Vol. 133: 167–177, 1996 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published March 28

Short-term fluctuations in salinity: effects on planktonic invertebrate larvae

Courtney E. Richmond[1,*], Sarah A. Woodin[2]

[1]Marine Science Program, University of South Carolina, Columbia, South Carolina 29208, USA
[2]Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208, USA

ABSTRACT: Estuarine habitats are characterized by environmental changes on many time scales. While certain physical parameters change on a regular, predictable basis, rapid environmental fluctuations can also occur as a result of stochastic events. For example, rainstorms are frequent, unpredictable events that can cause dramatic changes in the estuarine environment, in short periods of time. As a result of storm precipitation and terrestrial freshwater runoff, estuarine salinity can drop in excess of 20‰ in 6 h. Our research focuses on the effects of these storm-induced salinity fluctuations on estuarine embryos and larvae in terms of growth, mortality, rate of development, and activity. We studied 2 species of estuarine invertebrates: the embryos and larvae of the mud snail *Ilyanassa obsoleta* and the larvae of the marine polychaete *Arenicola cristata*. In both species, greater reductions in salinity resulted in smaller larvae. Both the duration of salinity reduction and the age of embryos and larvae when exposed to a salinity reduction affected growth rates: longer and earlier (relative to embryonic or larval age) storms had a greater effect on growth rates, resulting in smaller larvae. Both species also exhibited changes in activity when exposed to salinity reductions: *I. obsoleta* larvae swam significantly less at 10‰, while *A. cristata* larvae displayed a delay in activities associated with the sequence of development from swimming trochophore, to crawling larva, to settled metamorphic juvenile. Our results show that stochastic events may have a significant impact on the growth and development of pre-settlement estuarine invertebrates, implying ecological effects on recruitment success.

KEY WORDS: Salinity · Larvae · Fluctuations · Growth · Activity · Mortality Stoichiometry

INTRODUCTION

Estuaries are dynamic habitats with predictable environmental changes linked to processes such as tidal flow and diurnal temperature regimes. Superimposed upon this regular pattern of change in physical parameters are stochastic events such as storms, which can bring about rapid, dramatic changes in the estuarine environment.

Ten years of data on salinity levels in a South Carolina (USA) estuary with little riverine input show large fluctuations following precipitation events. In the absence of storms, high salinity levels in this vertically well-mixed estuary range between the mid-20‰'s and mid-30‰'s, depending on both the season and recent weather patterns (Service & Feller 1992). Following a storm, salinity can drop to 10‰ or below, over the course of approximately 6 h (Long Term Ecological Research data, North Inlet Estuary, SC). Because a large proportion of the freshwater input into the North Inlet Estuary comes from terrestrial runoff and low salinity water from the adjacent Winyah Bay, the combination of a large precipitation event and subsequent terrestrial runoff and riverine input can reduce estuarine salinity levels for several days (Michener et al. 1988, Blood & Vernberg 1992). Such drops in salinity occur throughout the entire water column, due to the well-mixed nature of the North Inlet Estuary. With very large freshwater inputs, such reductions for the entire water column are also seen in estuarine systems that are normally stratified (Schroeder 1978, Browder 1985, Smetacek 1986, Allen & Turner 1989, Wilber 1992).

Larvae raised in low salinity seawater exhibit slowed growth rates, increased mortality, and a possible delay of metamorphosis (bivalves: Gunter 1955; gastropods:

*E-mail: richmond.courtney@scarolina.edu

Scheltema 1965, Rosenberg & Rosenberg 1973, Zimmerman & Pechenik 1991; crustaceans: Costlow et al. 1971). Larvae raised under conditions of low temperature or an insufficient food supply show similar effects: the larvae take longer to develop, and frequently delay metamorphosis in stressful physical conditions (bivalves: Lucas & Costlow 1979, Lima & Pechenik 1985; gastropods: Scheltema 1962, 1967, Scheltema & Williams 1982; polychaetes: Qian et al. 1990; multiple taxa: Pechenik 1987, 1990). In the majority of these tests, the larvae were exposed to steady-state experimental conditions, not pulse events such as are seen in estuaries. Studies of embryos developing inside benthic egg capsules have shown that organisms are only partially protected from changes in ambient salinity; while the rate of salinity reduction is reduced inside the egg capsules, the embryos still experience the salinity alterations (Pechenik 1982, 1983).

Because estuaries are constantly changing habitats, we became interested in how larvae and embryos are affected by fluctuations in their physical environment, particularly those caused by large precipitation events. How are larval growth and activity levels affected by the freshwater introduced by storms in the estuary? Do we see differential larval growth in response to storm length, larval age at storm onset, or the storm-reduced salinity level? What happens when embryos developing inside egg capsules are exposed to salinity fluctuations?

In this study, we addressed these questions using the larvae and embryos of the marine mud snail *Ilyanassa obsoleta* and the larvae of the marine polychaete *Arenicola cristata*. The veliger larvae of *I. obsoleta* hatch out of benthic egg capsules after approximately 8 d, and live and feed in the plankton for a period of approximately 11 d before metamorphosis (given the appropriate chemical cue) into the benthic juvenile form (Scheltema 1967, Pechenik 1975, Strathmann 1987). *A. cristata* has lecithotrophic larvae that are planktonic for 2 to 4 d after hatching out of a gelatinous egg mass. After this brief planktonic period, larvae begin searching for a benthic settlement site; benthic tube-building indicates settlement and the beginning of the juvenile period (Okuda 1946, Strathmann 1987). Both *A. cristata* and *I. obsoleta* are common estuarine species found throughout the North Inlet Estuary.

MATERIALS AND METHODS

Salinity change. In all experiments, with the exception of growth Expt 1 with *Arenicola cristata*, we changed experimental salinities in a stepwise fashion, with a salinity change each hour over a 6 h period. This method of gradual salinity change was done both during the initiation of our simulated storms, and at the termination of storms, when salinities were returned to the pre-storm, control levels. Our intention in changing salinity in a gradual manner was to mimic the salinity changes seen in the estuary after a natural storm event. All seawater used in these experiments came from the North Inlet Estuary: we filtered this water twice through a 3 µm mesh cotton filter We mixed this filtered estuarine seawater with distilled water to bring the mixture to the desired salinity level, using a temperature-compensated refractometer to measure salinity. Controls had the same schedule of seawater change as did the experimental treatments, except that salinity remained the same with each seawater change.

***Ilyanassa obsoleta*: larval growth experiment.** In this experiment, the following variables were altered in order to assess the impact of salinity fluctuations on larval growth: (1) storm severity (degree to which salinity is lowered); (2) duration of the storm; and (3) the timing of the storm relative to larval age. Control salinity was 25‰; all experimental treatments were held at a salinity of 25‰ both prior to and after salinity reductions. There were 2 experimental salinities: 10 and 15‰; and 2 storm durations: 2 d and 4 d storms. Finally, there were 2 different times that the 2 d storms were initiated, relative to larval age: 2 d post-hatch, and 4 d post-hatch. We tested all combinations of these variable levels, such that for each of the 2 experimental salinities, there were 3 storm types: a 2 d storm initiated on Day 2, post-hatch; a 2 d storm initiated on Day 4, post-hatch; and a 4 d storm that began on Day 2, post-hatch.

For each of the 7 treatments (including controls), there were 3 replicates, arranged randomly within 3 plastic sweater boxes at all times, with the exception of the 6 h period when we changed salinity. We kept the larvae in 400 ml plastic beakers, with 64 µm mesh bottoms, which were then submerged inside 1000 ml plastic beakers filled with seawater. A small hole cut into the 1000 ml beaker allowed us to drain the seawater out until the beaker was only half full; when this was done, any larvae within the inner, mesh-bottomed beaker were still submerged in seawater. In this way, we could replace the drained seawater without removing the larvae from submersion at any time. Whenever we changed the seawater in any beakers, we also changed the seawater in all other beakers, replacing the drained seawater with new seawater at the appropriate salinity level for that treatment. After replacement, all beakers were mixed, to avoid salinity gradations within a replicate. Throughout the experiment, larvae were maintained in a 12 h:12 h light:dark cycle.

We set up the experiment on Day 1, post-hatch; all larvae had hatched within the previous 24 h period. We allowed the larvae to acclimate to the experimental beakers in 25‰ seawater for 24 h, before initiation of salinity reductions (those storms that began on Day 2, post-hatch). There were 100 larvae for each of the 3 replicate beakers. At each measurement date, we measured a subsample of 10 larvae from each beaker, and then returned these larvae to their original beakers; thus, the same larvae were not measured each time. Larval size was the maximum dimension of the larval shell, measured by ocular micrometer. These measurements were made on Days 1, 4, 6 and 13, post-hatch; Day 4 is a mid-storm measurement; Day 6 is a measurement taken after all storms were completed; and the measurement on Day 13 is 1 wk after the end of all storms. We fed larvae from an algal culture of *Isochrysis galbana* each day, such that algal cell densities were 6.25×10^6 cells ml^{-1} (Days 1 and 2, post-hatch), 7.9×10^6 cells ml^{-1} (Days 3 to 10), and 16.8×10^6 cells ml^{-1} (Days 10 to 13). At this daily feeding time, we also stirred the seawater in each 1000 ml beaker by swirling the inner beaker inside the outer beaker, both to evenly distribute the algal cells, and to ensure oxygenation of the seawater. We changed seawater every other day in all beakers, replacing seawater with that at the appropriate salinity for each experimental beaker. We observed *Isochrysis* in each of our experimental salinities, and confirmed that the algal cells are able to survive and swim actively for a minimum of 2 d in salinities as low as 5‰.

The sizes of larvae were analyzed by a nested analysis of variance, by date. Replicate beakers were nested under storm type, where 'storm type' represents the 7 distinct combinations of storm salinity, duration, and initiation timing, including controls. The beaker nested term was the error term for both salinity and storm components of the ANOVA, while the random error term was the error term for the beaker component of the ANOVA. The nested analysis of variance was followed by *a posteriori* Bonferroni multiple comparisons tests, at an experiment-wise error rate of $\alpha = 0.05$, using the appropriate error term for that component (Winer 1971). All data were examined for normality and homogeneity of variance. All analyses were done using PC-SAS, Version 6.03 (SAS Institute, Inc.).

***Ilyanassa obsoleta*: larval behavior experiment.** As in the growth experiment with *I. obsoleta* larvae, larvae were less than 24 h old (post-hatch) at initiation. We used one storm type, which began on Day 1, post-hatch, and continued through the end of the experiment, on Day 7, post-hatch. This storm had 3 experimental salinities: 5, 10 and 15‰. Control salinity was maintained at 25‰ throughout the course of the experiment.

We placed larvae into 24 well cell culture plates (1 plate $block^{-1}$), with 1 larva $well^{-1}$ (approximately 2 ml seawater $well^{-1}$). There were 4 blocks, with 6 replicate larvae salinity $^{-1}$ $block^{-1}$; we arranged replicates of the 4 treatments randomly within blocks. After set-up and an initial observation of all larvae, we began the salinity reductions, changing salinity in an hourly, stepwise fashion over a 6 h period. This hourly salinity change was accomplished by pipetting individual larvae into seawater at the appropriate salinity. On Days 1, 2, 3, 5 and 7, post-hatch, we observed the behavior of every larva under a dissecting microscope. The 2 behaviors recorded were: presence of activity, as evidenced by a velar ciliary beat, and larval swimming. Activity was recorded as either present or absent, avoiding any subjective judgment of activity levels. Each day, we changed the seawater in each well (replacing it with seawater of the appropriate salinity), and fed each larva in excess from an algal culture of *Isochrysis galbana* such that the algal cell density was 0.76×10^6 cells ml^{-1}.

We analyzed the behavioral data as percentages of larvae exhibiting each behavior in each treatment and block, within observation dates, using distribution-free multiple-comparisons nonparametric statistics, based on Kruskal-Wallis rank sums (Hollander & Wolfe 1973). The experiment was designed to be analyzed by repeated measures analysis of variance, but the data were non-normal and transformations did not correct this problem. Therefore, we used a nonparametric test instead.

***Ilyanassa obsoleta*: egg capsule experiments.** This experiment was conducted twice, in an identical manner. We collected adult *I. obsoleta* from the North Inlet Estuary and kept them in a laboratory environmental chamber until the snails began to lay benthic egg capsules. The egg capsules used in these experiments were laid within a 24 h period. The capsules we used were laid by a minimum of 10 different females; all capsules collected were combined and mixed before we assigned them to treatments.

The design of these experiments was similar to that of the larval growth experiments with *Ilyanassa obsoleta*, only with storms occurring while embryos were developing inside the egg capsules. There were 7 treatments, including a control treatment (31‰ in the first experiment, 29‰ in the second) and 2 storm salinities (10 and 15‰), with 3 storm types for each of these 2 storm salinities. Storm types consisted of variations in storm initiation dates and durations; at each salinity (10 and 15‰), the 3 storm types were: (1) a 2 d long storm, initiated on Day 2, post-hatch; (2) a 2 d long storm, initiated on Day 4, post-hatch; and (3) a 4 d long storm, initiated on Day 2, post-hatch. We placed 10 egg capsules into each of 6 replicate dishes $treatment^{-1}$:

egg capsules were collected from broods laid by upwards of 10 females, mixed, and then randomly partitioned among treatments. As with all our experiments, we changed salinity gradually over a 6 h period, at storm initiation and termination. Any time a treatment required a salinity change, we changed the seawater in all dishes, replacing it with seawater at the appropriate salinity for each treatment. While the egg capsule walls reduce the rate of exchange between the intracapsular fluid and ambient seawater, the intracapsular fluids should reach equilibrium with ambient seawater within approximately 30 min (Pechenik 1982, 1983). The salinity of the intracapsular fluids would then have equilibrated with the ambient seawater between each hourly seawater change during storm initiation and termination.

Once all salinity reductions were completed, and all dishes were restored to the control salinity level, we observed the capsules daily for occurrence of hatching: our data for age at hatching were the dates hatching began in each of the 6 replicate dishes storm type^{-1}. Once hatching began in a dish, we changed the seawater in that dish daily, removing all hatched larvae, in order to determine the first day on which a minimum of 100 larvae hatched. When this occurred, we then counted 100 larvae out of the dish, and transferred them to a new culturing dish containing seawater at the control salinity level (31‰ in the first experiment, 29‰ in the second). The following day, when larvae were 1 d old, we measured the longest shell length of a subset of 10 larvae, using a dissecting microscope and ocular micrometer. We measured 1 dimension of shell length (the longest distance across) after establishing the linear relationship between shell length and width (distance across the shell perpendicular to the length) (r^2 = 0.99). We analyzed these data by nested analysis of variance, with replicate dishes nested under storm type. The dishes' nested term was the error term for the storm type component of the ANOVA, while the random error term was the error term for the dishes' component. All analyses were done using PC-SAS, Version 6.03 (SAS Institute).

Arenicola cristata*: larval growth experiments.** ***Experiment 1: We used 1 storm type (3 d long), and 1 storm salinity (15‰) in this experiment. Control salinity was maintained at 29‰ throughout the experiment. All of the larvae used had hatched within the previous 24 h period. We maintained larvae inside 400 ml plastic beakers with 64 µm mesh Nitex bottoms; these beakers were then submerged within 1000 ml plastic beakers, the latter of which were filled with seawater. There were 4 replicate beakers per treatment, arranged randomly except during the 6 h period of salinity change at the storm beginning and end. All beakers contained seawater at the control salinity level before and after salinity reductions. The method of changing salinity in this experiment was different from that used in the growth experiment with *Ilyanassa obsoleta*: each of the 2 treatments (storm and control) had its own head tank of seawater, which simultaneously delivered seawater of the desired salinity into all 4 replicate beakers. In this way, we were able to change salinity in a continuous, gradual manner, over the course of a 6 h period. Whenever the seawater was changed in the storm treatment beakers, we also replaced the seawater in the control beakers with seawater at 29‰. We mixed the seawater within each beaker by swirling the inner beaker inside the outer beaker on an hourly basis. We also took hourly salinity measurements with a hand-held, temperature-compensated refractometer to establish that salinity changes were occurring at the appropriate rate.

The storm began on Day 1, post-hatch, and ended on Day 4, post-hatch. We measured a subsample of 10 larvae from each beaker on Days 1, 3 and 5, post-hatch. After measuring each subsample of larvae, the 10 individuals were returned to their experimental beakers to continue in the experiment. On Day 1, we measured larvae using the video digitizing software package MorphoSys, Version 1.26 (1990, C. A. Meacham, T. Duncan, University Herbarium, University of California, Berkeley, USA) with a video camera attached to a dissecting microscope. This software enabled us to measure larval area, which was the area encompassed by the outline of each individual larva. Because larvae crawling on vessel walls and juveniles in tubes cannot be digitized with our equipment, we could not use MorphoSys to estimate larval areas after Day 1. We confirmed a linear relationship between larval area as measured by MorphoSys, and larval area as calculated from direct measurements of larval length and width using a microscope and ocular micrometer (r^2 = 0.87). On Days 3 and 5, we calculated larval area by measuring the length and width of individual larvae, using an ocular micrometer, then multiplying these dimensions for a rectangular estimate of area. Although these estimates on Days 3 and 5 do not represent actual measurements of larval area, they do allow us to look at relative areas when comparing larvae within a measurement date. Larval size was analyzed by a nested analysis of variance by date, using PC-SAS, Version 6.03 (SAS Institute, Inc.). Beakers were nested under salinity: the beaker nested term was used as the error term for the salinity component of the ANOVA, while the random error term was the error term for the beaker component of the ANOVA. All data were examined for normality and homogeneity of variance.

Experiment 2: The data used in this experiment are measurements on larvae/juveniles in the behavior ex-

periment described below. We measured all larvae/juveniles (6 individuals block^{-1}; 3 blocks salinity^{-1}) on Days 1, 4, 6, 8, 10 and 12, post-hatch, using an ocular micrometer. As in Expt 1, we estimated larval/juvenile areas from measurements of larval/juvenile lengths and widths. Because larvae in this experiment were often in positions where we were unable to take measurements, we were unable to analyze the results using a repeated measures design. Instead, we analyzed these data using distribution-free multiple-comparisons nonparametric statistics, based on Kruskal-Wallis rank sums (Hollander & Wolfe 1973). Rank sums were calculated for the mean larval size per treatment and block, within observation dates.

***Arenicola cristata*: larval behavior experiment.** The design of this experiment was very similar to that of our behavior experiment with *Ilyanassa obsoleta*. In this experiment, there were 4 salinity levels: 5, 10, 15 and 32‰. We placed larvae into 24-well cell culture plates (blocks), with 1 larva well^{-1}. There were 5 blocks (culture plates) in this experiment, with 6 replicate individuals salinity level^{-1} block^{-1}; treatments were randomly distributed within blocks. Initial salinity level was 32‰ in all cell wells.

We set up the experiment when larvae were less than 24 h old, post-hatch (Day 0). We then began the 6 h period of salinity reduction to 'storm' levels on Day 1, post-hatch; these storm salinities were maintained throughout the duration of the experiment. We changed salinity as described in the behavior experiment with *Ilyanassa obsoleta*. After Day 2, post-hatch, we changed the seawater daily, replacing it with seawater of the appropriate experimental salinity. We fed larvae/juveniles every day from an algal culture of *Isochrysis galbana*, such that food was not limiting (algae was added such that densities were $>0.20 \times 10^6$ cells ml^{-1}).

The behaviors we observed were: swimming, crawling (pre-settlement, prior to building tubes), and settlement (benthic tube-building). We made behavioral observations of each individual larva/juvenile on Days 1, 2, 3, 4, 9 and 11 post-hatch; at each observation period, we looked at every individual in the experiment. Because some larvae exhibited more than one behavior during an observation, larval behaviors may add up to greater than 100% for certain treatments (see Table 4). Conversely, some of the larvae in 5 and 10‰ salinities were not counted as exhibiting any of the 4 behaviors scored; therefore, the behaviors for these treatments on some days may not add up to 100%. These larvae were generally near-death, but were not exhibiting any of the scored behaviors. The data were analyzed using distribution-free multiple-comparisons nonparametric statistics, based on Kruskal-Wallis rank sums (Hollander & Wolfe 1973) on the percentages of larvae exhibiting each behavior within each treatment and block, within observation dates. This experiment was designed as a repeated measures analysis of variance, but due to positions of individuals within the cell well plates, all individuals could not be seen on all days. For this reason, we used the same nonparametric test as was used for the *Ilyanassa obsoleta* behavioral data.

RESULTS

Ilyanassa obsoleta: larval growth experiments

The salinity level, timing relative to larval age, and length of storms affected larval growth in *Ilyanassa obsoleta* larvae (Fig. 1). At initiation, there were no significant differences in larval sizes among treatments (Fig. 1a). By Day 6, post-hatch, when all storms were over and all treatments were once again at the control salinity level, we began to see trends in the relative sizes of larvae in each treatment. On this day, larvae given 10‰ 4 d storms were significantly smaller than larvae in all other treatments, while larvae given 15‰ early 2 d storms were significantly larger than larvae in all other treatments except controls (Fig. 1b, Bonferroni multiple comparisons test, experiment-wise error rate of $\alpha = 0.05$). On Day 13, post-hatch, 1 wk after all storms were completed, the longer-term effect of salinity reductions on larval growth became evident. As on Day 6, larvae were significantly smaller than all other treatments in 4 d long, 10‰ salinity reductions (Fig. 1c, Bonferroni multiple comparisons test, experiment-wise error rate of $\alpha = 0.05$). Control larvae were significantly larger than the larvae in all storm treatments. Larvae exposed to late 2 d storms at 10‰ were significantly smaller than all treatments except for those larvae given 4 d, 10‰ storms (Fig. 1c, $\alpha = 0.05$). Within salinities, larvae in late 2 d storms were significantly smaller than those in early 2 d storms (Fig. 1c, $\alpha = 0.05$). The overall long-term pattern of growth was that larvae given storms that were either later, longer, or at a lower salinity were smaller at almost 2 wk of age, 1 wk after termination of all salinity manipulations.

Ilyanassa obsoleta: larval behavior experiments

At initiation of the experiment, there were no significant differences in activity levels between treatments, in terms of both velar beat and swimming (Table 1, Day 1, pre-storm). However, by the end of the 6 h period of salinity reduction on Day 1, larvae were swimming significantly less in 5‰ seawater than in

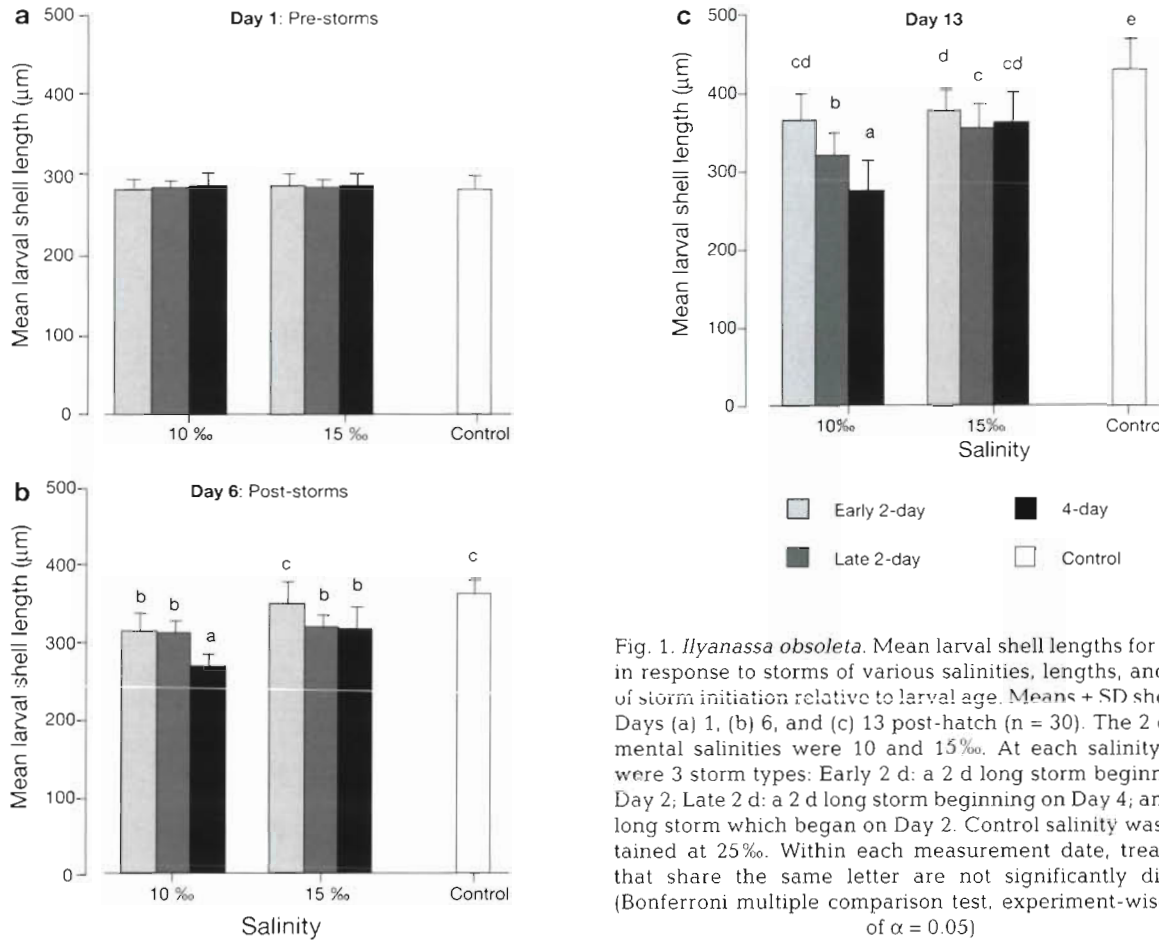

Fig. 1. *Ilyanassa obsoleta*. Mean larval shell lengths for larvae, in response to storms of various salinities, lengths, and times of storm initiation relative to larval age. Means + SD shown on Days (a) 1, (b) 6, and (c) 13 post-hatch (n = 30). The 2 experimental salinities were 10 and 15‰. At each salinity, there were 3 storm types: Early 2 d: a 2 d long storm beginning on Day 2; Late 2 d: a 2 d long storm beginning on Day 4; and a 4 d long storm which began on Day 2. Control salinity was maintained at 25‰. Within each measurement date, treatments that share the same letter are not significantly different (Bonferroni multiple comparison test, experiment-wise error of $\alpha = 0.05$)

all other treatments (Table 1a, Day 1, post-storm). At this time, larvae at 5‰ continued to beat their velar cilia, as did larvae from the other salinity levels; it was not until Day 2, post-hatch that larvae at this salinity exhibited a significant reduction in velar beat (Table 1b). In these first 2 d, there were no significant differences in activity between larvae in 10‰, 15‰, and control treatments.

Larvae exposed to 5‰ salinity reductions experienced 100% mortality by Day 5, post-hatch (Table 1); the majority of larvae at this salinity did not survive past Day 3, post-hatch. Larvae survived salinity reductions at 10‰, but showed a reduction in swimming behavior, relative to 15‰ storms and controls, by Day 3 (Table 1a); at this time, the larvae were found on the bottom of test containers, with velums fully opened

Table 1. *Ilyanassa obsoleta*. Mean percentage of larvae exhibiting (a) swimming, or (b) velar beat, during salinity reductions (4 blocks, 6 larvae block^{-1} salinity treatment^{-1}). Means ± SD shown, n = 24. Storms began on Day 1, post-hatch, and continued through Day 7. Storm salinities were 5, 10, and 15‰; control salinity was 25‰. Treatments that share the same letter, within days, were not significantly different (multiple-comparisons nonparametric statistics, $\alpha = 0.05$). Means are based on percentage of larvae exhibiting each behavior, within each block. Where enumerated as 'dead', the larval bodies had begun to decompose

Larval age (days post-hatch)	Salinity 5‰	10‰	15‰	25‰
(a) Swimming				
1 (Pre-storm)	100 (±0) A	100 (±0) A	100 (±0) A	100 (±0) A
1 (Post-storm)	17 (±14) A	96 (±8) B	96 (±8) B	100 (±0) B
2	0 (±0) A	79 (±16) B	75 (±21) B	79 (±21) B
3	0 (±0) A	68 (±20) B	83 (±14) C	71 (±28) BC
5	Dead	39 (±16) A	63 (±28) B	58 (±17) B
7	Dead	38 (±25) A	63 (±25) B	53 (±15) B
(b) Velar beat				
1 (Pre-storm)	100 (±0) A	100 (±0) A	100 (±0) A	100 (±0) A
1 (Post-storm)	92 (±17) A	100 (±0) A	100 (±0) A	100 (±0) A
2	67 (±19) A	100 (±0) B	100 (±0) B	100 (±0) B
3	14 (±19) A	100 (±0) B	100 (±0) B	96 (±8) B
5	Dead	95 (±10) A	100 (±0) A	100 (±0) A
7	Dead	88 (±25) A	100 (±0) A	100 (±0) A

and velar cilia beating. This continued through Days 5 and 7, post-hatch; 15‰ storm treatments and controls were not significantly different throughout the experiment (Table 1a).

Ilyanassa obsoleta: egg capsule experiments

In both of our experiments exposing *Ilyanassa obsoleta* egg capsules to salinity reductions, larvae given 10‰, 4 d long salinity reductions hatched later than larvae in all other treatments (Table 2a, Bonferroni multiple comparisons test, experiment-wise error rate of $\alpha = 0.05$). In addition, larvae given late 2 d long storms were significantly smaller 1 d after hatching than for all other treatments, in both experiments (Table 2b, Bonferroni multiple comparisons test, experiment-wise error rate of $\alpha = 0.05$). There are several trends in the data which are not significant, but are interesting; for example, control larvae were always the first to hatch (Table 2a), and had the largest mean size of all treatments (Table 2b). Also, within salinities, larvae given 4 d long storms hatched later than larvae in both early and late 2 d long storms. Finally, within a storm type (both initiation timing and storm duration), egg capsules given storms at 15‰ always hatched before those at 10‰.

In Expt 1, there was no correlation between the age at which egg capsules began hatching and the size of the larvae one day after hatching ($F = 0.323$, $p \leq 0.59$, df = 6, $r^2 = 0.06$) However, in Expt 2, the age of egg capsules at hatching was a good predictor of the size of new hatches: larvae that hatched early were larger than those that hatched later ($F = 9.241$, $p \leq 0.03$, df = 5, $r^2 = 0.65$) (these results for Expt 2 do not incorporate the 10‰ 4 d treatment, due to insufficient data for sizes at hatching).

Of the egg capsules exposed to 10‰, 4 d long storms in Expt 2, larvae hatched in 4 of the 6 replicate dishes; the dates these 4 dishes hatched were used in the analysis of age at hatching (Table 2a). However, of these 4 dishes that had hatches, only 1 dish had 100 larvae hatch, the minimum required to continue the experiment to take larval measurements. For this reason, we did not use the measurements taken from the 10‰ 4 d treatment in Expt 2 in the statistical analysis of size at hatching (Table 2b).

Table 2. *Ilyanassa obsoleta*. Mean (a) age (days) and (b) size (µm) of embryos at first hatching, in response to salinity reductions (10 egg capsules dish^{-1}, 6 replicate dishes storm treatment^{-1}). Means ± SD shown; (a) n = 6, (b) n = 60. Storm salinities were 10 and 15‰; control salinity was 31‰ in Expt 1, and 29‰ in Expt 2. 'Storm timing' indicates the age of egg capsules when salinity reductions were administered; early storms were on Days 2 to 4, late storms were on Days 4 to 6, and 4 d long storms were on Days 2 to 6. Treatments that share the same letter, within each experiment, were not significantly different (Bonferroni multiple comparisons test; experiment-wise error at $\alpha = 0.05$)

Salinity (storm timing)	Hatch age Expt 1 Mean (±SD)	Hatch age Expt 2 Mean (±SD)
(a) Age at hatching		
10‰ (early 2 d)	10.2 (±0.4) B	10.0 (±1.1) BC
10‰ (late 2 d)	9.8 (±0.4) BC	11.2 (±0.7) B
10‰ (4 d)	12.7 (±0.8) A	13.8[a] (±1.9) A
15‰ (early 2 d)	9.2 (±0.4) BC	8.7 (±0.8) CD
15‰ (late 2 d)	8.7 (±0.8) CD	9.3 (±0.8) CD
15‰ (4 d)	9.7 (±0.8) BC	10.3 (±0.8) BC
Control (no storm)	7.8 (±0.4) D	7.8 (±0.7) D
(b) Size at hatching		
10‰ (early 2 d)	306 (±9) B	284 (±11) AB
10‰ (late 2 d)	289 (±8) D	260 (±39) D
10‰ (4 d)	311 (±16) AB	259[b] (±23)
15‰ (early 2 d)	298 (±7) C	285 (±10) AB
15‰ (late 2 d)	291 (±10) D	270 (±13) C
15‰ (4 d)	298 (±11) C	279 (±15) B
Control (no storm)	312 (±10) A	290 (±10) A

[a] Mean age (a) for 10‰ 4 d storms in Expt 2 is based on 4 replicates

[b] Mean size (b) at hatching for 10‰ 4 d storms in Expt 2 is based on size of hatches from one replicate; the mean and SD are for these 10 larvae measured. These data are not included in the statistical analysis

Arenicola cristata: larval growth experiments

In Expt 1, *Arenicola cristata* larvae exposed to 15‰ salinity reductions were not significantly different in size from controls on Day 1, post-hatch (Fig. 2a, $p = 0.9631$, $F = 0.00$, MS = 0.00000018, df = 1) or on Day 3, post-hatch (Fig. 2a, $p = 0.4381$, $F = 0.69$, MS = 0.00025, df = 1). By Day 5, however, larvae given 15‰ salinity reductions were significantly smaller than control larvae (Fig. 2a, $p = 0.0009$, $F = 37.50$, MS = 0.00578, df = 1). In Expt 2, larvae were significantly smaller in 15‰ storms than controls by Day 6, post-hatch, and this size discrepancy continued through the end of the experiment, on Day 12 (Fig. 2b).

Arenicola cristata: larval behavior experiments

Larvae exposed to salinity reductions at both 5 and 10‰ experienced 100% mortality during the course of the experiment (Table 3). Those in 5 and 10‰ salinity seawater did not survive past Day 2, post-hatch (1 d into salinity reductions). After Day 2, there was no significant difference in mortality between larvae given 15‰ and control storms.

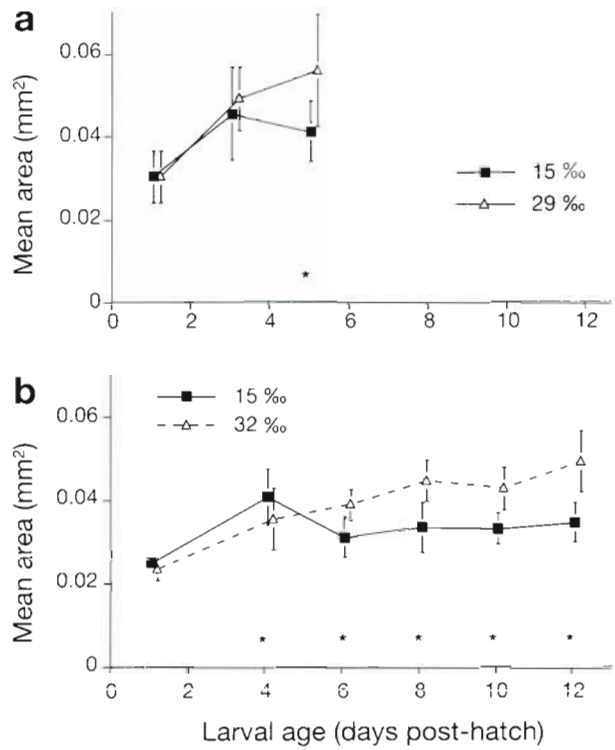

Fig. 2. *Arenicola cristata*. Mean size of larvae, in response to reduced salinities. Means ± SD shown; (a) n = 40, (b) n = 30. (a) Results of Expt 1 (3 beakers treatment^{-1}). Salinity reductions began on Day 1, post-hatch, and continued through Day 4, post-hatch. The asterisk above Day 5 indicates a significant difference between treatments (1-way, nested ANOVA). (b) Results of Expt 2 (5 blocks, 6 larvae treatment^{-1} block^{-1}). Salinity was reduced in the storm treatment on Day 1, post-hatch, and remained at the reduced level through Day 12. Values shown are means ± SD for each salinity, within days. Asterisks above Days 4, 6, 8, 10, and 12 indicate significant differences among treatments (multiple-comparisons nonparametric statistics, $\alpha = 0.05$)

DISCUSSION

Storm events can greatly affect estuarine environments, creating sizable, stochastic alterations in physical parameters on top of the normal estuarine pattern of diurnal and lunar fluctuations. Parameters such as temperature, nutrient concentration, and salinity can change drastically within hours as a result of a large precipitation event (Schroeder 1978, Browder 1985, Smetacek 1986, Allen & Turner 1989, Blood & Vernberg 1992, Wilber 1992). In our experiments, we addressed the question of how larvae and embryos are affected by storm-related fluctuations in salinity levels. Our findings are consistent with several previous studies which have shown that larvae grown in reduced salinity levels grow at significantly slower rates (Scheltema 1965). When we exposed larvae of both *Ilyanassa obsoleta* and *Arenicola cristata* to salinity fluctuations that mimicked those seen after a precipitation event in a well-mixed estuary, greater reductions in salinity yielded smaller larvae (Figs. 1 & 2). The same was true of *I. obsoleta* embryos inside egg capsules; those exposed to storms at 10‰ developed more slowly and hatched later than those given 15‰ salinity reductions (Table 2).

Three components of storms that potentially affect larvae are the degree to which the storm changes salinity, the duration of the salinity reduction, and the timing of the storm relative to larval age. In our experiments with *Ilyanassa obsoleta*, storm length and timing relative to larval age significantly altered larval growth and behavior (Fig. 1, Table 1). Our results indicate that it is the combination of all 3 variables: salinity reduction, storm length, and timing of the storm relative to larval age, that ultimately determines larval response to a storm event. Because of this, both the

There was an overall delay in the sequence of behavior leading to larval settlement in larvae given 15‰ salinity reductions, relative to controls. Larvae in this reduced salinity swam significantly longer and crawled significantly longer than controls (Table 4a, b), while the larvae in control seawater initiated settlement (benthic tube-building) significantly earlier than those given 15‰ storms (Table 4c). This 'lag' in settlement behavior between the experimental and control salinity reductions was present at least through Day 4, post-hatch; by Day 9, all surviving larvae had settled.

Table 3. *Arenicola cristata*. Mean percentage of larvae dead or moribund, in response to salinity reductions (5 blocks, 6 larvae treatment^{-1} block^{-1}). Data shown for larvae in 5 and 10‰ signify larval death, while larvae in 15 and 32‰ were either dead or moribund. Storms began on Day 1, post-hatch, and continued through Day 12. Storm salinities were: 5, 10, and 15‰; control salinity was maintained at 32‰. Treatments that share the same letter, within days, were not significantly different (multiple-comparisons nonparametric statistics, $\alpha = 0.05$). Means ± SD shown (n = 30). Where enumerated as 'dead', the larval bodies had begun to decompose. 'Moribund' individuals appeared dead but had not yet begun to decompose

Larval age (days post-hatch)	Salinity 5‰	10‰	15‰	32‰
1	30 (±14) A	4 (±9) BC	7 (±10) B	0 (±0) C
2	90 (±15) A	11 (±10) B	8 (±12) B	0 (±0) C
3	Dead	Dead	8 (±12) A	3 (±7) A
4	Dead	Dead	8 (±12) A	4 (±9) A
9	Dead	Dead	14 (±22) A	5 (±11) A
11	Dead	Dead	11 (±15) A	5 (±11) A

Table 4. *Arenicola cristata*. Mean percentage of larvae (a) swimming, (b) crawling, or (c) settled, in response to salinity reductions. Storms began on Day 1, post-hatch, and continued through Day 12. Storm salinities were: 5, 10, and 15‰; control salinity was maintained at 32‰. Data show means ± standard deviations (n = 30). Treatments that share the same letter, within days, are not significantly different (multiple-comparisons nonparametric statistics, experiment-wise error rate of $\alpha = 0.05$). Where enumerated as 'dead', the larval bodies had begun to decompose

Larval age (days post-hatch)	Salinity 5‰	10‰	15‰	32‰
(a) Percentage swimming				
1	0 (±0) A	0 (±0) A	21 (±23) B	40 (±14) C
2	0 (±0) A	0 (±0) A	22 (±7) B	10 (±15) C
3	Dead	Dead	23 (±25) A	0 (±0) B
4	Dead	Dead	0 (±0) A	0 (±0) A
9	Dead	Dead	0 (±0) A	0 (±0) A
11	Dead	Dead	0 (±0) A	0 (±0) A
(b) Percentage crawling				
1	7 (±9) A	66 (±24) B	85 (±20) C	53 (±18) D
2	0 (±0) A	55 (±20) B	85 (±9) C	77 (±15) D
3	Dead	Dead	52 (±31) A	21 (±18) B
4	Dead	Dead	75 (±31) A	0 (±0) B
9	Dead	Dead	4 (±9) A	0 (±0) A
11	Dead	Dead	0 (±0) A	0 (±0) A
(c) Percentage settled				
1	0 (±0)	0 (±0)	0 (±0)	0 (±0)
2	0 (±0)	0 (±0)	0 (±0)	0 (±0)
3	Dead	Dead	5 (±11) A	69 (±12) B
4	Dead	Dead	17 (±26) A	82 (±20) B
9	Dead	Dead	82 (±20) A	95 (±11) A
11	Dead	Dead	78 (±27) A	95 (±11) A

size of a storm event and the age of larvae during the event may be important in the field for larval growth and survival. Although we did not test larval growth in *Arenicola cristata* in response to simulated storms of varying lengths or timings, we were able to show that these larvae, like *I. obsoleta*, grow at significantly reduced rates when exposed to fluctuations in salinity levels (Fig. 2).

Larval behavioral responses to salinity change differed in the 2 species. In 10‰, *Ilyanassa obsoleta* larvae swam significantly less (Table 1a), though their velums were fully opened and their velar cilia were beating (Table 1b). This behavior may indicate larval energy conservation by reducing activity, while continuing to feed; the velum is used for feeding as well as for swimming and gas exchange (Zimmerman & Pechenik 1991). This behavior could also result in a descent in the water column, effectively increasing the salinity in the larval environment in a stratified estuarine system. In a well-mixed water column such as the North Inlet system, however, this behavior would not serve to change environmental conditions for the larvae. The behavioral changes seen in the larvae of *Arenicola cristata* were either associated with incipient mortality (5 or 10‰) (Table 3), or with a delay in the sequence of behavioral processes associated with the assumption of a benthic existence (15‰) (Table 4a–c). At 15‰, larvae took significantly longer to stop swimming and initiate crawling, a behavior associated with searching for a suitable benthic settlement site; larvae also initiated settlement, indicated by benthic tube-building, significantly later (Table 4c). While *A. cristata* larvae do not undergo a dramatic metamorphosis, this extension of the larval period is the equivalent of a delay in metamorphosis, an effect which has been documented for the larvae of several species of marine and aquatic organisms in response to stressful environmental conditions (Pechenik 1987).

Few studies have addressed how fluctuating versus constant physical conditions affect organisms during early developmental and larval stages. The results of these studies all share one conclusion: organisms are more tolerant of stressful environments when changes in physical conditions are gradual, occurring over a period of several hours, rather than abrupt (Barnes 1953, Cawthorne 1978, Sastry & Ellington 1978, Lucas & Costlow 1979, Rosenberg & Costlow 1979, Pechenik 1982, 1983). Organisms that are encapsulated during embryonic development benefit from a decrease in the rate of intrusion of ambient salinity changes into the intracapsular fluid; the walls of egg capsules of some species are only semi-permeable, slowing the flux of water transfer through this membrane (Pechenik 1982, 1983). Encapsulated embryos may be more tolerant of severe changes in their environment because of this protection in time. Because even dramatic changes in physical parameters in the estuary occur over several hours, rather than instantaneously, larvae and encapsulated embryos may be more likely to survive environmental stresses than previously predicted by steady-state experiments. This implies that many larvae may be experiencing developmental slowing or delays in the field. The duration of the stressful physical event (*Mulinia lateralis*; Kennedy et al. 1974), and the timing of the event relative to larval age or stage (*Rhithropanopeus harrisii*; Rosenberg & Costlow 1979) play significant roles in determining larval survival, growth, and developmental rates. The results of this study show how the combination of all of these variables —

fluctuating conditions, and stressful events of varying intensities, durations, and timing — can influence larval and embryonic growth and development, as well as larval behavior. With *Ilyanassa obsoleta* larvae, for example, 2 d long storms initiated on Day 4, post-hatch had significantly greater effects on growth rate than 2 d long storms initiated on Day 2, post-hatch (Fig. 1c).

These results have obvious implications for the larvae of estuarine invertebrates, and may explain some of the documented variability in recruitment (Service & Feller 1992). A delay of metamorphosis could have several negative effects on larvae, including an increased chance of encountering planktonic predators (Gunter 1955, Browder 1985, Allen & Turner 1989), additional exposure to unfavorable, storm-related physical conditions that could be increasingly detrimental to larval growth and survival, or an increase in the probability of larvae being 'lost' to estuarine systems as a result of increased flux due to freshwater input. This last possibility may be less important for estuarine larvae, at least in the case of well-mixed estuaries such as the North Inlet Estuary in South Carolina, a study spanning several years examined the zooplankton entering and exiting the estuary, and failed to find any significant export of invertebrate larvae other than those decapod larvae with specific mechanisms enabling them to exit the estuary (Stancyk & Feller 1986). Developmental delays also have the potential to be a favorable response to salinity fluctuations: by delaying settlement, larvae may in fact increase their chances of being transported to a part of the estuary where salinity pulses are less severe.

For larvae that survive a storm event and live through metamorphosis to at least the juvenile stage, there may still be a lasting effect in the juvenile or adult stages (Qian et al. 1990). Several studies on amphibians have assessed some of the ecological consequences of stressful environments during larval development. In the salamander *Ambystoma talpoideum*, for example, there is a direct relationship between environmental changes during the larval stage and fitness traits at the adult stage, such as adult size, survival, age at first reproduction, and fecundity (Semlitsch 1987, Semlitsch et al. 1988, Semlitsch & Wilbur 1988). The larvae of these salamanders hatch into an ephemeral environment — temporary ponds — which, depending on the drying time of the pond, lead to either early or late metamorphosing larvae. Larval characteristics, such as time to metamorphosis and body size at metamorphosis, were shown to have a lasting effect on several adult life history traits. Other studies have suggested that there may be a differential effect of delayed metamorphosis on non-feeding, lecithotrophic larvae, and feeding, planktotrophic larvae; larvae with yolky energy reserves may be even more adversely affected by delayed metamorphosis, due to a combination of depleted energy reserves and a smaller size at metamorphosis (Miller & Hadfield 1990). Still other studies have failed to find any effects on the juvenile fitness of larvae that delayed metamorphosis (Pechenik & Eyster 1989: *Crepidula fornicata*, planktotrophic larvae; Pechenik & Cerulli 1991: *Capitella* sp. I, lecithotrophic larvae).

Given the unpredictable, dynamic nature of the estuarine habitat, larvae will not be affected by only one variable, such as magnitude of the freshwater input flux. As we have shown here, the timing, duration, and magnitude of a physical change all significantly affected such variables as larval size and activity (Figs. 1 & 2, Tables 1 to 4). When salinity levels can fluctuate on the order of 20‰ over the course of only hours following a large storm event in a well-mixed estuary such as the North Inlet Estuary (Blood & Vernberg 1992), we can assume that larvae in the field are affected. One such effect is evident in the long-term data record for the North Inlet Estuary; much of the short-term fluctuations in species abundances in this estuary have been attributed to short-term climatic fluctuations (Michener et al. 1988). As we learn more about the response of these estuarine organisms to stochastic changes in their physical environment, we may begin to understand some of the underlying forces driving longer-term patterns of population dynamics.

Acknowledgements. Comments by B. Coull, J. Eckman, S. Stancyk, C. H. Peterson, and 2 anonymous reviewers improved the manuscript. We thank Dorian Houser, Yvonne Hui, Michelle Johnson, and Anne Kitchell for aiding in the experimental execution. We also thank the Baruch Institute for providing access to collection sites and laboratory facilities. C.E.R. thanks S. Lindsay and L. Blackburn for their constant support. Support was provided by NSF grant OCE-8900212 to S.A.W. This is contribution no. 1041 from the Belle W. Baruch Institute for Coastal Research.

LITERATURE CITED

Allen RL, Turner RE (1989) Environmental influences on the oyster industry along the west coast of Florida. J Shellfish Res 8(1):95-104

Barnes H (1953) The effect of lowered salinity on some barnacle nauplii. J Anim Ecol 22:328-330

Blood ER, Vernberg FJ (1992) Characterization of the physical, chemical and biological conditions and trends in Winyah Bay and North Inlet Estuaries: 1970-1985, Vol II. SC Sea Grant Consortium, Charleston, SC, p I-III-117

Browder JA (1985) Relationship between pink shrimp production on the Tortugas grounds and water flow patterns in the Florida Everglades. Bull mar Sci 37:839-856

Cawthorne DF (1978) Tolerance of some cirripede nauplii to fluctuating salinities. Mar Biol 46:321-325

Costlow JD Jr, Bookhout CG, Monroe R (1971) The effect of salinity and temperature on larval development of

Sesarma cinereum (Bosc) reared in the laboratory. Biol Bull mar biol Lab, Woods Hole 118:183–202
Gunter G (1955) Mortality of oysters and abundance of certain associates as related to salinity. Ecology 36:601–605
Hollander M, Wolfe DA (1973) Nonparametric statistical methods. John Wiley & Sons, Inc, New York
Kennedy VS, Rosenburg WH, Zion HH, Castagna M (1974) Temperature-time relationships for survival of embryos and larvae of *Mulinia lateralis* (Mollusca:Bivalvia). Mar Biol 24:137–145
Lima G, Pechenik JA (1985) The influence of temperature on growth rate and length of larval life of the gastropod *Crepidula plana* Say. J exp mar Biol Ecol 90:55–71
Lucas JS, Costlow JD Jr (1979) Effects of various temperature cycles on the larval development of the gastropod mollusc *Crepidula fornicata*. Mar Biol 51:111–117
Michener WK, Allen DM, Blood ER, Hiltz TA, Kjerfve B, Sklar FH (1988) Climatic variability and salt marsh ecosystem response: relationship to scale. In: Greenland D, Swift LW Jr (eds) Climate variability and ecosystem response. Proceedings of a long-term ecological research workshop. USDA Forest Service, General Technical Rep SE-65, Asheville, NC, p 27–37
Miller SE, Hadfield MG (1990) Developmental arrest during larval life and life-span extension in a marine mollusc. Science 248:356–358
Okuda S (1946) Studies on the development of Annelida Polychaeta I. J Fac Sci Hokkaido, Imp Univ Ser 6 (Zoology) 9(2):115–219
Pechenik JA (1975) The escape of veligers from the egg capsules of *Nassarius obsoletus* and *Nassarius trivittatus* (Gastropoda, Prosobranchia). Biol Bull mar biol Lab, Woods Hole 149:580–589
Pechenik JA (1982) Ability of some gastropod egg capsules to protect against low-salinity stress. J exp mar Biol Ecol 63:195–208
Pechenik JA (1983) Egg capsules of *Nucella lapillus* (L.) protect against low-salinity stress. J exp mar Biol Ecol 71. 165–179
Pechenik JA (1987) Environmental influences on larval survival and development. In: Giese AC, Pearse JS, Pearse VB (eds) Reproduction of marine invertebrates, Vol IX. Blackwell Scientific, Palo Alto, p 551–608
Pechenik JA (1990) Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? Ophelia 32(1–2):63–94
Pechenik JA, Cerulli TR (1991) Influence of delayed metamorphosis on survival, growth, and reproduction of the marine polychaete *Capitella* sp. I. J exp mar Biol Ecol 151:17–27
Pechenik JA, Eyster LS (1989) Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. Biol Bull 176:14–24
Qian PY, McEdward LR, Chia FS (1990) Effects of delayed settlement on survival, growth, and reproduction in the spionid polychaete, *Polydora ligni*. Invertebr Reprod Dev 18(3):147–152
Rosenberg R, Costlow JD Jr (1979) Delayed response to irreversible non-genetic adaptation to salinity in early development of the brachyuran crab *Rhithropanopeus harrisii*, and some notes on adaptation to temperature. Ophelia 18(1):97–112
Rosenberg R, Rosenberg K (1973) Salinity tolerance in three Scandinavian populations of *Littorina littorea* (L.) (Gastropoda). Ophelia 10:129–139
Sastry AN, Ellington WR (1978) Lactate dehydrogenase during the larval development of *Cancer irroratus*: effect of constant and cyclic thermal regimes. Experientia 34: 308–309
Scheltema RS (1962) Environmental factors affecting length of pelagic development in the gastropod, *Nassarius obsoletus*. Am Zool 2:445
Scheltema RS (1965) The relationship of salinity to larval survival and development in *Nassarius obsoletus* (Gastropoda). Biol Bull mar biol Lab, Woods Hole 129: 340–354
Scheltema RS (1967) The relationship of temperature to the larval development of *Nassarius obsoletus* (Gastropoda). Biol Bull mar biol Lab, Woods Hole 132(2):253–265
Scheltema RS, Williams IP (1982) Significance of temperature to larval survival and length of development in *Balanus eburneus* (Crustacea: Cirripedia). Mar Ecol Prog Ser 9: 43–49
Schroeder WW (1978) Riverine influence on estuaries: a case study. In: Wiley ML (ed) Estuarine interactions. Academic Press, New York, p 347–364
Semlitsch RD (1987) Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. Ecology 68(4):994–1002
Semlitsch RD, Scott DE, Pechmann JHK (1988) Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69(1):184–192
Semlitsch RD, Wilbur HM (1988) Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. Copeia 4:978–983
Service SK, Feller RJ (1992) Long-term trends of subtidal macrobenthos in North Inlet, South Carolina. Hydrobiologia 231:13–40
Smetacek VS (1986) Impact of freshwater discharge on production and transfer of materials in the marine environment. In: Skreslet S (ed) The role of freshwater outflow in coastal marine ecosystems. Springer-Verlag, Berlin, p 85–106
Stancyk SE, Feller RJ (1986) Transport of non-decapod invertebrate larvae in estuaries: an overview. Bull mar Sci 39(2): 257–268
Strathmann MF (1987) Reproduction and development of marine invertebrates of the Northern Pacific Coast: data methods for the study of eggs, embryos, and larvae. University of Washington Press, Seattle
Wilber DH (1992) Associations between freshwater inflows and oyster productivity in Apalachicola Bay, Florida. Estuar coast Shelf Sci 35:179–190
Winer BJ (1971) Statistical principles in experimental design, 2nd edn. McGraw-Hill, New York
Zimmerman KM, Pechenik JA (1991) How do temperature and salinity affect relative rates of growth, morphological differentiation, and time to metamorphic competence in larvae of the marine gastropod *Crepidula plana*? Biol Bull 180:372–386

This article was presented by C. H. Peterson (Senior Editorial Advisor), Morehead City, North Carolina, USA

Manuscript first received: January 30, 1995
Revised version accepted: August 15, 1995