Effects of Developmental Acclimation on Adult Salinity Tolerance in the Freshwater-Invading Copepod *Eurytemora affinis*

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ABSTRACT

Invasive species are commonly thought to have broad tolerances that enable them to colonize new habitats, but this assumption has rarely been tested. In particular, the relative importance of acclimation (plasticity) and adaptation for invasion success are poorly understood. This study examined effects of short-term and developmental acclimation on adult salinity tolerance in the copepod *Eurytemora affinis*. This microcrustacean occurs in estuarine and salt marsh habitats but has invaded freshwater habitats within the past century. Effects of short-term acclimation were determined by comparing adult survival in response to acute versus gradual salinity change to low salinity (fresh water). Effects of developmental acclimation on adult tolerance were determined using a split-brood 4 × 2 factorial experimental design for one brackish-water population from Edgartown Great Pond, Massachusetts. Twenty full-sib clutches were split and reared at four salinities (fresh, 5, 10, and 27 practical salinity units [PSU]). On reaching adulthood, clutches from three of the salinity treatments (no survivors at fresh) were split into low- (fresh) and high- (40 PSU) salinity stress treatments, at which survival was measured for 24 h. Short-term acclimation of adults did not appear to have a long-term affect on low-salinity tolerance, given that gradual transfers to fresh water enhanced survival relative to acute transfers in the short term (after 7 h) but not over a longer period of 8 d. Developmental acclimation had contrasting effects on low- versus high-salinity tolerance. Namely, rearing salinity had a significant effect on tolerance of high-salinity (40 PSU) stress but no significant effect on tolerance of low-salinity (freshwater) stress. In addition, there was a significant effect of clutch on survival under freshwater conditions, indicating a genetic component to low-salinity tolerance but no significant clutch effect in response to high salinity. While developmental acclimation might enhance survival at higher salinities, the minimal effect of acclimation and significant effect of clutch on low-salinity tolerance suggest the importance of natural selection during freshwater invasion events.

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Introduction

Acclimation is a nonheritable change in an individual's physiology in response to environmental conditions (Freeman and Herron 1998). Rigorous tests of acclimation are important for determining factors that limit geographic ranges of populations (Davis and Shaw 2001). For instance, determining the contribution of developmental plasticity versus selection during habitat shifts could yield insights into mechanisms of invasion success (Lee 2002). A common assumption is that invasive species possess broad physiological tolerances that enable them to colonize new habitats (Baker 1965; Wolff 2000), but this assumption has rarely been tested (Lee 2002).

Invasions of fresh water by brackish and marine species have been increasing in recent years due to human activity and characterize invasion pathways of many aquatic invaders in Europe and North America (Lee 1999; Lee and Bell 1999; Ricciardi and MacIsaac 2000). The main objective of this study was to determine effects of short-term and developmental acclimation on adult salinity tolerance for a rapidly colonizing species, the copepod *Eurytemora affinis*. While some studies examined effects of salinity on development rate and tolerance of invertebrates (Roller and Stickle 1994; Walker and Clare 1994; Wright et al. 1996), effects of developmental salinity on adult performance or tolerance have rarely been tested.

Eurytemora affinis is a copepod sibling species complex (Lee 2000; Lee and Frost 2002) that occurs primarily in brackish lakes and estuaries (2–25 practical salinity units [PSU]) and hypersaline salt marshes (25–40 PSU) in North America, Asia, and Europe. However, within the past 100 yr, populations have invaded freshwater lakes and reservoirs (~0 PSU) independently within and among genetically distinct clades throughout its range (Lee 1999). Thus, except for one clade in the Pacific Northwest of North America, which has no known freshwater descendants (Lee 2000), each genetically divergent clade contains populations residing in diverse habitats, including salt marshes, estuaries, and freshwater lakes.

The specific goals of this study were (1) to determine effects of short-term acclimation by comparing effects of acute versus gradual changes in salinity on survival, (2) effects of developmental acclimation on adult tolerance of low and high salinity, and (3) effects of clutch (genotype) on tolerance of low and high salinity. Effects of acute (15–0 PSU) and gradual changes (same change via 2 PSU/h decrements) in salinity were compared to determine whether rate of salinity change affects survival and to identify which decremental change in salinity exacts the greatest mortality. The treatment with gradual salinity change allows for short-term acclimation, while the acute treatment does not. Effects of developmental acclimation were examined by splitting and rearing 20 clutches across three salinities and then measuring tolerances of the adults at high-salinity (40 PSU) and low-salinity (freshwater) treatments. The ability to acclimate during development would suggest that rearing salinity could enhance the ability to tolerate novel habitats and facilitate invasion events. Effect of clutch on adult tolerance was determined by performing an ANOVA within and among clutches in response to salinity stress. A significant effect of clutch would indicate a genetic component affecting salinity tolerance, providing substrate on which natural selection could act during habitat invasion events.

Material and Methods

Study Population

Eurytemora affinis (Poppe 1880) was collected in May 1996 from Edgartown Great Pond, Martha's Vineyard, Massachusetts. This population occurs in a large brackish lake separated from the ocean by a sandbar. A few hundred copepods were collected by towing a 100-μm plankton net. The sample used for this study was collected when the lake was at a salinity of 11 PSU. This population is more tolerant of fresh water than other saline populations tested (Lee 1999) and was chosen to determine the maximal extent to which a saline population could acclimate to low-salinity conditions.

Acute and Gradual Transfers to Low Salinity

Response to acute and gradual salinity change were compared to determine whether rate of salinity change affects survival in response to low salinity (fresh water). The prediction was that short-term acclimation during gradual transfers to low salinity would result in greater survival than during acute transfers. For this experiment, the population was reared at 15 PSU for at least two generations using the same temperature, light cycle, and food and water source described in the next section. Three treatments were used: (1) gradual transfers, in which salinity was changed from 15 to 0 PSU at a rate of approximately 2 PSU/h for 7 h, (2) acute transfers, in which salinity was changed suddenly from 15 to 0 PSU (fresh water), but the water was changed every hour for 7 h (to control for handling), and (3) controls, in which salinity remained constant at 15 PSU, but the water was changed every hour for 7 h. After the initial 7 h, salinity remained a constant at 0 PSU in the gradual and acute treatments and at 15 PSU in the control for a period of 8 d. There were three replicates per treatment, and 50 copepods per replicate. Mortality was measured every hour for the first 7 h and then daily for 8 d.

Conditions during Development

Populations were maintained at 10 PSU in a 13°C environmental chamber for at least two generations prior to the experiments. Twenty egg sacs (full-sib clutches) were excised from adult females with a pin and quartered into four salinity treatments (ca. 4–11 eggs each) of 0 (lake water), 5, 10, and 27 PSU. The treatment salinity of 10 PSU was chosen as the control because it was proximate to the salinity at which the population was found. Water of varying salinity was made from mixtures of water from Lake Union or Lake Washington, Seattle (0 PSU, fresh lake water), and Puget Sound, Washington (27 PSU). Conductivity of Lake Union and Lake Washington ranges from 100 to 400 μS/cm, which is approximately equivalent to 0.05–0.2 PSU.

Eggs were placed in 20-mL scintillation vials maintained half full with caps left ajar to allow oxygen exchange. Vials were kept in a 13°C environmental chamber maintained on an 8D : 16L light cycle. To avoid altering salinities of treatments and to provide algal cells that would not burst due to osmotic shock, copepods in saline water were fed a mixture of estuarine algae (*Rhodomonas* sp., *Isochrysis galbana*, *Thalassiosira pseudonana*), while those in fresh water were fed a mixture of freshwater algae (*Scenedesmus* sp., *Ankistodesmus* sp., *Chlamydomonas* sp.). Because different food sources were used, two separate controls were maintained on either estuarine or freshwater algae to test favorable growth on both food sources. Developing copepods were fed in excess every 2 or 3 d. Every 10 d, 50% of the water volume was replaced. Visual inspection of vials was performed daily. Individuals were classified as adults when males developed geniculate right antennules and females developed large winglike processes on the posterior end of their prosome (body).

Testing Effects of Developmental Acclimation

To determine effects of developmental acclimation on adult salinity tolerance, tolerances of low- (0 PSU) and high- (40 PSU) salinity stress were measured for adults reared at low (5 PSU), control (10 PSU), and high (27 PSU) salinities (Fig. 1). This experiment investigated tolerance of acute salinity stress in an effort to examine a trait relevant for sudden invasion events. Survival in response to stress salinities was recorded for

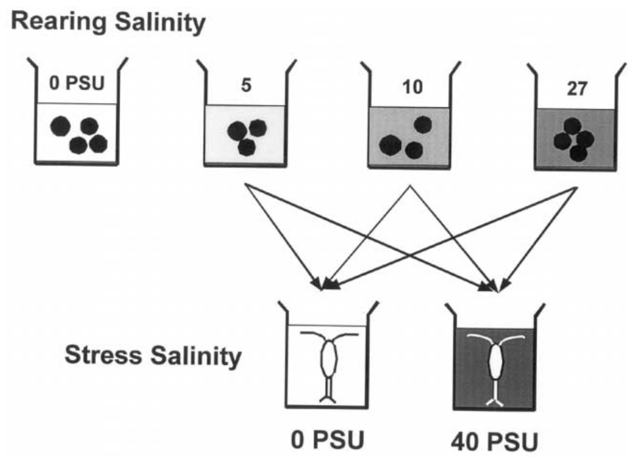

Figure 1. Experimental design to test effects of developmental acclimation on adult salinity tolerance. Twenty clutches were split into four treatments ranging from salinities of 0 (fresh water) to 27 PSU. On reaching adulthood, survivors from each treatment were subjected to either high-salinity (40 PSU) or low-salinity (0 PSU) stress. Survival time was measured at the stress salinities up to 24 h.

up to 24 h. The test salinities used were ecologically relevant, given that 0 PSU and 40 PSU represent two extremes at which *E. affinis* has been found (Lee 1999). Individuals from the same clutch were split into high- and low-salinity stress treatments to remove clutch (genetic) effects from each observation. A χ^2 contingency analysis and Wilcoxon test were used to compare response of individuals to high- and low-salinity stress treatments. An ANOVA was performed to examine effects of clutch on survival time under low-salinity (0 PSU) and high-salinity (40 PSU) stress conditions. A significant clutch effect would indicate a genetic component affecting response to salinity stress.

Results

Response to Acute and Gradual Transfers to Low Salinity

The transition to zero salinity induced high mortalities regardless of whether the transfer was incremental or acute (Fig. 2). For the acute transfer, most of the mortality occurred in the first hour during the initial transfer from 15 to 0 PSU (73% ± 2%, *unfilled square*), followed by a slow decline in survivors thereafter (Fig. 2). For the gradual transfer from 15 to 0 PSU, most (61% ± 5%) of the mortality occurred at the seventh hour during the final 2 to 0 PSU transition (Fig. 2, *unfilled diamond*), while low levels of mortality were spread out evenly during the preceding salinity transitions. At 7 h, with both treatments at 0 PSU, cumulative mortality was significantly lower in the gradual treatment (Fig. 2, *unfilled shapes*; Student's $t = -7.54$, df = 4, $P = 0.0017$). However, the difference in cumulative mortality converged after 8 d and was not significant (Student's $t = 1.99$, df = 4, $P = 0.12$).

Effects of Developmental Acclimation on Adult Tolerance

Developmental acclimation had contrasting effects on low- and high-salinity tolerances of adults (Fig. 3). While high developmental salinity (27 PSU) had a significant effect on adult tolerance of high-salinity stress (40 PSU), the impact of low developmental salinity (5 PSU) on tolerance of low-salinity stress (0 PSU) was small. Copepods reared at high salinity (27 PSU) survived high-salinity stress (40 PSU) significantly longer than those reared at low salinity (5 PSU; Fig. 3, *filled squares*; Fig. 4, *filled bars*; contingency analysis at 24 h, $\chi^2 = 15.90$, $P < 0.0001$; Wilcoxon test at 24 h, $\chi^2 = 11.67$, $P = 0.0006$). In contrast, developmental salinity (5 vs. 27 PSU) had no significant effect on survival time in response to low-salinity stress (Fig. 3, *unfilled squares*; Fig. 4, *unfilled bars*; contingency analysis at 24 h, $\chi^2 = 3.68$, $P = 0.45$; Wilcoxon test at 24 h, $\chi^2 = 0.12$, $P = 0.73$). The test did not include copepods reared at 0 PSU because none survived past metamorphosis. The diet of freshwater algae was probably not responsible for developmental failure in fresh water, given that controls (10 PSU) survived well on both freshwater and saltwater algae (time to metamorphosis: 13.20 ± 0.79 d on saltwater algae, 13.75 ± 1.46 d on freshwater algae; Student's $t = -0.29$, df = 16, $P = 0.78$).

Survival times for the 0 and 40 PSU stress treatments were not correlated within clutches (Kendall's $\tau = 0.068$, $N = 16$ clutches, $P = 0.74$), indicating lack of genetic correlation between low- and high-salinity tolerances. Response to salinity stress varied considerably among individuals (Fig. 4). Effect of clutch (genotype) on survival time was significant for the 0

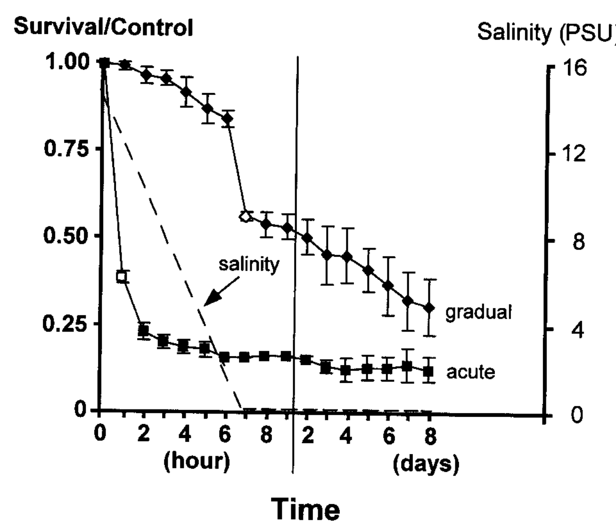

Figure 2. Survival in response to gradual and acute changes in salinity over a period of 8 d. Values are mean ± SE for three replicates, relative to controls maintained at 15 PSU. Unfilled shapes represent the transition to 0 PSU (fresh water). The dashed line represents salinity change for the gradual transfer.

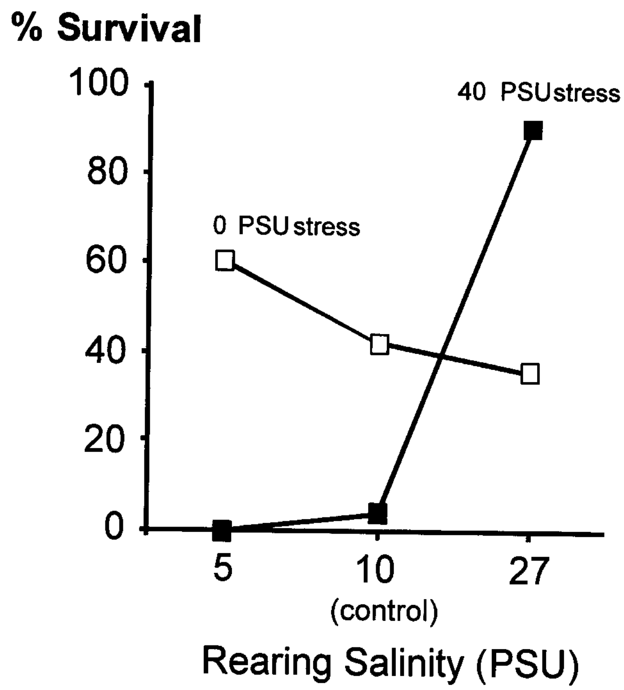

Figure 3. Test of salinity tolerance of adults reared at three salinities. Tolerance of high-salinity (40 PSU) and low-salinity (0 PSU) stress for adult copepods reared at 5, 10, and 27 PSU. Tolerance was measured as percentage survival out of total number of adults (sample sizes are shown in Fig. 4) after a 24-h assay at the stress salinities.

PSU stress treatment (ANOVA, $F = 3.39$, $df = 13$, $P = 0.0047$) but not for the 40 PSU stress treatment (ANOVA, $F = 0.94$, $df = 10$, $P = 0.51$). Effect of rearing salinity on clutch effect could not be determined due to small number of survivors within clutches at 5 and 27 PSU.

Discussion

This study examined effects of short-term and developmental acclimation on adult salinity tolerance in order to gain insights into mechanisms that enable rapid range expansions. Most striking were the differences in effect of developmental acclimation on response to low- versus high-salinity stress and differences in clutch effect at the two stress salinities.

Response to Acute and Gradual Transfers to Low Salinity

Gradual transfers to fresh water enhanced survival relative to acute transfers in the short-term (after 7 h), but differences converged over time after 8 d (Fig. 2). Convergence between the two treatments suggest that short-term acclimation of adults does not have a long-term effect on low-salinity tolerance. Initial significant differences (after 7 h) between the acute and gradual treatments might be attributed to effects of osmotic shock in the acute treatment. It would be interesting to determine whether the same or different individuals were being selected for in the acute and gradual treatments, given that tolerance to acute versus gradual salinity change might represent distinct traits.

Effects of Developmental Acclimation on Adult Tolerance

Developmental acclimation greatly enhanced tolerance of high- but not of low-salinity stress (Fig. 3), suggesting that *Eurytemora affinis* is "euryhaline" with respect to higher salinities (10–40 PSU) but not to lower salinities (fresh water). In addition, clutch had a significant effect on response to low- but not to high-salinity stress, suggesting that tolerance of low-salinity stress is genetically determined but that tolerance of higher salinities is not (at least up to 40 PSU). Experiments for two other brackish-water populations of *E. affinis* (from Grays Harbor salt marsh, Washington, and Columbia River estuary, Oregon) yielded similar results (C. E. Lee and C. H. Petersen, unpublished data). Limitations of this experiment included not

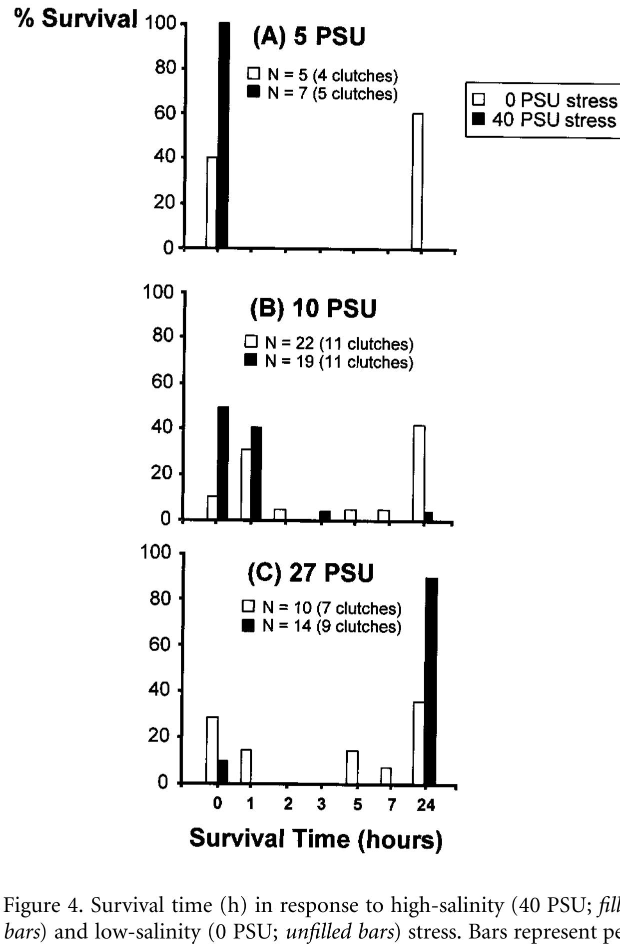

Figure 4. Survival time (h) in response to high-salinity (40 PSU; *filled bars*) and low-salinity (0 PSU; *unfilled bars*) stress. Bars represent percentage survival to a particular time (h) out of the total number of individuals (*N*). Survival was measured for split broods reared at 5 (*A*), 10 (*B*), and 27 (*C*) PSU.

examining effects of development acclimation on performance, fitness, or longer-term salinity tolerance and not accounting for temperature-salinity interactions.

The lack of significant effect of developmental acclimation on freshwater tolerance suggests that plasticity would not have a buffering effect on evolutionary change (Wake et al. 1983), allowing low-salinity tolerance to evolve. Other studies have shown that brackish-water populations of *E. affinis* cannot acclimate to freshwater conditions in a single generation (Lee 1999; Lee and Petersen 2002) and that transfers to fresh water result in only a 1% survival over two generations (Lee 1999). Genetic variation for low-salinity tolerance indicates genetic substrate for natural selection. In contrast, acclimation to higher salinities over the short term (Roddie et al. 1984) or during development (this study) occurs more readily in *E. affinis*. This ability to acclimate to higher salinities exists not only for brackish-water populations but also for *E. affinis* populations adapted to fresh water (Ishikawa et al. 1999; Lee et al., in press).

The inability of a brackish-water population to acclimate to fresh water, either through adult or developmental acclimation, could have several causes. The ability to acclimate might have been compromised by lower robustness of copepods reared at low salinity. For instance, the population used in this study exhibits lower survival and longer development times when reared at low salinity (5 PSU) relative to higher salinities (10 and 27 PSU; Lee and Petersen 2002). Alternatively, differences in osmoregulatory mechanisms at low and high salinities might have resulted in differences in stress response. Osmoregulatory mechanisms for copepods are not known, but for decapod crustaceans and some other invertebrates, high- and low-salinity tolerances are acquired through inherently different means (Charmantier et al. 1998). High-salinity tolerance relies on the accumulation of compatible solutes, amino acids that increase internal osmotic pressure, while leaving the biochemical milieu unaltered (Burton and Feldman 1982; Yancey et al. 1982). In contrast, low-salinity tolerance in decapod crustaceans requires increases in ion uptake and mechanisms to prevent ionic loss (Towle 1990). These differences in ion uptake capacity might require mutational changes in ion uptake proteins or changes in transcriptional regulation. Such changes might require adaptive (genetic) rather than acclimatory modifications.

Natural Selection during Freshwater Invasions

The inability of saline populations of *E. affinis* to acclimate to freshwater conditions poses problems for invading freshwater habitats. Although freshwater populations of *E. affinis* were recently derived from estuarine and salt marsh populations (Lee 1999), they are physiologically distinct from their ancestral saline populations, having experienced a heritable shift in salinity tolerance (Lee et al., in press). Results from this and other studies (Lee 1999; Lee and Petersen 2002) challenge the notion that euryhalinity, or broad salinity tolerance, could explain the distribution of *E. affinis* in freshwater habitats (Wolff 2000). The lack of significant effect of developmental acclimation on freshwater tolerance and the significant clutch effect in response to low-salinity stress suggest that natural selection plays an important role in the derivation of freshwater populations. The inability of developmental acclimation to generate a freshwater population in a single generation further supports this possibility (Lee and Petersen 2002). High levels of variation among clutches in response to low salinity, in terms of survival, development time, genotype-by-environment interaction (Lee and Petersen 2002), and acute salinity stress (this study), reflect ample substrate upon which natural selection could act.

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