Compensatory growth in tadpoles after transient salinity stress

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Abstract. Many freshwater habitats worldwide are being degraded by an anthropogenic increase in salinity. Although salt concentrations are known to fluctuate with variable freshwater inflows, we know little about what effects this may have on freshwater organisms. Using a species of frog, *Litoria ewingii*, we measured tadpole growth both during and after salt stress to determine their capacity to compensate or recover from this stress. During exposure to ecologically relevant concentrations of salt (5%, 10% and 15% seawater), tadpoles grew slower and were significantly smaller than those in our freshwater control (0.4% seawater). Upon return to fresh water, previously salt-exposed tadpoles grew faster than those in the control group, and by the eighth day of the 'recovery' period, no longer differed significantly in size. The results of our study demonstrate a capacity for tadpoles to compensate for a period of environmental stress by temporarily increasing growth rate when the stress abates.

Additional keywords: anthropogenic disturbance, compensatory growth, ecotoxicology, tree frog.

Introduction

Environmental change resulting from human disturbance is having far-reaching consequences for ecosystems worldwide (Vitousek *et al*. 1997; Halpern *et al*. 2008). In particular, many freshwater systems are being degraded by increased salinisation brought about by activities such as the planting of shallow-rooted crops, land clearing and unsustainable irrigation practices (Hart *et al*. 2003; Mahajan and Tuteja 2005). These activities lead to the mobilisation of salt in the groundwater and soil profile (Williams 2001) and when these salts make their way to the surface, they can leach into rivers, wetlands and streams, causing previously 'fresh' (<0.3–0.5 g NaCl L^{-1}, $\sim0.1\%$ seawater) water to turn 'saline' (0.5–10 g NaCl L^{-1}, up to $\sim30\%$ seawater). Negative effects of rising salinity on aquatic communities have been found to also originate from the salt used to de-ice roads (Sanzo and Hecnar 2006; Karraker *et al*. 2008; Collins and Russell 2009).

Most studies focusing on the impacts of salinity on freshwater organisms have tended to be toxicological in nature (e.g. Chinathamby *et al*. 2006). From these, it is generally accepted that elevated salinity can impact survival by interfering with key physiological and metabolic processes, such as osmoregulation and growth (Gomez-Mestre *et al*. 2004). Less well understood is the capacity for animals to 'recover' from salt exposure. This is a biologically meaningful question because salt concentrations in the environment may fluctuate depending on seasonal rainfall and/or rates of evaporation over a timeframe shorter than the developmental period of many freshwater organisms.

Compensatory growth is a period of heightened growth when favourable conditions are restored after a phase of growth depression (Fraser *et al*. 2007). This type of growth has been well documented in a wide range of taxa, including insects, fish, frogs, birds and mammals (for a review, see Metcalfe and Monaghan 2001). Many of these studies have demonstrated the capacity for compensatory growth in response to food shortages (e.g. Blake and Chan 2006; Capellán and Nicieza 2007). Far fewer have documented compensatory growth in response to physical environmental stressors, such as salinity (Yan *et al*. 2005).

Anurans (frogs and toads) are renowned for their plasticity in behavioural and morphological traits (Hoverman and Relyea 2008; Schoeppner and Relyea 2009). For example, several species will increase their developmental rate in response to ponds drying (Laurila and Kujasalo 1999; Altwegg 2002). Evidence suggests that tadpoles can also metamorphose at greater body mass in moderately elevated salinity levels (Chinathamby *et al*. 2006) in an apparently hormetic response (i.e. an increase in performance due to overcompensation to a stressor). Understanding how animals respond and possibly 'recover' from an environmental stressor is potentially as important as understanding the 'lethal limits' of exposed organisms, especially when coupled with management practices targeting affected habitats.

The brown tree frog (*Litoria ewingii*) is a small anuran amphibian native to south-eastern Australia, and is a species that elevated salinity may already have excluded from certain wetland habitats (Smith *et al*. 2007). Several species of frog have been shown to have a reduced growth rate under salinity stress (e.g. Christy and Dickman 2002; Chinathamby *et al*. 2006) but it is unknown whether tadpoles are able to compensate for this reduced growth if environmental conditions subsequently improve. We hypothesised that salt-exposed
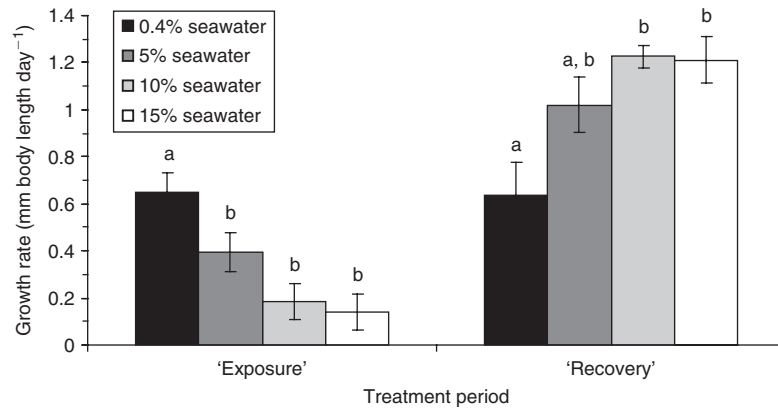
Fig. 1. Growth rates (mm body length day^{-1}) of tadpoles during the 'exposure' and 'recovery' periods. Different letters indicate significantly different groups within each period.

L. ewingii tadpoles would have the developmental plasticity to compensate for reduced growth during transient salinity stress, by increasing growth rate when the stress abated, relative to control (freshwater) tadpoles.

Materials and methods

General methods

Frog eggs were collected from freshwater pools in Silvan, Victoria, Australia (37°50'S, 145°26'E, salinity level = 0.3% seawater, which is equivalent to 0.11 g NaCl L^{-1}). Once hatched, tadpoles were housed in individual tanks (length × width × height = 13 cm × 13 cm × 7 cm) containing 600 mL of fresh water (i.e. at 0.4% seawater, equivalent to 0.14 g NaCl L^{-1}). Tadpoles were fed frozen endive *ad libitum*, and were kept at a constant temperature of 21°C on a 12-h light : dark cycle. We conducted regular partial water changes and removed uneaten food to maintain water quality.

Experimental methods

Four days after hatching, tadpoles were randomly assigned to one of four treatment groups (n = 10 animals per group). Tadpoles allocated to the 'freshwater' treatment were kept in 0.4% seawater (i.e. a concentration typical of freshwater systems unaffected by increased salinity). Tadpoles in the 'saline' treatments were maintained in 5%, 10% or 15% seawater (equivalent to 1.75, 3.53 and 5.25 g NaCl L^{-1} respectively). These salinities fall below the lethal limit for this species (Chinathamby *et al.* 2006) and are environmentally meaningful in reflecting the range of salinities recorded in degraded frog habitats (Smith *et al.* 2007). Water was prepared using Coralife marine salt (pH buffered 8.2–8.3; ESU Inc., Carson, CA, USA) in which Na$^+$ and Cl$^-$ are the dominant ions but which contains a combination of other ions found in naturally occurring saline water. Salt concentrations were checked with a conductivity sensor (Hannah H198130 conductivity meter; Hanna Instruments, Woonsocket, RI) every 4 days and kept within 0.01 g NaCl L^{-1} of the desired concentration by addition of salt or distilled water as appropriate.

To prevent osmotic shock, tadpoles were gradually introduced to salt-water treatments by incrementally increasing the level of salt by 5% seawater (1.75 g NaCl L^{-1}) per day and kept in their respective treatments until Day 16. Tadpoles were then returned to fresh water by incrementally reducing salt concentrations by 5% seawater per day, and kept in fresh water until Day 32. We measured tadpole body length at the beginning, middle and end of both the 'exposure' period (Day 0, Day 8 and Day 16) and the 'recovery' period (Day 16 (after the first incremental reduction in salt concentration), Day 24 and Day 32). Tadpole body length (snout to tail tip) was measured from a digital photograph taken directly above each tadpole and compared with a 1-cm reference scale captured in the photo.

Statistical analysis

All data were checked for normality and heterogeneity of variances. We used an ANOVA to determine differences in growth rate (mm body length day^{-1}) and body length (mm) between groups after the end of the 'exposure' and 'recovery' periods, time to metamorphosis (days) and mass at metamorphosis (g). We used a Mantel–Cox log rank of the Kaplan–Meier survival statistic to detect differences between groups in survival to metamorphosis. Any tadpoles that metamorphosed were 'right censored' for analysis (SYSTAT Software Inc., Richmond, CA).

Results

We found a significant effect of salinity on tadpole growth during both the 'exposure' and 'recovery' periods. There was no significant difference in body length between groups at the beginning of the salinity treatment period ($F_{3,36} = 0.882$, $P = 0.459$), but growth rates (mm body length day^{-1}) differed significantly during salt exposure (Fig. 1; $F_{3,36} = 3.804$, $P = 0.02$), such that tadpoles in the freshwater control group grew faster than those in the 5% seawater ($t = -2.925$, $P = 0.0297$), 10% seawater ($t = -4.539$, $P < 0.001$) and 15% seawater ($t = -4.715$, $P < 0.001$) groups. By the end of the salinity treatment (Day 16) the control group was significantly larger (Tukey's *post hoc* test) than the 5% seawater ($t = -3.665$, $P = 0.004$), 10% seawater ($t = -5.706$, $P < 0.001$) and 15% seawater ($t = -6.533$, $P < 0.001$) groups.

When salt-exposed tadpoles were returned to fresh water after the 'exposure' period, growth rate was significantly faster (Tukey's *post hoc* test) in the 10% seawater ($t = -3.766$,
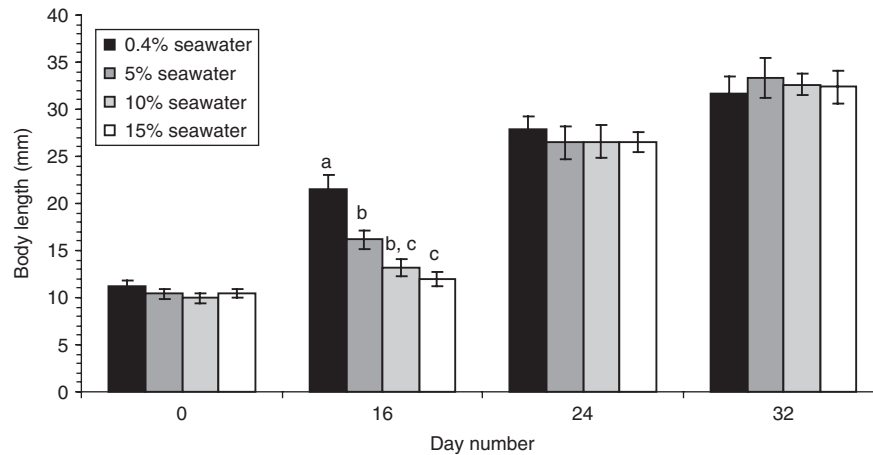
Fig. 2. Mean (±1 s.e.) body length (mm) on Days 0, 16, 24 and 32 of tadpoles in the different salinity groups. Salinity treatments ended at Day 16. Different superscript letters indicate significantly different groups.

Table 1. Days (mean ± s.e.) taken to reach metamorphosis and metamorphic mass (mean ± s.e.) of tadpoles in the different salinity groups
Different superscript letters indicate significantly different groups within each column

Salinity (% seawater)	Mass at metamorphosis (g)	Time to metamorphosis (days)
0.4%	0.22 ± 0.04	49.50 ± 0.83[a]
5%	0.19 ± 0.02	55.33 ± 2.08[ab]
10%	0.19 ± 0.01	59.14 ± 3.38[b]
15%	0.18 ± 0.02	61.80 ± 1.93[b]

$P = 0.004$) and 15% seawater ($t = -3.369$, $P = 0.011$) groups than the control group during the same time period (Fig. 1). There was no significant difference in body length between any of the groups by Day 24 ($F_{3,29} = 1.092$, $P = 0.368$) or at the end of the 'recovery' period (Day 32, $F_{3,28} = 0.159$, $P = 0.923$, Fig. 2). There was no significant difference between groups in survival to metamorphosis (survival analysis, $\chi^2_3 = 3.2$, $P = 0.36$) or metamorphic mass ($F_{3,24} = 0.45$, $P = 0.72$, Table 1), but tadpoles previously exposed to 10% seawater (Tukey's *post hoc* test, $t = 3.511$, $P = 0.0009$) and 15% seawater ($t = 4.029$, $P = 0.003$) took significantly longer to reach metamorphosis than the control group (Table 1).

Discussion

A negative relationship between increasing salt concentration and anuran growth rates is concordant with earlier studies on anuran larvae (Christy and Dickman 2002; Rios-López 2008) including those on *L. ewingii* (Chinathamby *et al*. 2006). The duration of salt exposure in our study was much shorter than that of Chinathamby *et al*. (2006), hence we detected sublethal effects of salt on growth but did not find differences in survival or metamorphic mass. The negative sublethal effects of salinity may have been due to the increased costs of osmoregulation (Gomez-Mestre and Tejedo 2003), which leaves fewer resources for growth compared with animals in fresh water. We did not quantify feeding by tadpoles in the different groups, so it is possible that feeding rates differed and that this also reduced energy available for growth. However, when we removed the salinity stress, the 10% and 15% seawater groups showed a pronounced compensatory growth about twice that of control animals, which allowed them to reach the same body length as control animals in 8 days. Thus, regardless of whether elevated salinity resulted in a reallocation of energy to various competing needs or a reduction in energy intake, or both, we showed that animals can potentially compensate for a sublethal salinity challenge and 'catch up' their lost growth in a short time. Intriguingly, although mass at metamorphosis was not different between groups, the 10% and 15% seawater groups took longer to metamorphose. Their development must have slowed after the period of compensatory growth, but our data do not enable us to identify how or when this happened.

Growth of *L. ewingii* tadpoles slows as they approach metamorphosis, at which time they begin to lose mass (Chinathamby *et al*. 2006) and these trade-offs between developmental rate, body size and time to metamorphosis are complex. It may be that a period of accelerated growth comes at a cost later in life. For example, Gervasi and Foufopoulos (2008) found that tadpoles exposed to desiccation had shorter developmental times, but after metamorphosis had weaker cellular immune systems and lower total leucocyte numbers. This trade-off between increased growth and potential life history costs will be a fruitful area for continued research. Nevertheless, a transient period of compensatory growth can be advantageous, especially in the light of the fact that small body size is known to expose tadpoles to a greater risk of predation (Williamson and Bull 1999) and salinity itself can decrease the ability of tadpoles to escape predators (Squires *et al*. 2008). In species where breeding is seasonal, it may also be of benefit to grow quickly to not risk missing a breeding season. Thus, compensatory growth may provide a potentially useful mechanism to overcome the immediate effects of elevated salt levels in aquatic habitats subjected to freshwater inflows.

Tadpoles show plasticity in growth rate in response to a range of environmental stressors (Laurila and Kujasalo 1999; Altwegg 2002), particularly as a consequence of the ephemeral nature of freshwater pools or ponds. Altwegg (2002), for example, showed that tadpoles of European pool frogs, *Rana lessonae*, were able

to increase their developmental rate in response to drying of ponds. Other studies have shown the potential for compensatory growth in *Rana temporaria* in response to acid and food deprivation (Räsänen *et al.* 2002; Capellán and Nicieza 2007), in *R. sylvatica* in response to high density conditions (Jasienski 2008) and in *Hyperolius spinigularis* in response to predation risk (Vonesh and Bolker 2005). However, ours is the first study showing that tadpoles can compensate for lack of growth during salt stress. Under natural conditions, as a pool dries, salt concentrations steadily rise as water evaporates. Heavy rains may provide relief from saline conditions and it would be adaptive for tadpoles to take advantage of these more favourable conditions while they last, particularly if the salinity of the environment fluctuates repeatedly during the developmental period of the tadpole life stage. Information from these types of studies may be useful in targeting certain species for protection and in managing high-value wetlands that have become salinised. More broadly, our study underscores the importance of examining the non-lethal impacts of exposure to aquatic contaminants. Doing so may provide pivotal insights into the capacity for organisms to cope with the increasing environmental stressors resulting from anthropogenic disturbance.

Acknowledgements

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