

Salinity Tolerance and Brackish Habitat Utilization in the Common Australian Frog *Crinia signifera*

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ABSTRACT.—Ecosystem disturbance through urbanization and agriculture, coupled with anthropogenic climate change, poses a pervasive threat to ecosystem health. Such landscape disturbance can manifest as salinization, particularly in Australia. Increasing salinization of both soils and waterways has the potential to render habitats unsuitable for amphibians. However, some species exist naturally in brackish and saline habitats, which suggests the capacity to adapt to salinization. To assess this adaptability in amphibians, we examined current brackish habitat utilization by a common Australian Froglet, *Crinia signifera*, and determined the tolerance of eggs and tadpoles of this species to acute and chronic exposure to brackish water. *Crinia signifera* lay eggs in brackish water and, through laboratory experiments, we determined that short-term survival in water up to 7.5 parts per thousand (ppt) salinity is possible but may vary among populations. Chronic exposure experiments demonstrated that *C. signifera* can successfully complete larval development to metamorphosis in brackish water (up to 5.0 ppt). Data presented here, together with previously published reports of salinity tolerance in other Australian frog species, collectively demonstrate a capacity for adaptation to landscape salinization. This has probably been mediated by an evolutionary history in the saline landscapes so common in Australia.

Landscape-level ecosystem disturbance through urbanization and agriculture is well recognized and ongoing (Rundel et al., 2013). Coupled with anthropogenic climate change and the resultant landscape-scale effects on vegetation (Guerin et al., 2013), a pervasive threat is posed to ecosystem health generally. Such landscape changes include salinization of soils and aquatic ecosystems (Jolly et al., 2008). In the northern hemisphere, salinization of these ecosystems is often because of the application of road-deicing salts (e.g., Kaushal et al., 2005). In the southern hemisphere, and specifically Australia, mass clearance of vegetation following European settlement, coupled with modification of hydrological systems, has led to increasing dryland salinity and salinization of riparian and wetland ecosystems (Allison et al., 1990; Jolly et al., 2008). Salts may also be introduced by irrigation practices, particularly when poor-quality water is used in soils with low hydraulic conductivity (Rengasamy, 2006). Up to 30% of the land area of Australia is thought to be subjected to salinization (Rengasamy, 2006), which has the capacity to impact aquatic faunal communities (Horrihan et al., 2007; Jolly et al., 2008). Indeed, Australia's geological history, including frequent epeiric seas and resultant saline soils (McKenzie et al., 2004), make the study of salinity impacts on organisms highly relevant.

Many frog species require aquatic ecosystems for breeding, development of eggs and larvae, and other life history requirements. This is thought to principally occur in fresh water (usually defined as either <1 ppt chloride (Cl⁻) or in Australia as <3 ppt; Williams, 2001), as the permeable skin and jelly egg casings of amphibians make them particularly vulnerable to osmotic stress. Indeed, repeated studies of the effects of road-deicing salts on amphibians have shown that exposure to salt water can lead to increased mortality, developmental deformities, physiological stress, and the alteration of growth and development (e.g., Sanzo and Hecnar, 2006; Collins and Russell, 2009; Chambers, 2011; Harless et al., 2011, 2013a,b, 2014; Karraker and Gibbs, 2011). In spite of this, several instances of

growth and development to metamorphosis occurring in waters over 1 ppt salinity ('brackish') have been recorded around the world (Hopkins and Brodie, 2015).

A recent review (Hopkins and Brodie, 2015) documented saline habitat utilization in 144 species of amphibians worldwide, including 124 anurans. Frogs successfully breed in habitats ranging from coastal tidal pools (e.g., *Fejervarya limnocharis* in Taiwan; Wu and Kam, 2009) and salt marshes (e.g., *Pelobates cultripes* in France; Thirion, 2014) to brackish desert pools (e.g., *Epidalea calamita* in Spain; Gomez-Mestre and Tejedo, 2003) and ponds polluted by road-deicing salts (e.g., *Lithobates sylvaticus* in North America; Karraker et al., 2008). In Australia, at least 13 different species have been documented inhabiting purportedly saline habitats, although the vast majority has not been examined in any detail (Table 1). Globally, only about a quarter of those species found in saline habitats have been comprehensively studied in the field and lab and experimentally tested for salinity tolerance (Hopkins and Brodie, 2015), and the proportion studied well in Australia is even less. Finally, we know best about the adaptability of adult amphibians to saline habitats but very little about that of eggs and larvae, which are often more sensitive to osmotic stress (Table 1; Hopkins and Brodie 2015). Thus, despite saline habitat utilization being apparently more common in amphibians than originally thought, and numerous Australian species found in saline habitats throughout the continent, there remains some significant gaps in our understanding of the adaptive potential of the vast majority of species (Hopkins and Brodie, 2015).

In this study, we aimed to address these gaps in our knowledge by investigating brackish habitat utilization and salinity tolerance in the common Eastern Froglet, *Crinia signifera*. This species is widely distributed throughout southern temperate Australia (Tyler and Knight, 2011) and is considered one of the most common frog species in urban areas of this region. Furthermore, this species has been observed breeding in brackish habitats (Table 1). Incidentally, in the course of other research in the Adelaide area (CRW, pers. obs.), we have frequently heard male advertisement calls along brackish waterways as well as observing tadpoles in these habitats.

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TABLE 1. Australian amphibians that have been found in saline habitats, the salinity of the habitat, and experimentally determined salinity tolerance of the species prior to this study. Reproduced in part, with permission and some modification, from Hopkins and Brodie (2015). Where the species may have been found in multiple salinities, the highest environmental and/or tolerance level of salinity is listed.

Species (life-stage) ^a	Habitat	Salinity of habitat (ppt)	Experimental tolerance (ppt)	References
<i>Rhinella marina</i> (A)	Coastal brackish pools, beach, mangrove swamps	Not measured (described as "brackish")	16	van Beurden and Grigg, 1980; Liggins and Grigg, 1985
<i>Litoria aurea</i> (A, L)	Coastal brackish estuary, ponds, and saline lagoons; inland wetland impacted by secondary salinization	7.3	5.6	Pyke et al., 2002; Kearney et al., 2012
<i>Litoria caerulea</i> (A, L)	Pond adjacent to coastal salt lagoon	6	Unknown	Pyke et al., 2002
<i>Litoria cyclorhyncha</i> (A, L)	Inland saline creek; coastal brackish pond	37.4	Unknown	Janicke and Roberts, 2010; Taylor et al., 2018
<i>Litoria dentata</i> (A, L)	Pond adjacent to coastal salt lagoon	6	Unknown	Pyke et al., 2002
<i>Litoria peronii</i> (A, L)	Pond adjacent to coastal salt lagoon	6	Unknown	Pyke et al., 2002
<i>Litoria tyleri</i> (A, L)	Pond adjacent to coastal salt lagoon	6	Unknown	Pyke et al., 2002
<i>Limnodynastes dumerili</i> (L)	Inland wetlands affected by secondary salinization	4	Unknown	Smith et al., 2007
<i>Limnodynastes peronii</i> (A, L)	Pond adjacent to coastal salt lagoon	6	Unknown	Pyke et al., 2002
<i>Limnodynastes tasmaniensis</i> (L)	Inland wetland affected by secondary salinization	3.9	Unknown	Smith et al., 2007
<i>Neobatrachus sudelli</i> (L)	Inland wetland affected by secondary salinization	2.64	Unknown	Smith et al., 2007
<i>Neobatrachus pictus</i> (A)	Observed in the mudflats of an inland saline wetland (not in the water)	14.24	Unknown	Taylor et al., 2018
<i>Crinia riparia</i> (A)	Inland brackish creek	1.75	Unknown	Odendaal and Bull, 1982
<i>Crinia signifera</i> (A)	Inland brackish creek; brackish coastal tide pool;	0.85 (creek; tide pool salinity not listed)	Unknown	Odendaal and Bull, 1982; Mokany and Shine, 2003

^a A = Adult, L = larvae, E = egg.

However, our knowledge of habitat utilization in this common species remains somewhat anecdotal, and little to no experimental work has been conducted to determine salinity tolerance in this species (but see Byrne et al., 2015; examination of salinity tolerance of *C. signifera* sperm).

In order to understand the capacity of frogs to adapt to ongoing environmental change, in this case salinization, we aimed to establish the current utilization of brackish habitat by *C. signifera* and to determine the tolerance of tadpoles of this species to acute and chronic exposure to brackish water. If brackish habitat utilization was verified, we then sought to determine whether *C. signifera* can successfully complete larval development to metamorphosis in brackish water and whether any growth and development trade-offs for salinity tolerance may exist. Given the presence of multiple *C. signifera* populations in the region, we also sought to determine whether interpopulation differences in salinity tolerance may exist in this species as it can in other amphibians (Gomez-Mestre and Tejedo, 2003; Hopkins et al., 2017).

MATERIALS AND METHODS

Study Populations and Collection Method.—Three populations of *C. signifera* in the Adelaide region were studied. Each was separated by several kilometers of urbanized landscape, so we surmised that genetic exchange between each group was probably minimal (Fig. 1). We measured conductivity and temperature at each site and calculated salinity in Cl⁻ ppt on each occasion that frog eggs were collected using a Hanna Instruments HI98129 high range salinity meter (Hanna Instruments, Keysborough, Victoria, Australia).

Consistent with previous field observations, we found eggs of *C. signifera* submerged in the water, individually or in small

groups of up to 15, attached to littoral reeds and grasses (Williamson and Bull, 1994; Wilson et al., 2015). Collections were typically made at the edges of the water body, and all collections for a given experiment using eggs from one location were made on the same day. Collections for different locations occurred on different days. The eggs and attached vegetation were collected and placed into a plastic bag containing source water. We then placed bags into insulated containers for return to the laboratory for growth experiments.

At Darlington (Fig. 1), we collected *C. signifera* from a small creek in a hillside landscape nearby a major arterial suburban road. This water salinity level was measured variously as 0.38–0.82 ppt over two separate visits, indicating fresh water. This earthen channel was approximately 40–80 cm deep. Vocally advertising *C. signifera* were conspicuous at this site.

At Globe Derby Park (Fig. 1), we collected *C. signifera* from brackish ground pools formed by rainfall run-off and groundwater on a low-lying earthen roadway. The pools were vegetated with salt tolerant low-lying plants and filamentous algae. The *C. signifera* eggs were found on the edge of a gravel road in a large pool of water. Eggs were laid attached to submerged grass and filamentous algae in groups of up to five individuals. The water was approximately 20–40 cm deep. This location had an abundance of advertising adult frogs along with noticeable tadpole and egg abundance of both *C. signifera* and spotted marsh frogs (*Limnodynastes tasmaniensis*). The water salinity level measured at 1.79 ppt.

At Mawson Lakes (Fig. 1), we collected *C. signifera* from a creek running through a suburban landscape with a water depth approximately 20–40 cm. The creek had earthen sides but degraded and sparse littoral vegetation, functioning as a storm water drain for the surrounding suburb. The water was



FIG. 1. Location information for *C. signifera* populations studied here along with salinity measurements at the time of egg collection.

considered fresh, with salinity measured at 0.50 ppt. Advertising *C. signifera* were conspicuous at this site.

Laboratory Experiments and Salt Solution Preparation.—Tadpoles from all three *C. signifera* populations were tested for their acute and chronic exposure tolerance to various salinity levels in a series of laboratory experiments at the University of South Australia in Adelaide. Eggs were collected from the field and hatched into laboratory aquaria, separately for each population. Hatchlings were then used in experiments.

For both acute and chronic experiments, we made treatment solutions of 0.75, 2.0, 5.0, and 7.5 ppt by combining Ocean Nature sea salt (Aquasonic, Wauchope, New South Wales) with reverse osmosis (RO) water. These salinities encompassed both the range of salinities in which we found *Crinia* in the wild as well as known salinities found in most other brackish habitats inhabited by frogs in Australia (Table 1). A salinity meter (Hanna Instruments HI 931101) was used to confirm salinities, and pH was tested and corrected for each treatment (to a neutral pH of approximately 7) to control for any acidity changes caused by the addition of sea salt (Hopkins et al., 2013b).

Experiment 1: Effect of Acute Salinity Exposure on Survivorship.—Survivorship of tadpoles was recorded in either 0.75, 2.0, 5.0, or 7.5 ppt for 7 days. Each newly hatched tadpole (developmental stage 24 or 25 [Gosner, 1960]) was retrieved from a communal aquarium. Seventy-milliliter plastic specimen containers were filled with 45 mL of randomly assigned salt solution, and individual tadpoles were placed in each container in a constant temperature room set to 25°C with 12 : 12 L : D lighting. Each tadpole was allocated randomly to a salt solution treatment, resulting in a total sample size of 15 replicates per treatment for

the Darlington population and 11 per treatment each for the Mawson Lakes and Globe Derby populations. The meniscus line, specimen number, and the current date were recorded onto the container, but not the treatment, so that tadpole survival observations were recorded blind to salinity concentration.

Each day we checked the tadpoles for mortality by using a paintbrush to gently prod them for a motor response. Dead tadpoles were removed and preserved in 70% ethanol. Containers were checked for water evaporation daily, and RO water was used to replenish the water levels to the meniscus line to maintain salinity through time (Hopkins et al., 2014). If a tadpole died or had been in the salt solutions for 7 days, they were then staged (Gosner, 1960) using a dissecting microscope. Any abnormal characteristics (i.e., developmental deformities, after Hopkins et al., 2013a) were recorded. Surviving tadpoles were euthanized by chilling to 4°C before freezing (after Shine et al., 2015). Tadpoles were then transferred to a 1.5-mL tube for preservation in 70% ethanol.

Experiment 2: Effect of Chronic Salinity Exposure on Growth and Development.—We conducted an experiment to determine the effects of salt concentration on long-term growth and development on two populations of *C. signifera*; from Globe Derby Park and Darlington (Fig. 1). Experimental set up was similar to Experiment 1, albeit with some variations. The number of salt concentration treatments was reduced: 0.75, 2.0, and 5.0 ppt only (the 7.5 ppt treatment was not used owing to poor survivorship in the Darlington population in Experiment 1; see Results). To permit free swimming and growth of larger tadpoles, the container size was increased from 70 mL to 1.2 L. Tadpoles were checked daily for survivorship and water topped up to the

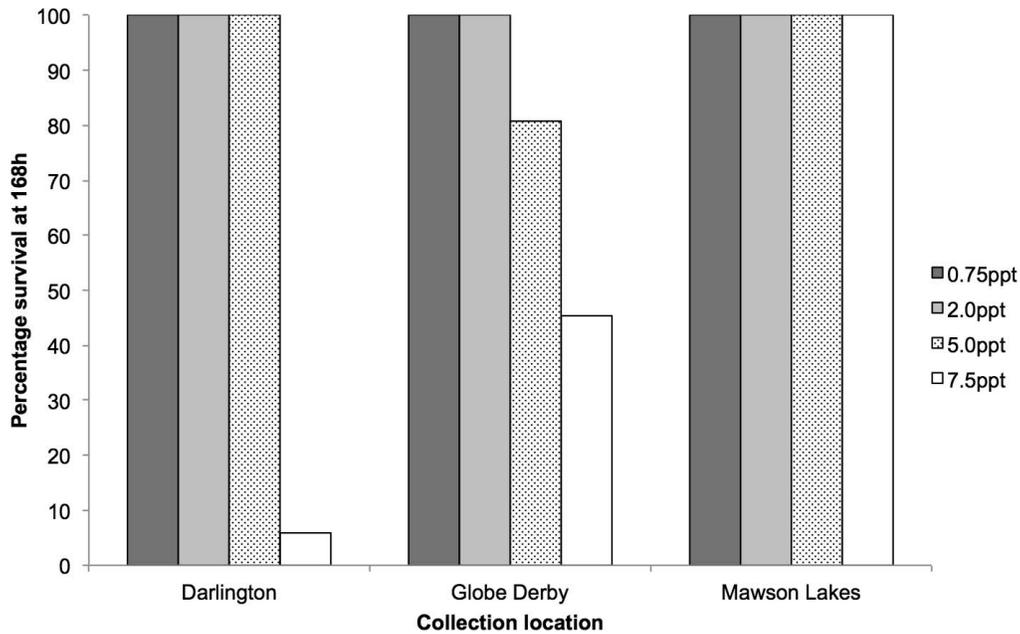


FIG. 2. Survival percentage of Stage 25 tadpoles of *C. signifera* in brackish solutions after 168 h of exposure.

meniscus line. Tadpoles were also staged weekly (Gosner, 1960), and all water was changed weekly (with water of the same salinity) to prevent fouling. Each week individual tadpoles were fed a 1-cm diameter disc of organically grown lettuce. Once the tadpoles had reached stage 43 (metamorphosis) they were euthanized, weighed, and preserved in 70% ethanol.

We observed 59 tadpoles in chronic exposure growth and development experiments; 46 from Darlington (16 at 0.75 ppt, 15 at 2.0 ppt, 15 at 5.0 ppt) and 13 from Globe Derby Park (4 at 0.75 ppt, 5 at 2.0 ppt, 4 at 5.0 ppt). We terminated the experiment at 60 days.

Statistical Analyses.—For acute exposure experiments, logistic regression was used to identify significant determinants of tadpole death, with salt treatment, population, and their interactions tested as main effects. We applied a Firth's correction (Firth, 1993) to the logistic regression using the R package *logistf* (Heinze and Ploner, 2018) because of quasi-separation of data caused by some treatment–population combinations having either 100% survival or mortality. For surviving larvae in the acute experiment, we examined possible effects on development through conducting linear regressions (*lm* function) of the effects of treatment, population, and their interactions, on change in developmental stage since hatching (developmental stage at 7 days – developmental stage at hatching). These data were \log_{10} transformed to meet assumptions of normality and homogeneity of variance. Kaplan Meier survival functions were calculated for each treatment and population through time for chronic exposure experiments. The log rank test was then used to compare survival functions between salt treatments only because of very uneven sample sizes among populations. As growth and development was nonnormally distributed in the chronic experiment, and normality could not be achieved through transformations, the Kruskal Wallis test was used to compare wet weights of metamorphs and time to metamorphosis among populations. Statistical analyses were performed in Stata ver. 14.2 (Stata Corp. College Station, USA) and R Studio version 1.1.463 (R Core Team, 2018) with significance set at $\alpha = 0.05$.

RESULTS

Experiment 1: Acute Salinity Exposure.—There was a significant effect of salt treatment ($\chi^2_3 = 48.67$, $P < 0.0001$), but not population ($\chi^2 = 0.03$, $df = 2$, $P = 0.99$), on tadpole survivorship, with higher average mortality in 7.5 ppt compared to all other treatments (contrasts $P < 0.0001$ 7.5 ppt vs. all other salt treatments). Firth's-corrected logistic regression also revealed a significant treatment \times population interaction ($\chi^2 = 71.31$, $df = 11$, $P = 0.0001$). Tadpoles from all three locations demonstrated 100% survivorship over 1 wk at salinities up to 2.0 ppt (Fig. 2). Strong survivorship (80–100%) was also recorded by all populations for the 5.0 ppt treatment. However, significant divergence in survivorship was noted at the 7.5 ppt treatment, with the Mawson Lakes population showing complete survivorship at 7.5 ppt compared with only 45% survivorship for the Globe Derby park population and 6% for Darlington. There was no significant interaction between population and treatment on change in development ($F_{6,114} = 0.44$, $P = 0.85$) during the acute experiment, and so this interaction term was removed from the model. There was a significant effect of population ($F_{2, 120} = 77.46$, $P < 0.0001$) on short-term development; each population developed at a significantly different rate than the others (Tukey-adjusted multiple comparisons among populations $P < 0.0001$ for all pairs; mean \pm SE change in development stage for Darlington = 2.64 ± 0.13 ; Mawson Lakes = 1.02 ± 0.09 , Globe Derby = 0.57 ± 0.15), but this difference was unaffected by salt treatment. There was only a very marginal effect of salt treatment ($F_{3, 120} = 2.31$, $P = 0.08$) on development. On average, tadpoles developed slightly less in 7.5 ppt (mean \pm SE change in development = 1.03 ± 0.24) compared to 5 ppt (1.53 ± 0.20), 2 ppt (1.65 ± 0.19), or 0.75 ppt (1.49 ± 0.21); however, these differences were not statistically significant. No developmental abnormalities were observed.

Experiment 2: Chronic Salinity Exposure.—Tadpoles grew and developed to metamorphosis at all three concentrations tested. Survival did not differ between Globe Derby Park and Darlington (across all treatments combined) over the 60-day observation period (log rank test of differences between populations $\chi^2 =$

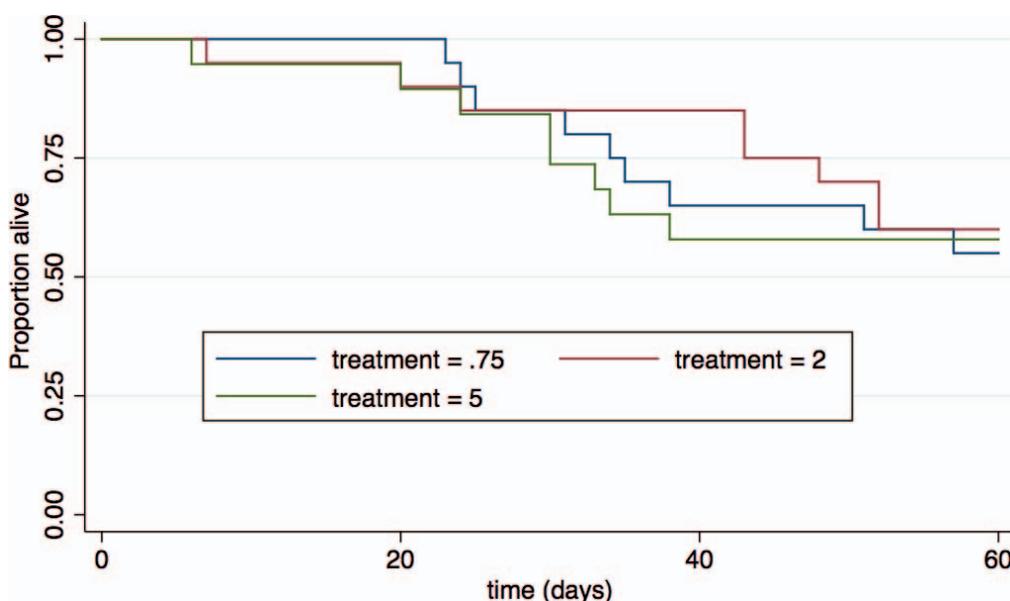


FIG. 3. Kaplan Meier survivorship functions of *C. signifera* tadpoles grown for 60 days in various salinity treatments (parts per thousand of salt).

0.22, $P = 0.637$), so we pooled data for Globe Derby ($n = 13$) with those from Darlington ($n = 46$) for a total of 59 individuals.

A total of 34 out of 59 tadpoles (58%) survived the 60-day experiment (Fig. 3). Survivorship did not differ among the three salinity concentrations (log rank test $\chi^2 = 0.18$, $P = 0.914$). Of those that survived, 19 achieved metamorphosis during the experiment across all three treatments after an average of 49.1 days (± 6.1 days standard deviation [SD]). No developmental abnormalities were observed.

Rates of metamorphosis amongst the three salinity treatments were similar, and neither time to metamorphosis nor wet weight at metamorphosis differed significantly among treatments (Table 2).

DISCUSSION

We found that a common Australian frog, *C. signifera*, can successfully breed in brackish water and that its tadpoles can complete metamorphosis in salinity levels comparable with other salt-tolerant frogs. For instance, the tolerances described here—acute exposure tolerance to 7.5 ppt and chronic exposure tolerance to 5.0 ppt—are akin to previous findings for the Bell Frog, *Litoria aurea*, which completed metamorphosis at 5.6 ppt (Kearney et al., 2012). More broadly, a recent meta-analysis of 39 studies of anuran salt tolerance found that the average 50% lethal concentration (LC₅₀) for tadpoles in sodium chloride (NaCl) is 5.5 ppt (95% Bayesian credible interval = 4.24–6.25 ppt; Albecker and McCoy, 2017), placing our results in line with other frogs. This contrasts sharply with a caudate amphibian, however (e.g., rough-skinned newt larvae had up to 40% mortality in only 2.0 ppt Cl⁻; Hopkins et al., 2014). Interestingly,

Kearney et al. (2012) found that *L. aurea* displayed better survivorship and size at higher salinities compared with lower ones, indicating a type of salinity specialization, and we find a similar, but nonsignificant, trend in *C. signifera*; chronic survival tended to be greater in 2.0 ppt than in 0.75 ppt in our study. *Crinia signifera* does appear to be more tolerant of higher salinities than does another common South Australian frog, the Tree Frog *Litoria ewingii*, which had slower growth at higher salinity levels (4.2 ppt and 5.6 ppt) (Chinathamby et al., 2006; Kearney et al., 2014). These results expand our knowledge of salinity tolerance in the common *Crinia signifera* and establish new high salinity levels that permit development to metamorphosis. Our work characterizes *C. signifera* as a being preadapted to further landscape salinization and as an excellent model organism for testing hypotheses regarding salt tolerance in frogs.

We found some evidence that salinity tolerance at an acute (but not necessarily chronic) exposure varies between *C. signifera* populations, which is in line with other studies indicating that amphibian populations may diverge in their abilities to tolerate salt water (Gomez-Mestre and Tejedo, 2003; Hopkins et al., 2016, 2017). A recent study of sperm motility in *C. signifera* from nine different populations indicated that sperm performed better in the osmolalities that better matched their source population (Byrne et al., 2015), also indicating possible local adaptation. Counter-intuitively, however, our results do not match this general pattern or local adaptation; the population with the highest recorded salinity in our study (Globe Derby) had lower salt tolerance at 5 ppt and 7.5 ppt than did a population with much lower salinity (Mawson Lakes). However, our abilities to generalize regarding interpopulation

TABLE 2. 60-day values for growth and development for *C. signifera*.

Salinity (ppt)	Metamorphosis rate out of n (%)	Metamorphosis time as mean days (SD)	Metamorphosis wet weight as mean milligrams (SD)
0.75	6/20 (30%)	49.5 (8.6)	82.0 (13.7)
2.0	9/20 (45%)	59.3 (4.6)	78.5 (22.0)
5.0	4/19 (21%)	58.6 (12.3)	70.1 (30.4)
Statistical result		$\chi^2 = 3.53$, $P = 0.17$	$\chi^2 = 0.21$, $P = 0.901$

variation in our study may be confounded by three important factors: 1) a relatively small sample size of some populations in the chronic experiment compared to others, thus precluding robust tests of population-level variation at this exposure duration; 2) an accurate characterization of the salinities at our collection sites throughout time—serial measurements at our collection locations may be required to better characterize how these vary in salinity temporally; and 3) the need for more sampling locations from a variety of habitats with varying salinities to more accurately determine if local adaptation to environmental salinity has occurred.

Crinia signifera, similar to some other Australian amphibians, may be preadapted to salinization through a long evolutionary history of exposure to such environments (Janicke and Roberts, 2010). Much of temperate and semiarid Australia is generally characterized by episodic rainfall and an unreliable supply of fresh water. Furthermore, the flatness of Australia's geomorphology, inland drainage patterns, geological history of epeiric seas, and the strong influence of maritime weather patterns (particularly in southern regions) mean that this continent is a remarkably effective salt accumulator, resulting in large areas of sodosols (saline soils; McKenzie et al., 2004). Consequently, there are many Australian freshwater ecosystems (fed by drainage of saline soils) that have endured a long history of naturally fluctuating salinity levels (Williams, 2001). This history of episodic exposure of life forms to salinity is likely to have applied selective pressures resulting in many species evolving broad salinity tolerances (Williams, 2001). Australian frogs would seem to prove no exception to this rule.

Understanding the salt tolerance of *C. signifera* will allow us to predict how this species and possibly other amphibians within Australia will cope in regard to future landscape salinization, exacerbated by climate change and drought. Knowledge of *C. signifera* salinity tolerances across a much broader range of populations will assist in understanding salinity tolerance evolution and the ability of Australian frogs to adapt to environmental pressures (Hopkins and Brodie, 2015).

It remains important to now clarify the degree of salinity tolerance in other Australian frog species and to investigate the evolutionary processes that may have led to this tolerance (Hopkins and Brodie, 2015). More fully characterizing inter- and intrapopulation variation in salt tolerances across life stages will be particularly insightful in understanding the potential for local adaptation through natural selection (Hopkins et al., 2013a; Byrne et al., 2015). In addition, a more detailed understanding of the mechanistic basis of tolerance is needed, as are investigations of physiological trade-offs incurred through the acquisition of salinity tolerance in a variety of species. For instance, understanding any physiological correlates with salinity tolerance (e.g., corticosterone levels, immune function, fecundity trade-offs), and the ecological benefits of life in brackish waters (escape from predation, resource availability), will further illustrate the true impacts of landscape salinization on salt-tolerant frogs. Furthermore, it is known that increasing salt concentrations may render amphibians susceptible to other stressors such as pesticides (Wood and Welch, 2015) and temperature (Hopkins et al., 2017). Indeed, there is a growing body of evidence that there is a range of physiological stressors experienced by amphibians which increases their vulnerability to disease (Rohr et al., 2004). Understanding how both native and introduced amphibians (such as the salt tolerant and highly invasive 'Cane' or 'Marine' Toad, *Rhinella marina*; Table 1) tolerate increased salt concentra-

tions is important for predicting the impacts of landscape change on frog communities. The increasing salinization of fresh water throughout Australia and the world (e.g., Williams, 2001) adds further urgency to such studies.

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