Short- and medium-term exposure to salinity alters response to predation, activity and spatial movements in tadpoles

Léa Lorrain-Soligon*, Léa Koch, Akiko Kato, François Brischoux

Centre d'Etudes Biologiques de Chizé, CEBC UMR 7372 CNRS — La Rochelle Université, Villiers en Bois, France

Current environmental changes can drastically affect aquatic freshwater ecosystems. Salinization is one such change that affects freshwater species due to osmotic costs, which induce a wide variety of behavioural responses, including altered response to predation. This is particularly important in larval anurans, known to modulate their morphology, physiology and behaviour in response to salinity. Although these responses are known for long-term exposure, the effects of shorter exposure to salinity, relevant for coastal biodiversity as a result of sea water intrusions and precipitation variation, have rarely been assessed despite their importance in understanding the speed at which impacts on biodiversity occur. In this study, we experimentally acclimated larvae of the spined toad, *Bufo spinosus*, for 2 weeks to freshwater or to moderate levels of salinity (4 g/litre). The response to predation, activity and spatial movements of each individual were then assessed both at the salinity of acclimation and after a change in salinity. We demonstrated that medium-term exposure to salinity decreased activity, decreased growth and increased mortality. In contrast, sudden changes in salinity increased the escape distance of tadpoles in response to a predation stimulus but decreased activity. These results suggest that exposure to both stressors (predation cues and varying salinities) led to increased activity presumably linked to a stress response. However, individuals exposed to a change in salinity or to moderate salinity for medium durations expressed similar behavioural responses, indicating that the detrimental effects of high salinity can occur rapidly. Future studies should investigate the dynamics of osmolality in larval anurans exposed to brackish water to assess whether these behavioural shifts are linked with hydromineral dysregulation, and how long after exposure this occurs.

Current environmental changes can dramatically affect freshwater ecosystems (Angeler et al., 2014; Jackson et al., 2016; Woodward et al., 2010). Salinization, the increase in salinity in water bodies, is one such change that affects many wetlands worldwide (Cunillera-Montcusí et al., 2022; Herbert et al., 2015; Kaushal et al., 2018). Salinization can result from natural (primary salinization) or human-induced processes (secondary salinization; Herbert et al., 2015). Secondary salinization is linked to the alteration of the hydrobiological cycle (Neubauer & Craft, 2009) in response to several different causes such as sea level rise (Llovel et al., 2019; Nicholls & Cazenave, 2010; Werner & Simmons, 2009), increase in frequency and intensity of marine storms (IPCC, 2022), changes in precipitation and riverine flow (Herbert et al., 2015; Martínez-Megías & Rico, 2021; Neubauer & Craft, 2009), application of road de-icing salt (Denoël et al., 2010; Hall et al., 2017; Hintz & Relyea, 2019; Sanzo & Hecnar, 2006), or water and land uses linked to anthropogenic activities (Alcérreca-Huerta et al., 2019; Ghalambor et al., 2021; Herbert et al., 2015; Leal Filho et al., 2022; Mulamba et al., 2019; Tweedley et al., 2019). All these processes have led to unprecedented rates of salinization worldwide (Cunillera-Montcusí et al., 2022; Hintz et al., 2022; Kaushal et al., 2018), which is currently threatening biodiversity (Herbert et al., 2015).

Freshwater species are, with some exceptions (Cañedo-Argüelles et al., 2013; Hopkins & Brodie, 2015; Walker et al., 2023), generally intolerant to relatively high salinity levels (Kumar & Afaq, 2022). Indeed, maintaining osmolality in saline environments is metabolically costly (Evans & Kültz, 2020; Lillywhite & Evans, 2021; Peña-Villalobos et al., 2013; Pistole et al., 2008; Rivera-Ingraham & Lignot, 2017; Schultz & McCormick, 2012), and alters energetic allocation to other organismal functions such as gas exchange (Kidder III et al., 2006), growth (Alkhamis et al., 2022; Cañedo-Argüelles et al., 2013), energy acquisition (Alkhamis et al., 2022; Cañedo-Argüelles et al., 2013;

* Corresponding author.
  E-mail address: llorrain.lea@gmail.com (L. Lorrain-Soligon).

Herbert et al., 2015; Pinder et al., 2005), and body reserves through changes in food conversion rate (Luz et al., 2008) and protein and lipid contents (Wang et al., 2022).

Because of these changes in energetic allocation, exposure to salinity also affects the behaviour of individuals. Indeed, in a large variety of taxa, chronic exposure to increased salinity has been shown to induce a wide variety of behavioural responses such as reduced or supressed appetite (Ashley et al., 2021; Davenport & Ward, 1993), reduced food intake (Hannam et al., 2003; Luz et al., 2008), and reduced activity levels (Lorrain-Soligon, Bichet, et al., 2022; Wood & Welch, 2015; Zhou et al., 2022) all of which could strongly affect food acquisition. In addition, exposure to salinity has been shown to negatively affect self-maintenance behaviours (e.g. preening in birds, Hannam et al., 2003), to increase the frequency of specific behaviours (e.g. sneezing and head shaking to expel the secretions of salt glands, Rocha et al., 2016), and to affect social interactions (Zhou et al., 2022). Ultimately, exposure to salinity can induce behavioural responses such as saltwater avoidance and increased freshwater-seeking behaviour (Ashley et al., 2021; Gutiérrez, 2014; Lorrain-Soligon, Robin, et al., 2022) which are expected to influence species distributions (Brischoux et al., 2012, 2021).

Importantly, exposure to salinity can also interact with, and thus influence the responses to, other environmental stressors (Velasco et al., 2019). This seems particularly relevant for environmental stressors that can ultimately affect organismal fitness such as predation. Indeed, increased salinity has been shown to reduce the intensity of antipredator responses (Hoover et al., 2013). Such alteration of antipredator responses can thus strongly affect survival (Liu & Steiner, 2017). While organisms tend to avoid both saltwater and predator cues (Tietze & Gerald, 2016), salinity avoidance appears more important than predator avoidance when options are restricted (i.e. selection of freshwater with predation cues rather than saltwater without predation cues, Tietze & Gerald, 2016). Nevertheless, the combined effects of salinity and predation remain poorly studied, despite some evidence that predator-induced stress can induce stronger effects than other environmental stressors (Relyea & Mills, 2001).

Amphibians are particularly sensitive to salinity due to their highly permeable skin serving as a surface for respiration and for ion and water transport (Martin & Nagy, 1997; Shoemaker & Nagy, 1984), their limited ability to maintain normosmolality (Katz, 1989) and their larval dependence on the aquatic environment (Wells, 2007). As such, larval anurans are particularly well suited to study behavioural responses to salinity exposure. Indeed, salinity may depress thyroid hormones in tadpoles (Gomez-Mestre et al., 2004), and lead to changes in osmoregulatory hormones such as corticosterone and aldosterone (Tornabene, Crespi, et al., 2022; Tornabene, Hossack, et al., 2021). Exposure to salinity has also been shown to reduce development (Gomez-Mestre et al., 2004; Haramura, 2016; Hopkins et al., 2013; Lukens & Wilcoxen, 2020) and growth (Haramura, 2016; Lukens & Wilcoxen, 2020; Tornabene, Breuner, et al., 2021; Wood & Welch, 2015; Wu & Kam, 2009). In addition, larvae exposed to higher salinity express reduced activity and foraging behaviour (Chuang et al., 2022; Hall et al., 2017; Sanzo & Hecnar, 2006; Wood & Welch, 2015), lowered responses to stimuli (Karraker, 2007), poorer swimming performance (Haramura, 2016), erratic movements (Tornabene, Breuner, et al., 2021), and reduced swimming speed and distance (Denoël et al., 2010), all of which can affect their susceptibility to predation. Importantly, while these effects have been widely studied in individuals reared for their entire development in brackish water, few studies have examined the responses of individuals exposed for short events of increasing salinity (but see LoPiccolo, 2022). Yet, such studies are important to investigate the

effects of salinity variation and particularly sudden changes in salinity, which is particularly relevant in coastal and freshwater environments. In the latter, salinity can mostly increase due to runoff from roads where de-icing salt has been used (Brady, 2012; Brady et al., 2022; Denoël et al., 2010). In coastal environments, salinity can rapidly increase due to marine submersions (Lorrain-Soligon et al., 2021), sea water intrusion (Gopinath et al., 2015; Ranjbar & Ehteshami, 2019) and changes in precipitation and riverine flow (Herbert et al., 2015; Martínez-Megías & Rico, 2021; Neubauer & Craft, 2009). Importantly, in both environments, salinity can potentially rapidly decrease due to changes in precipitation regimes (Lorrain-Soligon, Robin, et al., 2023). Some of these processes induce changes in salinity over very short periods (within an hour, e.g. marine submersions and de-icing salt application), while others might induce changes in salinity across longer timescales (a few days, e.g. changes in precipitation and riverine flow).

In this study, to better understand the effects of salinity variation on amphibian behaviour, we experimentally acclimated larvae of the spined toad, *Bufo spinosus*, to freshwater (0 g/litre) or to moderate levels of salinity (4 g/litre) for 2 weeks. We then evaluated the response to predation (escape distance in response to stimuli) and different metrics of behaviour (mean speed, total distance travelled, active time, position relative to the centre of an arena and behavioural complexity) both at the salinity experienced during acclimation and after a sudden change of salinity. At the beginning and end of acclimation, individuals were also measured to compare growth rates. We predicted that 2 weeks of exposure to salinity should decrease growth rate and increase mortality due to the sensitivity of larval anurans to salinity (Albecker & McCoy, 2017; Gomez-Mestre et al., 2004). Additionally, we hypothesized it would decrease swimming ability (Denoël et al., 2010; Hall et al., 2017; Haramura, 2016; Karraker, 2007; Sanzo & Hecnar, 2006; Wood & Welch, 2015), which might decrease their responsiveness to predation. A sudden change in salinity during behavioural tests, as compared to a longer exposure to salinity, should increase activity due to osmotic stress but with no costs of osmoregulation (LoPiccolo, 2022).

METHODS

Study Species and Captures

The spined toad is a widespread amphibian species found in western Europe (Meek, 2022; Speybroeck et al., 2018). Breeding occurs in late winter, during which adults converge to reproductive ponds where they pair (amplexus) and lay their eggs. Egg laying, embryonic and larval development all occur in ponds. We caught 75 tadpoles from 20 to 24 April 2023 in a small pond (0.4248°W, 46.1459°N), where salinity was measured to be 0.0 g/litre using a conductimeter (YSI Professional Plus) at 15 cm depth. Sampling was focused specifically on a pond situated relatively far from the coastline and roads to assess the effect of salinity and its variation on individuals that were naïve to salt exposure. At least 18 different amplectant pairs were observed in this pond prior to sampling, suggesting that tadpoles involved in this experiment did not originate from the same family. Sampling was carried out along the banks of the pond, using transparent plastic boxes (14 × 7 cm and 6 cm deep). Tadpoles ranged from Gosner stage 30 to Gosner stage 33 (Gosner, 1960), during which no significant change in behaviour and morphology occurs (Cheron et al., 2021).

Treatments

Immediately after capture, individuals were brought to the laboratory (a thermally controlled room with temperature set at 17 °C and a controlled photoperiod of 12:12 h dark:light).

Individuals were acclimated for 4 days in an individual tank (13 × 18 cm and 18 cm high) with water from the pond of origin. After these 4 days, individuals were randomly allocated to two treatments to which they were acclimated for 2 weeks in individual 2-litre tanks (13 × 18 cm and 18 cm high) in which they remained throughout the experiment: control (0 g/litre salinity: 0.3 ± 0.0 g/litre, N = 31) and moderate salinity (brackish water, 4 g/litre salinity: 4.0 ± 0.06 g/litre, N = 44). We allocated more individuals to the moderate salinity treatment to take into account expected mortality in this experimental group (Albecker & McCoy, 2017; Bernabò et al., 2013; Chinathamby et al., 2006; Christy & Dickman, 2002; B. Jones et al., 2015; Karraker et al., 2008; Rios-López, 2008; Sanzo & Hecnar, 2006) and to maintain a sufficient sample size for final analyses. While 4 g/litre salinity might be elevated for some amphibian species (Hopkins & Brodie, 2015), adults in amplexus and larvae of *B. spinosus* have been found in up to 6 g/litre in the field (L. Lorrain-Soligon, personal observation; Lorrain-Soligon, Périsse, et al., 2023). Salinity treatments were obtained by dissolution of sea salt (NaCl). Our goal was to mimic the range of salinity found in coastal environments where the species is known to reproduce (Lorrain-Soligon, Périsse, et al., 2023). For the moderate salinity group, to reach final salinity, we progressively increased the salinity of the water at a rate of 1 g/litre a day to prevent osmotic shock, and because natural salinity fluctuations do not exceed ± 2 g/litre a day (Hsu et al., 2018). At the same time, we progressively replaced pond water by dechlorinated (aged) tap water. During the remaining procedure, water was changed once a week, and tadpoles were fed ad libitum with organic spinach. Individuals were checked twice a day for mortality.

Tadpole Size

Upon capture, individuals were individually placed in a petri dish, positioned upon a scale (graph paper), containing 2 cm of water. To measure total length, a picture was taken from above and measurements were performed using the software ImageJ (Schneider et al., 2012). Total length did not vary between the two treatments at the beginning of exposure (0 g/litre: 17.19 ± 0.32 mm SE; 4 g/litre: 17.88 ± 0.31 mm SE; linear model: estimate = 0.689, SE = 0.485, $t_{1.65}$ = 1.419, P = 0.161). After 2 weeks, individuals were measured once again following the same protocol to assess growth rates which were computed as the difference between size at the end and at the beginning of the experiment divided by size at the beginning of the experiment.

Tadpole Behaviour

We performed a comprehensive set of measures of behaviour at the end of the 2-week exposure to the two treatments: 0 g/litre and 4 g/litre (see Fig. 1). The tests were done across 6 days, and all individuals were tested in the morning (between 0900 and 1200 hours, six tadpoles at a time, three from each acclimation treatment), under controlled light intensity.

These measures were taken both at the salinity at which individuals were acclimated for 2 weeks (see Medium-term responses to salinity below) and following a sudden change in salinity (see Responses to salinity variation below).

Medium-term responses to salinity
First, to investigate the response to predation, we assessed swimming distance in response to a stimulus. Tadpoles were placed at the start of a 70 cm half pipe closed at the two extremities and filled with the salinity corresponding to the acclimation treatment (Fig. 1). Tadpoles were gently touched, and the escape distance was measured. We measured six successive escape distances. These tests lasted for <1 min and individuals were then transferred for the second set of behavioural tests (see
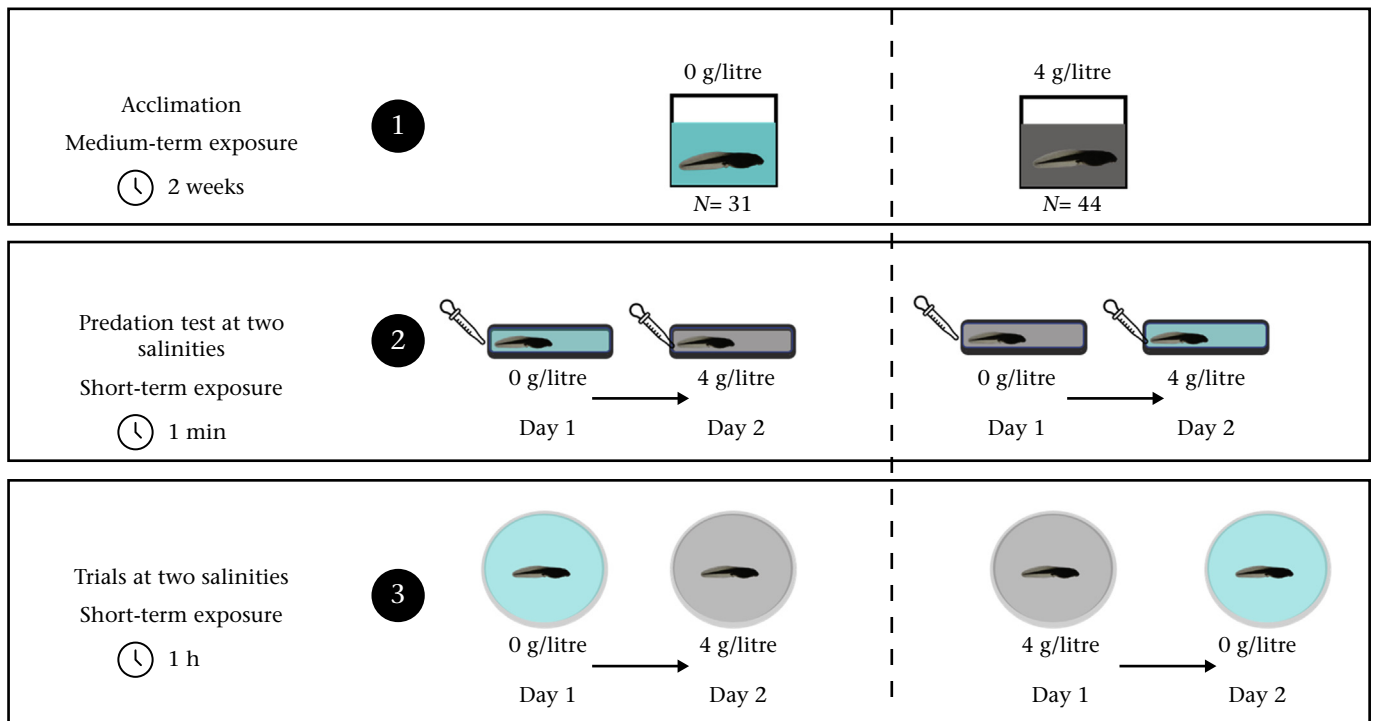
Figure 1. Experimental design used in this study. (1) Tadpoles were acclimated for 2 weeks in individual 2-litre tanks to control (0 g/litre salinity) or moderate salinity (brackish water, 4 g/litre salinity). (2) Their response to predation was assessed by measuring an individual's swimming distance in a pipe in response to a predation stimulus. (3) Behavioural responses of individuals to salinity were measured in an arena filled with water of the test salinity. Tadpoles were left to acclimate for 20 min, and then filmed f min. For steps 2 and 3, tadpoles acclimated at 0 g/litre were tested at 0 g/litre first and at 4 g/litre the day after, and conversely for tadpoles acclimated at 4 g/litre, to investigate the effects of a sudden change in salinity. Results were always analysed as an interaction between salinity of the acclimation (1) and salinity of the trial (2 or 3).

below). For analyses (see below), we used the mean distance (calculated from the six trials, or when individuals completed at least three successful trials).

Second, on the same day and immediately after the first test, to assess activity and spatial movements, tadpoles were placed in an individual arena (petri dish, diameter 13.5 cm, water level 1.5 cm) filled with the salinity corresponding to the acclimation treatment (Fig. 1). Tadpoles were left to acclimate for 20 min, and then filmed for 35 min, using a camera placed above the arena (GoPro HERO; GoPro, San Mateo, CA, U.S.A.). These trials thus lasted for approximatively 1 h. Videos were later analysed to extract behavioural data (see Video analyses below).

Responses to salinity variation

The following day, we switched salinity exposure for tadpoles, using the same protocols as previously described (Fig. 1). Tadpoles acclimated at 0 g/litre were tested at 4 g/litre, while individuals acclimated at 4 g/litre were tested at 0 g/litre (Fig. 1). To ensure that the order of the successive tests did not influence behaviour, we performed a previous escape distance trial (see Medium-term responses to salinity above) on a subsample of 10 individuals that were tested at 0 g/litre first and then 0 g/litre again the day after. We then computed mean escape distance (see paragraph below). We found that escape distance did not differ between day 1 and day 2 when individuals were exposed to the same treatment (linear mixed model: estimate $= -0.163$, SE $= 1.849$, $t_{1.109} = -0.088$, $P = 0.930$).

After these trials, individuals were released at their site of capture. Individuals in the moderate salinity treatment were progressively acclimated to freshwater before release, to avoid osmotic shock.

Video analyses

Videos were analysed with the software ToxTrac (Rodriguez et al., 2018). We used a white background to create a contrast that allowed the software to detect and monitor tadpoles. The successive positions of a tadpole (defined from the x- and y- calibrated coordinates within the petri dish) were then extracted per frame, and the distance moved between two consecutive frames was calculated. Using 'Tracking RealSpace' data, we determined the total duration of activity, the total distance travelled (relative to total length), mean swimming speed (relative to total length) and frequency of a tadpole being within 50 mm of the centre of the arena (based on the central point of the tadpole). As tadpoles can be more visible to predators in open areas (see Denoël et al., 2012 and references therein), we considered that swimming in the centre of the aquarium could be a 'risky' behaviour.

Additionally, we evaluated DFA (detrented fluctuation analysis) and DFAc (corrected detrented fluctuation analysis) using the fractal package (version 2.0-4, Constantine & Percival, 2017). DFA is a robust method used to estimate the degree to which time series are long-range dependent and self-affine (Cannon et al., 1997), which has already been used in the study of behaviour (Alados & Huffman, 2000; MacIntosh et al., 2011) including that of larval amphibians (Cheron et al., 2021, 2023). Following MacIntosh et al. (2013), to compute DFA, we coded behavioural sequences as binary time series [$z(i)$]: an immobile individual is coded as -1, while a mobile individual (an individual is considered moving if it moves more than 0.25 mm/s) is coded as 1, at 1 s intervals to length N (for 35 min). How DFA and DFAc were computed is thoroughly described in MacIntosh et al. (2013) and Peng et al. (1995). DFA is inversely related to the fractal dimension, a classical index of structural complexity (Mandelbrot, 1982), and thus smaller values reflect greater complexity.

Ethical Note

Individuals were exposed to a salinity level they might experience in nature (4 g/litre; tadpoles of this species can be found in up to 6 g/litre, L. Lorrain-Soligon, personal observation; Lorrain-Soligon, Périsse, et al., 2023), and individuals were progressively acclimated to this salinity level to avoid osmotic shock. Additionally, individuals were kept in optimal conditions, with large water volumes (2 litres) and food provided ad libitum. We did not perform invasive sampling, and we handled individuals only for transfer in behavioural tests. They were kept under laboratory conditions for 2 weeks and then released at their site of capture. This work was approved by the French authorities under permit APAFIS #33592-2021102610033982.

Statistical Analyses

All measures of distances or speed were corrected by individual total length, and were thus expressed relative to size (i.e. number of time individuals moved their total length) to provide a more meaningful measure of locomotor performance than absolute speed (Van Damme & Van Dooren, 1999). For all models computed, model accuracy was tested using the check_model function from the performance package allowing us to test for residuals distribution and normality (Lüdecke et al., 2020). For linear models (LMs) and linear mixed models (LMMs), when models did not fit the data, the response variable was log10+1 transformed. Binomial model accuracy was also tested by checking overdispersion.

First, we assessed differences in growth between treatments with an LM and mortality (as a binary component: 0 [mortality] or 1 [survival]) with a binomial generalized linear model (GLM) with acclimation treatment (0 or 4 g/litre) as an explanatory variable. Second, we assessed differences in mean escape distance during the predation test using an LMM. Lastly, we assessed differences in total duration of activity (log10+1 transformed), total distance travelled (log10+1 transformed), mean swimming speed (log10+1 transformed) and frequency of positioning at less than 50 mm from the centre of the arena (log10+1 transformed) with LMMs and behavioural complexity (DFA) with generalized linear mixed models (GLMMs) with a gamma distribution, due to a skewed distribution (Dobson & Barnett, 2018). For all models, we used the interaction between salinity acclimation treatment (0 or 4 g/litre) and salinity behavioural test treatment (0 or 4 g/litre) as explanatory variables, and individual as a random effect. For these models, the best variables were retained using a top-down selection, and only the retained variables are presented.

All data analysis were performed using R 3.6.3 (R Core Team, 2020) and RStudio v1.1.419 (RStudio Team, 2018).

RESULTS

Mortality and Tadpole Size

During acclimation (2 weeks), mortality was higher in individuals kept in brackish water compared to freshwater (four individuals died in freshwater, mortality rate $= 16\%$; 29 individuals died in brackish water, mortality rate $= 66\%$; estimate $= 2.569$, SE $= 0.623$, $z_{1.73} = 4.123$, $P < 0.001$). Growth rate (as a proportion of the initial size of individuals) was higher in freshwater compared to brackish water (freshwater: 0.212 ± 0.007 SE; saltwater: 0.061 ± 0.008 SE; estimate [proportion of the initial total length] $= 0.152$, SE $= 0.021$, $t_{1.46} = 7.393$, $P < 0.001$).

Tadpole Behaviour

Responses to predation stimuli

Mean escape distance (number of times an individual travelled its total length) was influenced by salinity during the trial (estimate $= 5.220$, SE $= 1.577$, $t_{1.59} = 3.309$, $P = 0.002$; Fig. 2) and by the interaction between acclimation treatment and salinity during the trial (estimate $= -7.597$, SE $= 2.213$, $t_{1.59} = -3.433$, $P = 0.001$; Fig. 2, Table A1), but not by salinity solely during acclimation (Estimate $= 2.433$, SE $= 2.230$, $t_{1.94} = 1.091$, $P = 0.278$). Mean escape distance was higher for individuals acclimated in freshwater and that performed the test in brackish water (Fig. 2).

Medium-term responses to salinity and responses to salinity variation

Total distance (log10+1 transformed) was influenced by salinity during acclimation (estimate $= -0.855$, SE $= 0.137$, $t_{1.113} = -6.230$, $P < 0.001$; Fig. 3a) and by the interaction between acclimation treatment and salinity during the trial (estimate $= 0.464$, SE $= 0.177$, $t_{1.58} = 2.623$, $P = 0.011$; Fig. 3a, Table A1), but not by salinity solely during the trial (estimate $= -0.191$, SE $= 0.125$, $t_{1.58} = -1.529$, $P = 0.132$). Overall, total distance was higher for individuals that were acclimated in freshwater and that performed the test in freshwater.

Mean active speed (log10+1 transformed) was influenced by salinity during acclimation solely (estimate $= -0.149$, SE $= 0.034$, $t_{1.58} = -4.396$, $P < 0.001$; Fig. 3b) and was lower for individuals raised in brackish water than those raised in freshwater. Total active time (log10+1 transformed) was influenced by salinity during acclimation (estimate $= -0.672$, SE $= 0.118$, $t_{1.114} = -5.690$, $P < 0.001$; Fig. 3c), marginally influenced by salinity during the trial (estimate $= -0.206$, SE $= 0.109$, $t_{1.58} = -1.888$, $P = 0.064$; Fig. 3c) and influenced by the interaction between acclimation treatment and salinity during the trial (estimate $= 0.429$, SE $= 0.154$, $t_{1.58} = 2.780$, $P = 0.007$; Fig. 3c, Table A1). Overall, total active time was higher for individuals that were acclimated in freshwater and that performed the test in freshwater. Frequency of positioning at less than 50 mm from the centre of the arena (log10+1 transformed) was influenced solely by salinity during acclimation
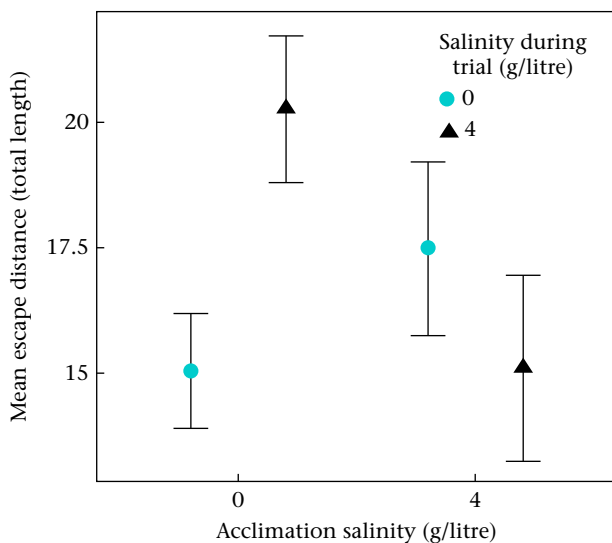
(estimate $= 0.478$, SE $= 0.118$, $t_{1.58} = 4.046$, $P < 0.001$; Fig. 3d), and was higher for individuals raised in brackish water than for those raised in freshwater (see Fig. 3d).

DFAc was influenced by salinity during acclimation (estimate $= -0.035$, SE $= 0.009$, $t = -4.021$, $P < 0.001$; Fig. 4), salinity during the trial (estimate $= -0.019$, SE $= 0.005$, $t = -3.712$, $P < 0.001$; Fig. 4) and by the interaction between acclimation treatment and salinity during the trial (estimate $= -0.029$, SE $= 0.007$, $t = 4.090$, $P < 0.001$; Fig. 4, Table A1). Overall, DFAc were the lowest (higher behavioural complexity) for individuals that were acclimated in freshwater and that performed the test in freshwater, and the highest (lower behavioural complexity) for individuals that were acclimated in brackish water and that performed the test in freshwater.

DISCUSSION

In this study, we demonstrated that larval spined toads that were naïve to salt exposure altered their behaviour in response to salinity. We showed that a 2-week exposure to moderate levels of salinity (4 g/litre) decreased growth rate, increased mortality and decreased escape distance (and thus reaction to predation), mean speed and behavioural complexity. However, when individuals were acclimated in freshwater and transferred to brackish water, escape distance increased, but mean speed and behavioural complexity decreased.

First, exposure to salinity for 2 weeks induced lower growth rates and higher mortality as expected from previous investigations (Albecker & McCoy, 2017; Gomez-Mestre et al., 2004; Tornabene, Breuner, et al., 2022). Additionally, we highlighted that this exposure to salinity negatively influenced behavioural performance (Denoël et al., 2010; Hall et al., 2017; Haramura, 2016; Karraker, 2007; Sanzo & Hecnar, 2006; Wood & Welch, 2015). Specifically, individuals exposed to salinity exhibited shorter escape distance and total distance travelled, slower swimming speed, lower activity time and decreased behavioural complexity, and were more often positioned closer to the centre of the arena. In natural environments, such alterations of key behavioural traits may led to reduced foraging (Bartumeus, 2007; Viswanathan et al., 2008), because these traits should be adaptive in unpredictable environments where they can increase the probability of encountering resources (Bartumeus, 2007; Viswanathan et al., 2008) and prevent predators from anticipating the prey's future position (Gazzola et al., 2021; Jones et al., 2011; Richardson et al., 2018). In addition, these alterations can also induce a higher risk of predation (Chovanec, 1992; Denoël et al., 2012; Eterovick et al., 2010; Laurila, 2000), as remaining closer to the edges is considered as less risky and spatial avoidance is expected in tadpoles exposed to a predation threat (Relyea, 2001). The effects we found on growth, mortality and behaviour are probably linked to higher costs of osmoregulation (Gomez-Mestre et al., 2004), mediated by modulations of specific hormonal mediators such as corticosterone and aldosterone (Tornabene, Crespi, et al., 2022; Tornabene, Hossack, et al., 2021). Importantly, we showed that these responses can be expressed over a relatively short period (2 weeks), shorter than those used in previous investigations (Denoël et al., 2010; Gomez-Mestre et al., 2004; Tornabene, Hossack, et al., 2021). This suggests that the energetic cost of osmoregulation can appear relatively rapidly at a larval stages (Gosner stages 30—33; see also Lorrain-Soligon et al., 2024) considered to be comparatively less sensitive than embryo or earlier larval stages (Albecker & McCoy, 2017; Chinathamby et al., 2006; Uchiyama & Yoshizawa, 1992). Future studies are required to investigate whether increasing salinity increases metabolic rates in larval anurans, as suggested in other species (Peña-Villalobos et al., 2013; Pistole et al., 2008).

Figure 2. Mean escape distance (relative to total size) in response to a predation stimulus, according to salinity during acclimation (2 weeks in freshwater [0 g/litre] or brackish water [4 g/litre]) and salinity during the trial (freshwater [0 g/litre] or brackish water [4 g/litre]). Data are represented as mean ± SE.

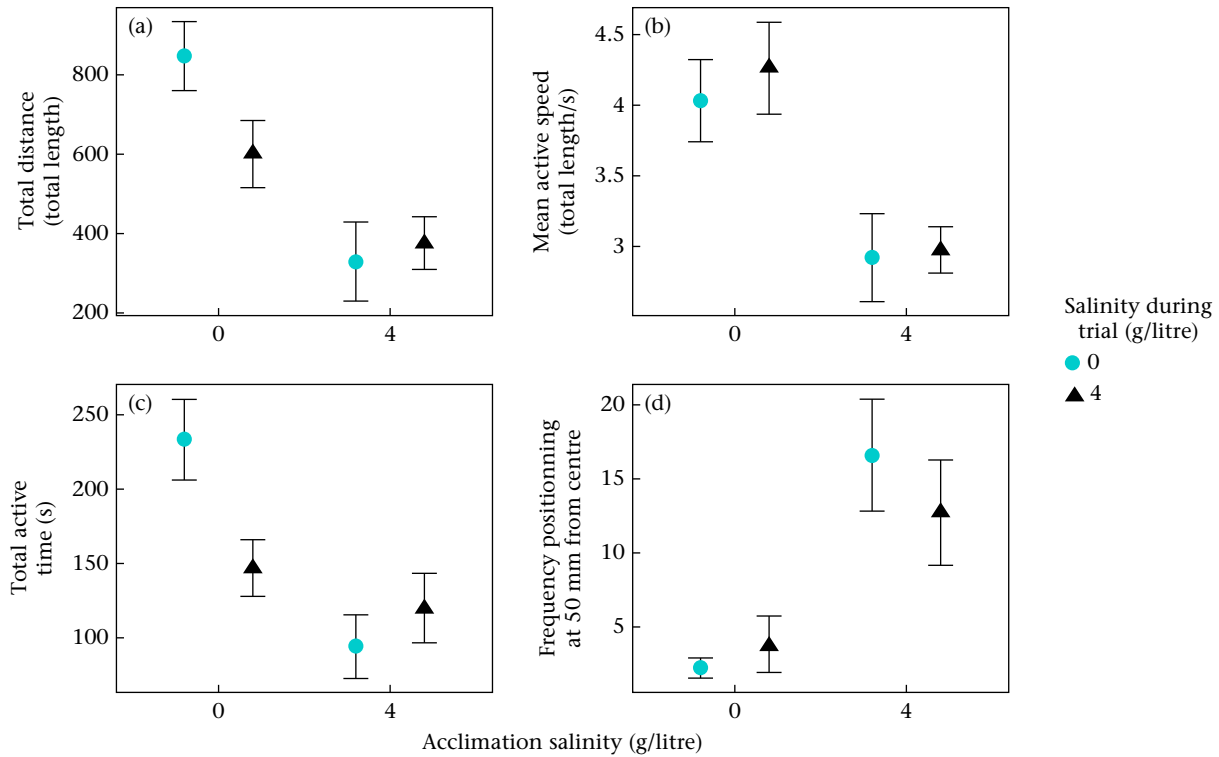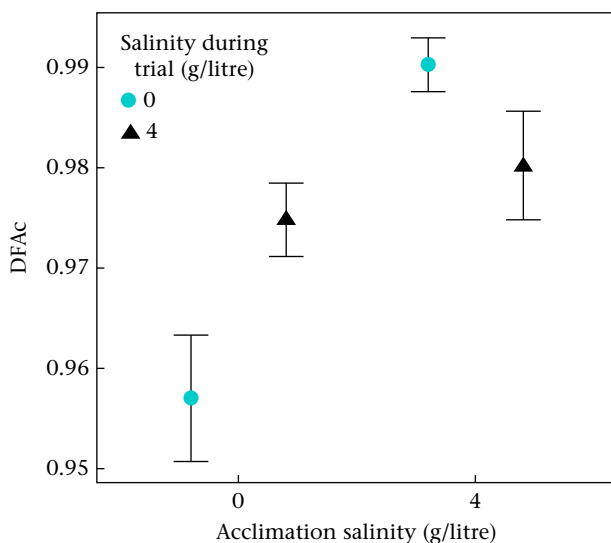**Figure 3.** (a) Total distance travelled (relative to total size), (b) mean active speed (relative to total size), (c) total active time and (d) frequency of positioning at less than 50 mm from the centre of the arena, according to salinity during acclimation (2 weeks in freshwater [0 g/litre] or brackish water [4 g/litre]) and salinity during the trial (freshwater [0 g/litre] or brackish water [4 g/litre]). Data are represented as mean ± SE.

Besides these effects during the acclimation phase of tadpoles to salinity, our experimental design allowed us to highlight different behavioural responses following a sudden change in salinity. First, we found that immediately after an increase in salinity, individuals reared in freshwater increased their escape distance in response to a predation stimulus. Conversely, individuals reared in brackish

Figure 4. DFAc (corrected detrented fluctuation analysis, an index of behavioural complexity), according to salinity during acclimation (2 weeks in freshwater [0 g/litre] or brackish water [4 g/litre]) and to salinity during the trial (freshwater [0 g/litre] or brackish water [4 g/litre]). DFAc is inversely related to the fractal dimension, a classical index of structural complexity (Mandelbrot, 1982), and thus smaller values reflect greater complexity. Data are represented as mean ± SE.

water did not modulate their behaviour during short-term exposure to either freshwater or a moderate level of salinity (4 g/litre), suggesting that a sudden decrease in water salinity did not trigger osmotic stress responses in these individuals, and that the costs of osmoregulation for individuals reared in brackish water (Gomez-Mestre et al., 2004) exceed the osmotic shock (or physiological relief) from being transferred to freshwater. These results suggest that, when exposed to a rapid increase in salinity, larval anurans can react more quickly to a predation threat, highlighting a higher response to predation cues in the high-salinity treatment for individuals reared in freshwater (Hoover et al., 2013; Troyer & Turner, 2015), presumably due to the presence of both stressors. Whether this response persists over longer timescales needs to be investigated.

Second, sudden exposure to salinity for individuals reared in freshwater led to a shorter distance travelled, slower mean speed and decreased behavioural complexity. These individuals thus expressed similar responses to those exposed during longer durations (Denoël et al., 2010; Hall et al., 2017; Haramura, 2016; Karraker, 2007; Sanzo & Hecnar, 2006; Wood & Welch, 2015), indicating that elevated salinity can have detrimental effects on larval amphibians after a very short exposure. Interestingly, and similar to our results on the response to predation, individuals reared in brackish water did not express different behaviour in response to exposure to fresh or brackish water. Our results thus point out that a relatively short exposure to salinity (2 weeks) can lead to irreversible effects on key behavioural traits, even when tadpoles were transferred to freshwater, which was expected to induce a physiological relief (or an additional stressor leading to an osmotic shock). As a result, even if salinity decreases in saline ponds as a result of increased precipitation (Lorrain-Soligon, Robin, et al., 2023), this could not be sufficient to dampen the effect of an elevated salinity during earlier larval development. Future studies

should investigate the dynamics of osmolality in larval anurans exposed to brackish water to assess whether these behavioural shifts are linked with hydromineral dysregulation as shown in other studies (Brady et al., 2022; Denoël et al., 2010; Lorrain-Soligon, Bichet, et al., 2022; Lorrain-Soligon, Robin, et al., 2022), and how long after exposure this occurs.

Conclusion

While chronic exposure to salinity has long been shown to alter the behaviour of larval anurans, we have shown that this effect can occur after very short exposure to salinity (1 h), and is maintained for longer exposure (2 weeks). This strongly suggests that osmolality and associated hydromineral dysregulation following exposure to salinity can be very rapid, which might threaten coastal biodiversity as a result of rapid salinity fluctuations.

Author Contributions

L.L.S. conceptualized the study; L.L.S. and L.K. collected the data; L.L.S. and A.K. analysed the data; L.L.S. and F.B. wrote the initial draft. All authors reviewed and edited the manuscript and approved the final version.

Data Availability

The data that support the findings of this study are available in the Supplementary material.

Declaration of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2024.03.023.

References

Alados, C. L., & Huffman, M. A. (2000). Fractal long-range correlations in behavioural sequences of wild chimpanzees: A non-invasive analytical tool for the evaluation of health. *Ethology, 106*(2), 105–116. https://doi.org/10.1046/j.1439-0310.2000.00497.x

Albecker, M. A., & McCoy, M. W. (2017). Adaptive responses to salinity stress across multiple life stages in anuran amphibians. *Frontiers in Zoology, 14*(1), 1–16. https://doi.org/10.1186/s12983-017-0222-0

Alcérreca-Huerta, J. C., Callejas-Jiménez, M. E., Carrillo, L., & Castillo, M. M. (2019). Dam implications on salt-water intrusion and land use within a tropical estuarine environment of the Gulf of Mexico. *Science of the Total Environment, 652*, 1102–1112. https://doi.org/10.1016/j.scitotenv.2018.10.288

Alkhamis, Y. A., Mondal, B., Mathew, R. T., Nagarajan, G., Rahman, S. M., Rahman, M. M., Alhajji, A., & Rahman, M. M. (2022). Periodic effects of salinity on compensatory expression of phenotypic traits in Nile tilapia (*Oreochromis niloticus*). *E Zoological Society of Pakistan*, 1–9. https://doi.org/10.17582/journal.pjz/2022011605012

Angeler, D. G., Allen, C. R., Birgé, H. E., Drakare, S., McKie, B. G., & Johnson, R. K. (2014). Assessing and managing freshwater ecosystems vulnerable to environmental change. *Ambio, 43*(1), 113–125. https://doi.org/10.1007/s13280-014-0566-z

Ashley, E. A., Davis, A. K., Terrell, V. K., Lake, C., Carden, C., Head, L., Choe, R., & Maerz, J. C. (2021). Effects of salinity on hatchling diamond-backed terrapin (*Malaclemys terrapin*) growth, behavior, and stress physiology. *Herpetologica, 77*(1), 45–55. https://doi.org/10.1655/Herpetologica-D-20-00028.1

Bartumeus, F. (2007). Lévy processes in animal movement: An evolutionary hypothesis. *Fractals, 15*(2), 151–162. https://doi.org/10.1142/S0218348X07003460

Bernabò, I., Bonacci, A., Coscarelli, F., Tripepi, M., & Brunelli, E. (2013). Effects of salinity stress on *Bufo balearicus* and *Bufo bufo* tadpoles: Tolerance, morphological gill alterations and Na+/K+-ATPase localization. *Aquatic Toxicology, 132–133C*, 119–133. https://doi.org/10.1016/j.aquatox.2013.01.019

Brady, S. P. (2012). Road to evolution? Local adaptation to road adjacency in an amphibian (*Ambystoma maculatum*). *Scientific Reports, 2*(1), Article 1. https://doi.org/10.1038/srep00235

Brady, S. P., Goedert, D., Frymus, L. E., Zamora-Camacho, F. J., Smith, P. C., Zeiss, C. J., Comas, M., Abbott, T. A., Basu, S. P., & DeAndressi, J. C. (2022). Salted roads lead to oedema and reduced locomotor function in amphibian populations. *Freshwater Biology, 67*(7), 1150–1161.

Brischoux, F., Lillywhite, H. B., Shine, R., & Pinaud, D. (2021). Osmoregulatory ability predicts geographical range size in marine amniotes. *Proceedings of the Royal Society B: Biological Sciences, 288*(1948), Article 20203191. https://doi.org/10.1098/rspb.2020.3191

Brischoux, F., Tingley, R., Shine, R., & Lillywhite, H. B. (2012). Salinity influences the distribution of marine snakes: Implications for evolutionary transitions to marine life. *Ecography, 35*(11), 994–1003. https://doi.org/10.1111/j.1600-0587.2012.07717.x

Cañedo-Argüelles, M., Kefford, B. J., Piscart, C., Prat, N., Schäfer, R. B., & Schulz, C.-J. (2013). Salinisation of rivers: An urgent ecological issue. *Environmental Pollution, 173*, 157–167. https://doi.org/10.1016/j.envpol.2012.10.011

Cannon, M. J., Percival, D. B., Caccia, D. C., Raymond, G. M., & Bassingthwaighte, J. B. (1997). Evaluating scaled windowed variance methods for estimating the Hurst coefficient of time series. *Physica A: Statistical Mechanics and its Applications, 241*(3–4), 606–626.

Cheron, M., Kato, A., Ropert-Coudert, Y., Meyer, X., MacIntosh, A. J. J., Raoelison, L., & Brischoux, F. (2023). Exposure, but not timing of exposure, to a sulfonylurea herbicide alters larval development and behaviour in an amphibian species. *Aquatic Toxicology, 254*, Article 106355. https://doi.org/10.1016/j.aquatox.2022.106355

Cheron, M., Raoelison, L., Kato, A., Ropert-Coudert, Y., Meyer, X., MacIntosh, A. J. J., & Brischoux, F. (2021). Ontogenetic changes in activity, locomotion and behavioural complexity in tadpoles. *Biological Journal of the Linnean Society, 134*(1), 165–176. https://doi.org/10.1093/biolinnean/blab077

Chinathamby, K., Reina, R. D., Bailey, P. C. E., & Lees, B. K. (2006). Effects of salinity on the survival, growth and development of tadpoles of the brown tree frog, *Litoria ewingii*. *Australian Journal of Zoology, 54*(2), 97–105. https://doi.org/10.1071/ZO06006

Chovanec, A. (1992). The influence of tadpole swimming behaviour on predation by dragonfly nymphs. *Amphibia-Reptilia, 13*(4), 341–349. https://doi.org/10.1163/156853892X00049

Christy, M., & Dickman, C. (2002). Effects of salinity on tadpoles of the green and golden bell frog (*Litoria aurea*). *Amphibia-Reptilia, 23*, 1–11. https://doi.org/10.1163/156853802320877582

Chuang, M., Cheng, Y.-J., Andersen, D., Borzée, A., Wu, C., Chang, Y.-M., Yang, Y.-J., Jang, Y., & Kam, Y. (2022). Increasing salinity stress decreases the thermal tolerance of amphibian tadpoles in coastal areas of Taiwan. *Scientific Reports, 12*, 9014. https://doi.org/10.1038/s41598-022-12837-7

Constantine, W., & Percival, D. (2017). *Fractal: A fractal time series modeling and analysis package*. R package version 2.0-4 https://CRAN.R-project.org/package=fractal.RPackageVersion,2–0.

Cunillera-Montcusí, D., Beklioğlu, M., Cañedo-Argüelles, M., Jeppesen, E., Ptacnik, R., Amorim, C. A., Arnott, S. E., Berger, S. A., Brucet, S., Dugan, H. A., Gerhard, M., Horváth, Z., Langenheder, S., Nejstgaard, J. C., Reinikainen, M., Striebel, M., Urrutia-Cordero, P., Vad, C. F., Zadereev, E., & Matias, M. (2022). Freshwater salinisation: A research agenda for a saltier world. *Trends in Ecology & Evolution, 37*(5), 440–453. https://doi.org/10.1016/j.tree.2021.12.005

Davenport, J., & Ward, J. F. (1993). The effects of salinity and temperature on appetite in the diamondback terrapin Malaclemys terrapin (Latreille). *Herpetological Journal, 3*(3), 95–98.

Denoël, M., Bichot, M., Ficetola, G. F., Delcourt, J., Ylieff, M., Kestemont, P., & Poncin, P. (2010). Cumulative effects of road de-icing salt on amphibian behavior. *Aquatic Toxicology, 99*(2), 275–280. https://doi.org/10.1016/j.aquatox.2010.05.007

Denoël, M., D'Hooghe, B., Ficetola, G. F., Brasseur, C., De Pauw, E., Thomé, J.-P., & Kestemont, P. (2012). Using sets of behavioral biomarkers to assess short-term effects of pesticide: A study case with endosulfan on frog tadpoles. *Ecotoxicology, 21*(4), 1240–1250. https://doi.org/10.1007/s10646-012-0878-3

Dobson, A. J., & Barnett, A. G. (2018). *An introduction to generalized linear models*. CRC Press.

Eterovick, P. C., Oliveira, F. F. R., & Tattersall, G. J. (2010). Threatened tadpoles of *Bokermannohyla alvarengai* (Anura: Hylidae) choose backgrounds that enhance crypsis potential. *Biological Journal of the Linnean Society, 101*(2), 437–446. https://doi.org/10.1111/j.1095-8312.2010.01501.x

Evans, T. G., & Kültz, D. (2020). The cellular stress response in fish exposed to salinity fluctuations. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 333*(6), 421–435. https://doi.org/10.1002/jez.2350

Gazzola, A., Balestrieri, A., Scribano, G., Fontana, A., & Pellitteri-Rosa, D. (2021). Contextual behavioural plasticity in Italian agile frog (*Rana latastei*) tadpoles exposed to native and alien predator cues. *Journal of Experimental Biology, 224*(9), Article jeb240465. https://doi.org/10.1242/jeb.240465

Ghalambor, C. K., Grosholz, E. D., Jeffries, K. M., Largier, J. K., McCormick, S. D., Sommer, T., Velotta, J., & Whitehead, A. (2021). Ecological effects of climate-driven salinity variation in the San Francisco estuary: Can we anticipate and manage the coming changes? *San Francisco Estuary and Watershed Science, 19*(2), 1–30. https://doi.org/10.15447/sfews.2021v19iss2art3

Gomez-Mestre, I., Tejedo, M., Ramayo, E., & Estepa, J. (2004). Developmental alterations and osmoregulatory physiology of a larval anuran under osmotic stress. *Physiological and Biochemical Zoology, 77*(2), 267–274. https://doi.org/10.1086/378143

Gopinath, S., Srinivasamoorthy, K., Saravanan, K., Suma, C. S., Prakash, R., Senthilnathan, D., Chandrasekaran, N., Srinivas, Y., & Sarma, V. S. (2015). Modeling saline water intrusion in Nagapattinam coastal aquifers, Tamilnadu, India. *Modeling Earth Systems and Environment, 2*(1), Article 2. https://doi.org/10.1007/s40808-015-0058-6

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica, 16*(3), 183–190.

Gutiérrez, J. S. (2014). Living in environments with contrasting salinities: A review of physiological and behavioural responses in waterbirds. *Ardeola, 61*(2), 233–256. https://doi.org/10.13157/arla.61.2.2014.233

Hall, E. M., Brady, S. P., Mattheus, N. M., Earley, R. L., Diamond, M., & Crespi, E. J. (2017). Physiological consequences of exposure to salinized roadside ponds on wood frog larvae and adults. *Biological Conservation, 209*, 98–106. https://doi.org/10.1016/j.biocon.2017.02.013

Hannam, K. M., Oring, L. W., & Herzog, M. P. (2003). Impacts of salinity on growth and behavior of American avocet chicks. *Waterbirds, 26*(1), 119–125. https://doi.org/10.1675/1524-4695(2003)026[0119:IOSOGA]2.0.CO;2

Haramura, T. (2016). Hatching plasticity in response to salinity levels in a rhacophorid frog inhabiting a coastal area. *Journal of Zoology, 299*(2), 125–131. https://doi.org/10.1111/jzo.12323

Herbert, E. R., Boon, P., Burgin, A. J., Neubauer, S. C., Franklin, R. B., Ardón, M., Hopfensperger, K. N., Lamers, L. P. M., & Gell, P. (2015). A global perspective on wetland salinization: Ecological consequences of a growing threat to freshwater wetlands. *Ecosphere, 6*(10), 1–43. https://doi.org/10.1890/ES14-00534.1

Hintz, W. D., Arnott, S. E., Symons, C. C., Greco, D. A., McClymont, A., Brentrup, J. A., Cañedo-Argüelles, M., Derry, A. M., Downing, A. L., Gray, D. K., Melles, S. J., Relyea, R. A., Rusak, J. A., Searle, C. L., Astorg, L., Baker, H. K., Beisner, B. E., Cottingham, K. L., Ersoy, Z., … Weyhenmeyer, G. A. (2022). Current water quality guidelines across North America and Europe do not protect lakes from salinization. *Proceedings of the National Academy of Sciences, 119*(9), Article e2115033119. https://doi.org/10.1073/pnas.2115033119

Hintz, W. D., & Relyea, R. A. (2019). A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. *Freshwater Biology, 64*(6), 1081–1097. https://doi.org/10.1111/fwb.13286

Hoover, Z., Ferrari, M. C., & Chivers, D. P. (2013). The effects of sub-lethal salinity concentrations on the anti-predator responses of fathead minnows. *Chemosphere, 90*(3), 1047–1052. https://doi.org/10.1016/j.chemosphere.2012.08.051

Hopkins, G. R., & Brodie, E. D. (2015). Occurrence of amphibians in saline habitats: A review and evolutionary perspective. *Herpetological Monographs, 29*(1), 1–27. https://doi.org/10.1655/HERPMONOGRAPHS-D-14-00006

Hopkins, G., French, S., & Brodie III, E. (2013). Increased frequency and severity of developmental deformities in rough-skinned newt (*Taricha granulosa*) embryos exposed to road deicing salts (NaCl & MgCl2). *Environmental Pollution (Barking, Essex: 1987), 173C*, 264–269. https://doi.org/10.1016/j.envpol.2012.10.002

Hsu, W. T., Wu, C. S., Hatch, K. A., Chang, Y. M., & Kam, Y. C. (2018). Full compensation of growth in salt-tolerant tadpoles after release from salinity stress. *Journal of Zoology, 304*(2), 141–149.

IPCC. (2022). In H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, & B. Rama (Eds.), *Climate change 2022: Impacts, adaptation, and vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge Univ. Press.

Jackson, M. C., Loewen, C. J., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. *Global Change Biology, 22*(1), 180–189. https://doi.org/10.1111/gcb.13028

Jones, K. A., Jackson, A. L., & Ruxton, G. D. (2011). Prey jitters; protean behaviour in grouped prey. *Behavioral Ecology, 22*(4), 831–836. https://doi.org/10.1093/beheco/arr062

Jones, B., Snodgrass, J. W., & Ownby, D. R. (2015). Relative toxicity of NaCl and road deicing salt to developing amphibians. *Copeia, 103*(1), 72–77. https://doi.org/10.1643/CP-13-082

Karraker, N. E. (2007). Are embryonic and larval green frogs (*Rana clamitans*) insensitive to road deicing salt? *Herpetological Conservation and Biology, 2*(1), 35–41.

Karraker, N. E., Gibbs, J. P., & Vonesh, J. R. (2008). Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. *Ecological Applications, 18*(3), 724–734. https://doi.org/10.1890/07-1644.1

Katz, U. (1989). Strategies of adaptation to osmotic stress in anuran Amphibia under salt and burrowing conditions. *Comparative Biochemistry and Physiology Part A: Physiology, 93*(3), 499–503. https://doi.org/10.1016/0300-9629(89)90001-7

Kaushal, S. S., Likens, G. E., Pace, M. L., Utz, R. M., Haq, S., Gorman, J., & Grese, M. (2018). Freshwater salinization syndrome on a continental scale. *Proceedings of the National Academy of Sciences, 115*(4), E574–E583. https://doi.org/10.1073/pnas.1711234115

Kidder III, G. W., Petersen, C. W., & Preston, R. L. (2006). Energetics of osmoregulation: II. Water flux and osmoregulatory work in the euryhaline fish, *Fundulus heteroclitus*. *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 305A*(4), 318–327. https://doi.org/10.1002/jez.a.252

Kumar, G., & Afaq, U. (2022). Freshwater biodiversity: Importance, threats, and management. In *Biodiversity of Freshwater Ecosystems* (pp. 1–20). Apple Academic Press.

Laurila, A. (2000). Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos, 88*(1), 159–168. https://doi.org/10.1034/j.1600-0706.2000.880118.x

Leal Filho, W., Nagy, G. J., Martinho, F., Saroar, M., Erache, M. G., Primo, A. L., Pardal, M. A., & Li, C. (2022). Influences of climate change and variability on estuarine ecosystems: An impact study in selected European, South American and Asian countries. *International Journal of Environmental Research and Public Health, 19*(1), Article 585. https://doi.org/10.3390/ijerph19010585

Lillywhite, H. B., & Evans, D. H. (2021). Osmoregulation by vertebrates in aquatic environments. In *Encyclopedia of Life Science* (pp. 1–14). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470015902.a0029291.

Liu, X., & Steiner, C. F. (2017). Ecotoxicology of salinity tolerance in *Daphnia pulex*: Interactive effects of clonal variation, salinity stress and predation. *Journal of Plankton Research, 39*(4), 687–697. https://doi.org/10.1093/plankt/fbx027

Llovel, W., Purkey, S., Meyssignac, B., Blazquez, A., Kolodziejczyk, N., & Bamber, J. (2019). Global ocean freshening, ocean mass increase and global mean sea level rise over 2005–2015. *Scientific Reports, 9*(1), Article 1. https://doi.org/10.1038/s41598-019-54239-2

LoPiccolo, V. (2022). Turning tadpoles: Effects of salinity and lateralization on *Hyla versicolor* escape behaviors. Master's Thesis. Montclair State University.

Lorrain-Soligon, L., Bichet, C., Robin, F., & Brischoux, F. (2022). From the field to the lab: Physiological and behavioural consequences of environmental salinity in a coastal frog. *Frontiers in Physiology, 13*, Article 919165. https://doi.org/10.3389/fphys.2022.919165

Lorrain-Soligon, L., Bizon, T., Robin, F., Jankovic, M., & Brischoux, F. (2024). Variations of salinity during reproduction and development affect ontogenetic trajectories in a coastal amphibian. *Environmental Science and Pollution Research, 31*, 11735–11748. https://doi.org/10.1007/s11356-024-31886-1

Lorrain-Soligon, L., Périsse, L., Robin, F., Jankovic, M., & Brischoux, F. (2023). The costs of living on the coast: Reduction in body size and size-specific reproductive output in coastal populations of a widespread amphibian. *Functional Ecology, 37*, 2703–2717. https://doi.org/10.1111/1365-2435.14413

Lorrain-Soligon, L., Robin, F., Bertin, X., Jankovic, M., Rousseau, P., Lelong, V., & Brischoux, F. (2023). Long-term trends of salinity in coastal wetlands: Effects of climate, extreme weather events, and sea water level. *Environmental Research, 237*, Article 116937. https://doi.org/10.1016/j.envres.2023.116937

Lorrain-Soligon, L., Robin, F., & Brischoux, F. (2022). Hydric status influences salinity-dependent water selection in frogs from coastal wetlands. *Physiology & Behavior, 249*, Article 113775. https://doi.org/10.1016/j.physbeh.2022.113775

Lorrain-Soligon, L., Robin, F., Rousseau, P., Jankovic, M., & Brischoux, F. (2021). Slight variations in coastal topography mitigate the consequence of storm-induced marine submersion on amphibian communities. *Science of the Total Environment, 770*, Article 145382. https://doi.org/10.1016/j.scitotenv.2021.145382

Lüdecke, D., Makowski, D., Waggoner, P., & Patil, I. (2020). Performance: Assessment of regression models performance. *Journal of Open Source Software, 6*(60), Article 3139. https://doi.org/10.21105/joss.03139

Lukens, E., & Wilcoxen, T. E. (2020). Effects of elevated salinity on Cuban treefrog *Osteopilus septentrionalis* aldosterone levels, growth, and development. *Marine and Freshwater Behaviour and Physiology, 53*(3), 99–111. https://doi.org/10.1080/10236244.2020.1772062

Luz, R. K., Martínez-Álvarez, R. M., De Pedro, N., & Delgado, M. J. (2008). Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. *Aquaculture, 276*(1), 171–178. https://doi.org/10.1016/j.aquaculture.2008.01.042

MacIntosh, A. J. J., Alados, C. L., & Huffman, M. A. (2011). Fractal analysis of behaviour in a wild primate: Behavioural complexity in health and disease. *Journal of the Royal Society Interface, 8*(63), 1497–1509. https://doi.org/10.1098/rsif.2011.0049

MacIntosh, A. J. J., Pelletier, L., Chiaradia, A., Kato, A., & Ropert-Coudert, Y. (2013). Temporal fractals in seabird foraging behaviour: Diving through the scales of time. *Scientific Reports, 3*(1), Article 1. https://doi.org/10.1038/srep01884

Mandelbrot, B. B. (1982). *The fractal geometry of Nature WH Freeman and Company* (Vol. 8). New York: W. H. Freeman.

Martínez-Megías, C., & Rico, A. (2021). Biodiversity impacts by multiple anthropogenic stressors in Mediterranean coastal wetlands. *Science of the Total Environment, 818*, Article 151712. https://doi.org/10.1016/j.scitotenv.2021.151712

Martin, K. L., & Nagy, K. A. (1997). Water balance and the physiology of the amphibian to amniote transition. In *Amniote Origins* (pp. 399–423). Elsevier.

Meek, R. (2022). Long-term changes in four populations of the spiny toad, *Bufo spinosus*, in Western France; Data from road mortalities. *Conservation, 2*(2), 248–261. https://doi.org/10.3390/conservation2020017

Mulamba, T., Bacopoulos, P., Kubatko, E. J., & Pinto, G. F. (2019). Sea-level rise impacts on longitudinal salinity for a low-gradient estuarine system. *Climatic Change, 152*(3), 533–550. https://doi.org/10.1007/s10584-019-02369-x

Neubauer, S. C., & Craft, C. B. (2009). Global change and tidal freshwater wetlands: Scenarios and impacts. In *Tidal freshwater wetlands* (pp. 253–266). Leiden, The Netherlands: Backhuys.

Nicholls, R. J., & Cazenave, A. (2010). Sea-level rise and its impact on coastal zones. *Science, 328*(5985), 1517–1520. https://doi.org/10.1126/science.1185782

Peña-Villalobos, I., Valdés-Ferranty, F., & Sabat, P. (2013). Osmoregulatory and metabolic costs of salt excretion in the Rufous-collared sparrow *Zonotrichia capensis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 164*(2), 314–318. https://doi.org/10.1016/j.cbpa.2012.10.027

Peng, C.-K., Havlin, S., Stanley, H. E., & Goldberger, A. L. (1995). Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *Chaos: An Interdisciplinary Journal of Nonlinear Science, 5*(1), 82–87. https://doi.org/10.1063/1.166141

Pinder, A. M., Halse, S. A., McRae, J. M., & Shiel, R. J. (2005). Occurrence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. *Hydrobiologia, 543*(1), 1–24. https://doi.org/10.1007/s10750-004-5712-3

Pistole, D. H., Peles, J. D., & Taylor, K. (2008). Influence of metal concentrations, percent salinity, and length of exposure on the metabolic rate of fathead minnows (*Pimephales promelas*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 148*(1), 48–52. https://doi.org/10.1016/j.cbpc.2008.03.004

R Core Team. (2020). *R: A language and environment for statistical computing.* Austria: Vienna: R Foundation for Statistical Computing. https://www.R-project.org/.

Ranjbar, A., & Ehteshami, M. (2019). Spatio-temporal mapping of salinity in the heterogeneous coastal aquifer. *Applied Water Science, 9*(2), 1–14. https://doi.org/10.1007/s13201-019-0908-x

Relyea, R. A. (2001). Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology, 82*(2), 523–540. https://doi.org/10.1890/0012-9658(2001)082[0523:MABPOL]2.0.CO;2

Relyea, R. A., & Mills, N. (2001). Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proceedings of the National Academy of Sciences, 98*(5), 2491–2496. https://doi.org/10.1073/pnas.031076198

Richardson, G., Dickinson, P., Burman, O. H. P., & Pike, T. W. (2018). Unpredictable movement as an anti-predator strategy. *Proceedings of the Royal Society B, 285*(1885), Article 20181102. https://doi.org/10.1098/rspb.2018.1112

Rios-López, N. (2008). Effects of increased salinity on tadpoles of two anurans from a Caribbean coastal wetland in relation to their natural abundance. *Amphibia-Reptilia, 29*(1), 7–18. https://doi.org/10.1163/156853808783431451

Rivera-Ingraham, G. A., & Lignot, J.-H. (2017). Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: Raising the questions for future research. *Journal of Experimental Biology, 220*(10), 1749–1760. https://doi.org/10.1242/jeb.135624

Rocha, A. R., Silva, R., Villegas, A., Sánchez-Guzmán, J. M., Ramos, J. A., & Masero, J. A. (2016). Physiological, morphological and behavioural responses of self-feeding precocial chicks copying with contrasting levels of water salinity during development. *PLoS One, 11*(10), Article e0165364. https://doi.org/10.1371/journal.pone.0165364

Rodriguez, A., Zhang, H., Klaminder, J., Brodin, T., Andersson, P. L., & Andersson, M. (2018). ToxTrac: A fast and robust software for tracking organisms. *Methods in Ecology and Evolution, 9*(3), 460–464. https://doi.org/10.1111/2041-210X.12874

RStudio Team. (2018). *RStudio: Integrated development for R.* RStudio. Disponível Em: http://www.Rstudio.com.

Sanzo, D., & Hecnar, S. J. (2006). Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). *Environmental Pollution, 140*(2), 247–256. https://doi.org/10.1016/j.envpol.2005.07.013

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Schultz, E. T., & McCormick, S. D. (2012). Euryhalinity in an evolutionary context. *Fish Physiology, 32*, 477–533. https://doi.org/10.1016/B978-0-12-396951-4.00010-4

Shoemaker, V. H., & Nagy, K. A. (1984). Osmoregulation in the Galápagos marine iguana, *Amblyrhynchus cristatus*. *Physiological Zoology, 57*(3), 291–300. https://doi.org/10.1086/physzool.57.3.30163717

Speybroeck, J., Beukema, W., Bok, B., & Van Der Voort, J. (2018). *Guide Delachaux des amphibiens & reptiles de France et d'Europe.* Delachaux et Nieslé.

Tietze, S. M., & Gerald, G. W. (2016). Trade-offs between salinity preference and antipredator behaviour in the euryhaline sailfin molly *Poecilia latipinna*. *Journal of Fish Biology, 88*(5), 1918–1931. https://doi.org/10.1111/jfb.12955

Tornabene, B. J., Breuner, C. W., & Hossack, B. R. (2021). Comparative effects of energy-related saline wastewaters and sodium chloride on hatching, survival, and fitness-associated traits of two amphibian species. *Environmental Toxicology and Chemistry, 40*(11), 3137–3147. https://doi.org/10.1002/etc.5193

Tornabene, B. J., Breuner, C. W., Hossack, B. R., & Crespi, E. J. (2022). Effects of salinity and a glucocorticoid antagonist, RU486, on waterborne aldosterone and corticosterone of northern leopard frog larvae. *General and Comparative Endocrinology, 317*, Article 113972. https://doi.org/10.1016/j.ygcen.2021.113972

Tornabene, B. J., Crespi, E. J., Breuner, C. W., & Hossack, B. R. (2022). Testing whether adrenal steroids mediate phenotypic and physiologic effects of elevated salinity on larval tiger salamanders. *Integrative Zoology, 18*(1), 27–44. https://doi.org/10.1111/1749-4877.12669

Tornabene, B. J., Hossack, B. R., Crespi, E. J., & Breuner, C. W. (2021). Corticosterone mediates a growth-survival tradeoff for an amphibian exposed to increased salinity. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 335*(8), 703–715. https://doi.org/10.1002/jez.2535

Troyer, R. R., & Turner, A. M. (2015). Chemosensory perception of predators by larval amphibians depends on water quality. *PLoS One, 10*(6), Article e0131516. https://doi.org/10.1371/journal.pone.0131516

Tweedley, J. R., Dittmann, S. R., Whitfield, A. K., Withers, K., Hoeksema, S. D., & Potter, I. C. (2019). Hypersalinity: Global distribution, causes, and present and future effects on the biota of estuaries and lagoons. In *Coasts and estuaries* (pp. 523–546). Elsevier.

Uchiyama, M., & Yoshizawa, H. (1992). Salinity tolerance and structure of external and internal gills in tadpoles of the crab-eating frog, *Rana cancrivora*. *Cell and Tissue Research, 267*(1), 35–44. https://doi.org/10.1007/BF00318689

Van Damme, R., & Van Dooren, T. J. M. (1999). Absolute versus per unit body length speed of prey as an estimator of vulnerability to predation. *Animal Behaviour, 57*(2), 347–352.

Velasco, J., Gutiérrez-Cánovas, C., Botella-Cruz, M., Sánchez-Fernández, D., Arribas, P., Carbonell, J. A., Millán, A., & Pallarés, S. (2019). Effects of salinity changes on aquatic organisms in a multiple stressor context. *Philosophical Transactions of the Royal Society B, 374*(1764), Article 20180011. https://doi.org/10.1098/rstb.2018.0011

Viswanathan, G. M., Raposo, E. P., & Da Luz, M. G. E. (2008). Lévy flights and superdiffusion in the context of biological encounters and random searches. *Physics of Life Reviews, 5*(3), 133–150. https://doi.org/10.1016/j.plrev.2008.03.002

Walker, R. H., Belvin, A. C., Mouser, J. B., Pennino, A., Plont, S., Robinson, C. D., Smith, L. B., Thapa, J., Zipper, C. E., Angermeier, P. L., & Entrekin, S. A. (2023). Global review reveals how disparate study motivations, analytical designs, and focal ions limit understanding of salinization effects on freshwater animals. *Science of the Total Environment*. , Article 164061. https://doi.org/10.1016/j.scitotenv.2023.164061

Wang, R., Shen, C., Feng, G., Zhuang, P., Huang, X., & Zhang, T. (2022). Gradually elevated salinities alter the haemolymph metabolic profile and nitrogenous wastes of the mature Chinese mitten crab, *Eriocheir sinensis* (Decapoda, Varunidae). *Crustaceana, 95*(8–9), 891–905. https://doi.org/10.1163/15685403-bja10243

Wells, K. D. (2007). *The Ecology and Behavior of Amphibians.* University of Chicago Press.

Werner, A. D., & Simmons, C. T. (2009). Impact of sea-level rise on sea water intrusion in coastal aquifers. *Groundwater, 47*(2), 197–204. https://doi.org/10.1111/j.1745-6584.2008.00535.x

Wood, L., & Welch, A. M. (2015). Assessment of interactive effects of elevated salinity and three pesticides on life history and behavior of southern toad (*Anaxyrus terrestris*) tadpoles. *Environmental Toxicology and Chemistry, 34*(3), 667–676. https://doi.org/10.1002/etc.2861

Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1549), 2093–2106. https://doi.org/10.1098/rstb.2010.0055

Wu, C.-S., & Kam, Y.-C. (2009). Effects of salinity on the survival, growth, development, and metamorphosis of *Fejervarya limnocharis* tadpoles living in brackish water. *Zoological Science, 26*, 476–482. https://doi.org/10.2108/zsj.26.476

Zhou, L., Liu, K., Zhao, Y., Cui, L., Dong, C., & Wang, Z. (2022). Increasing salinization of freshwater limits invasiveness of a live-bearing fish: Insights from behavioral and life-history traits. *Environmental Pollution, 308*, 119658. https://doi.org/10.1016/j.envpol.2022.119658

Appendix

Table A1
Post hoc analyses

Variable	Comparisons	Estimate	SE	df	t/z	P
Mean escape distance						
Test 0	Acclimation 0 - Acclimation 4	−2.43	2.23	93.8	−1.091	0.278
Test 4	Acclimation 0 - Acclimation 4	5.16	2.23	93.8	2.316	0.023
Acclimation 0	Test 0 - Test 4	−5.22	1.58	59	−3.309	0.002
Acclimation 4	Test 0 - Test 4	2.38	1.55	59	1.532	0.131
Total distance travelled						
Test 0	Acclimation 0 - Acclimation 4	0.855	0.137	113	6.23	<0.001
Test 4	Acclimation 0 - Acclimation 4	0.391	0.137	113	2.848	0.005
Acclimation 0	Test 0 - Test 4	0.191	0.125	58	1.529	0.132
Acclimation 4	Test 0 - Test 4	−0.273	0.125	58	−2.181	0.033
Total active time						
Test 0	Acclimation 0 - Acclimation 4	0.672	0.118	114	5.69	<0.001
Test 4	Acclimation 0 - Acclimation 4	0.243	0.118	114	2.056	0.042
Acclimation 0	Test 0 - Test 4	0.206	0.109	58	1.888	0.064
Acclimation 4	Test 0 - Test 4	−0.223	0.109	58	−2.043	0.046
DFAc						
Test 0	Acclimation 0 - Acclimation 4	0.035	0.009	Infinity	4.021	<0.001
Test 4	Acclimation 0 - Acclimation 4	0.006	0.009	Infinity	0.664	0.507
Acclimation 0	Test 0 - Test 4	0.019	0.005	Infinity	3.712	<0.001
Acclimation 4	Test 0 - Test 4	−0.010	0.005	Infinity	−2.056	0.040

Differences considered are the differences in mean escape distance, total distance, total active time and DFAc (a measure of behavioural complexity), according to salinity during acclimation (2 weeks in freshwater [0 g/litre] or brackish water [4 g/litre]) and to salinity during the trial (freshwater [0 g/litre] or brackish water [4 g/litre]).