Aquatic Toxicology 99 (2010) 275–280

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Cumulative effects of road de-icing salt on amphibian behavior

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ARTICLE INFO

Article history:
Received 3 April 2010
Received in revised form 1 May 2010
Accepted 7 May 2010

Keywords:
Amphibian decline
Behavior
Conservation
Ecotoxicology
Sodium chloride
Video-tracking

ABSTRACT

Despite growing evidence of the detrimental effect of chemical substances on organisms, limited research has focused on changes in behavioral patterns, in part due to the difficulties to obtain detailed quantitative data. Recent developments in efficient computer-based video analyses have allowed testing pesticide effects on model species such as the zebrafish. However, these new techniques have not yet been applied to amphibians and directly to conservation issues, i.e., to assess toxicological risks on threatened species. We used video-tracking analyses to test a quantitative effect of an environmental contaminant on the locomotion of amphibian tadpoles (*Rana temporaria*) by taking into account cumulative effects. Because recent research has demonstrated effects of de-icing salts on survival and community structure, we used sodium chloride in our experimental design (25 replicates, 4 concentrations, 4 times) to test for an effect at the scale of behavior at environmentally relevant concentrations. Analysis of 372 1-h video-tracks (5 samples/s) showed a complex action of salts on behavioral patterns with a dose and cumulative response over time. Although no effects were found on mortality or growth, the highest salt concentrations reduced the speed and movement of tadpoles in comparison with control treatments. The reduced locomotor performance could have detrimental consequences in terms of tadpoles' responses to competition and predation and may be an indicator of the low concentration effect of the contaminant. On one hand, this study demonstrates the usefulness of examining behavior to address conservation issues and understand the complex action of environmental factors and, more particularly, pollutants on organisms. On the other hand, our results highlight the need of new computerized techniques to quantitatively analyze these patterns.

1. Introduction

Behavioral ecotoxicology is an emerging field that aims to detect the effects of chemical substances before vital and reproductive functions are altered (Little and Finger, 1990; Brunelli et al., 2009). The field initially employed qualitative assessments of behavior, but now encompasses more quantitative analyses (Bayley, 2002). Locomotor behavior is a particularly suitable biomarker because it offers the opportunity to develop broad generic test protocols in most animal species (Bayley, 2002). Using grids and timers along with direct observations or video recordings permits the quantification of variables such as movement activity (Laurila et al., 2006), speed (Chen et al., 2009), space use (Saglio et al., 2001) and olfactory acuities (Mandrillon and Saglio, 2007) of individuals under contrasting conditions. The recent development of computer-based tools is opening opportunities for a very efficient way of quantifying behavioral patterns (Noldus et al., 2002; Kanen et al., 2005; Eddins et al., 2010). Some of these automatized procedures allow mapping the position of individuals through time with high accuracy and thus defining key features of their behavioral patterns and performance. Because of their recent development and high cost, these video-tracking methods remain little used and have never been applied to many taxa, such as amphibians. Their use has also been restricted primarily to neurological studies or standard ecotoxicological tests on model species such as the zebrafish or rodents, but not integrated directly into applied ecology and conservation. There is thus an open field of research to link video-tracked behavior to the risk of a plethora of chemical substances, which are released in the natural environment (Carlile, 2006) and which could affect species survival and reproduction with major consequences in terms of decline, extinction, and even shift in community structure (Walker et al., 2006).

Road de-icers are used in temperate regions worldwide when harsh climatic conditions affect road traffic. In most cases, the active agent is sodium chloride (Ramakrishna and Viraraghavan, 2005).

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0166-445X/$ – see front matter
doi:10.1016/j.aquatox.2010.05.007

The yearly world production of salt is tremendous: 189 Mtons, of which 10% is used for road de-icing (Environment Canada, 2001). From roads, salts can run off through a combination of aerosol sprays, overland flow and groundwater infiltration (Hautala et al., 1995; Blomqvist and Johansson, 1999; Marsalek, 2003; Karraker, 2008). This contamination can affect wetlands where chloride can accumulate, because of its high solubility, and reach values 100 times higher than in unimpacted water bodies, with concentrations of up to 25% of seawater (Godwin et al., 2003; Kaushal et al., 2005).

Although there is now much consideration of amphibian declines (Stuart et al., 2004), among which pollution by xenobiotics plays an important role (Bridges and Semlitsch, 2005; Relyea, 2005), it is only recently that emphasis has been placed on the effects of sodium chloride on amphibians (see review in Karraker, 2008). Because many amphibian species breed in agricultural or stormwater ponds, which are often located along or close to roads, they are expected to come into contact with road de-icing agents that are often applied just before their breeding seasons (Karraker, 2008; Snodgrass et al., 2008). Although sodium chloride is naturally present in ecosystems, it is found at unnatural concentrations in ponds because of road de-icing. However, despite recent evidence that such agents can decrease amphibian survival (Viertel, 1999; Dougherty and Smith, 2006; Karraker et al., 2008), affect communities because of different resistance to salts among species (Collins and Russell, 2009) and alter their physiology (Hillyard et al., 2007) at environmental concentrations, very few studies have focused on the effect of salt at such concentrations yet, making it difficult to develop a clear understanding of the risks caused by road de-icing agents (Karraker, 2008). In particular, behavioral patterns have not been examined, except for qualitative observations suggesting aberrant movements in exposed tadpoles (Sanzo and Hecnar, 2006; Collins and Russell, 2009).

The aim of this study was to model the concentration-cumulative effects of sodium chloride on behavioral patterns of tadpoles of a pond-breeding amphibian species, *Rana temporaria*. Sodium chloride affects the nervous system, and the skin of amphibians is particularly permeable to ions (Boutilier et al., 1992; Hillyard et al., 2007). Thus, we tested the hypothesis that environmental concentrations of salts would affect behavioral patterns. Previous ecotoxicological research showed that longer-term chronic treatments of de-icing salts are often needed to demonstrate effects on amphibians. We therefore expected a stronger response after a longer- than a shorter-term exposure. To quantitatively test these hypotheses, we used video-tracking techniques for the first time in amphibian research.

2. Materials and methods

2.1. Field and laboratory procedures

We chose to study the common frogs (*R. temporaria*) as a model species because it is often used in laboratory experiments, it is widespread all over Europe, and starts breeding immediately after the cold yearly period (i.e., in winter in lowlands), i.e., soon after the last applications of de-icing salts and in places possibly affected by them, such as in agricultural and stormwater ponds close to roads. The common frog is an explosive breeder which lay its eggs as a globular mass in water. The development of tadpoles takes a couple or a few months, depending on external conditions (Beebee and Griffiths, 2000; Jacob and Kinet, 2007).

Five egg clutches of common frogs were collected from a pond at Hasenell, Belgium (UTM: 32U 0293800 – 5613500) in March 2007. We sampled a pond that has never been in contact with de-icing agents to avoid possible biases due to adaptation. However, Turtle (2000) showed that amphibians caught from ponds exposed to salts had higher survivorship in ponds less exposed to salts. The clutches were carried to the laboratory in 6-l containers (40-min drive).

In the laboratory, clutches were cleaned of detritus and then placed in 31-l aquaria (40 cm × 87 cm × 9 cm high). After hatching, 100 tadpoles (Gosner stage 26) (Duellman and Trueb, 1986) were randomly distributed into four treatments (i.e., 25 replicates for each treatment): one control and 3 sodium chloride concentrations (NaCl: 500, 1000, and 1500 mg/l, corresponding to chloride concentrations of 300, 600, and 900 mg/l, respectively). These concentrations are found in wetlands where values up to 5000 mg/l due to road runoffs have been reported (Environment Canada, 2001). The closest published field study to our study site reported chloride concentrations of up to 2600 mg/l, i.e., 4333 mg/l of NaCl in ponds (Viertel, 1999). Each tadpole was kept individually in circular aquaria (diameter: 18 cm) filled with 2 l of reconstituted soft water following APHA (1985) recommendations: $NaHCO_3$: 48 mg/l, $CaSO_4 \cdot 2H_2O$: 30 mg/l, $MgSO_4 \cdot 7H_2O$: 61 mg/l, KCl: 2 mg/l. The position of the aquaria was randomized on shelves. Water was changed every 15 days. Spinach leaves (1 cm^2/tadpole) were given *ad libitum*. Photoperiod followed the natural cycle of the capture place, starting at 12 h light:12 h dark and ending at 15 h light:9 h dark at the end of the experiment (30-min increments of the day phase every 15 days). Room temperature was maintained at 16 °C.

2.2. Behavioral and statistical tests

Every 2 weeks over the 2-month study period (4 time replicates), tadpoles were transferred individually into observation aquaria identical to the maintenance ones, but filled with 0.5 l of reconstituted water. The aquariums were video-recorded, from above the water surface for 1 h. Video streaming data were automatically processed by video-tracking software (Ethovision 3.1, Noldus Information Technology, The Netherlands), which transforms tadpoles into pixels in the digitalized arena (i.e., the circular aquaria), and then gives the positions of tadpoles at defined times (every 0.2 s in our set-up) (Noldus et al., 2002). From these spatial coordinates, two behavioral variables were calculated: mean speed during movement and total distance moved. In total, 400 tracks (i.e., lines of connections between individual positions) and about seven million positions were obtained. After each session, the sizes of tadpoles (placed in Petri boxes filled with water) were measured under a stereoscopic microscope (accuracy: 0.5 mm). One of the 100 tadpoles died during the measurements and was consequently not included in our analyses. Only six other tadpoles died during the entire experiment (three in the control and three in the salt treatments), which permitted us to include 372 tracks for the behavioral analyses.

We used mixed models to evaluate the effects of NaCl concentration on behavioral parameters (mean speed, total distance moved), including concentration as a fixed factor and body size as a covariate. Clutch of origin was a random factor, to take into account potential differences in genetic parental effects or non-genetic maternal effects (Ficetola and De Bernardi, 2009). Behavioral parameters of each individual were measured on four different occasions. To build global models, evaluating the overall performance of individuals across the four measurements, we included individual identity (nested within concentration and clutch of origin) as a random factor, time of observation as a fixed factor, and the interaction between time of observation and NaCl concentration (Venables and Ripley, 2002). The global models, built considering the four observations, were followed by post hoc models to evaluate how NaCl affects behavior at the different time intervals. Tukey's HSD tests were used to assess differences between pairwise NaCl

Table 1
Mixed model evaluating the effect of NaCl, time and tadpole size on behavioral patterns.

	Distance moved			Speed		
	F	d.f.	*P*	*F*	d.f.	*P*
Factors						
Time	4.892	3, 266	**0.003**	4.993	3, 266	**0.002**
NaCl	2.04	3, 89.3	0.114	2.272	3, 89.5	0.086
NaCl × time	2.695	9, 266	**0.005**	1.980	9, 266	**0.042**
Covariate						
Size	9.250	1, 266	**0.003**	11.082	1, 266	**0.001**
Random factors						
Individual	5.123	85, 266	**<0.0001**	4.924	85, 266	**<0.0001**
Clutch	1.356	4, 85.9	0.256	1.949	4, 85.9	0.110

Significant values are highlighted in bold.

concentrations (Hothorn et al., 2008). Due to the deaths of a few individuals, sample size was not identical among treatments, therefore degrees of freedom were approximated using Satterwaite's (1946) rule. The residuals of models were normally distributed (Kolmogorov–Smirnov test, all $P > 0.05$). We used generalized linear mixed models with binomial error distribution to evaluate the effect of concentration on tadpole mortality, including clutch of origin as random factor (Bates and Maechler, 2009). We performed analyses in SPSS 17 and R 2.9 (www.r-project.org).

3. Results

Tadpoles moved an average distance of 44 m (SE: 0.9) at a speed of 1.7 cm/s (SE: 0.03). Time of observation (i.e., the number of days after the start of the experiment) had a significant effect on both distance and speed, indicating a change in behavioral patterns during development (Table 1). Furthermore, the interaction between NaCl concentration and time of observation had a significant effect on both behavioral parameters, which indicates that its effect varied during the repeated trials at 14-day intervals (Table 1), and that behavior changed with time differently between high and low concentration treatments (Fig. 1). Post hoc models showed that NaCl concentration had a significant negative effect on distance after 56 days of exposure (Table 2): controls moved significantly longer distances than the 1000 mg/l treatment (Tukey's test, $P = 0.015$) and the 1500 mg/l treatment ($P = 0.034$) (Fig. 1a). Furthermore, NaCl concentration had a significant effect on speed after 42 and 56 days of exposure (Table 2). After 42 days, controls moved significantly faster than the 1500 mg/l treatment ($P = 0.043$; Fig. 1b). After 56 days, controls moved significantly faster than the 1000 mg/l treatment ($P = 0.034$). The difference between controls and the 1500 mg/l treatment was close to significance ($P = 0.070$; Fig. 1b). In the control treatment, tadpoles showed a steady increase in the distances moved and speed of movements through time, from an average of 41.9 m at 1.6 cm/s to 52.4 m at 2 cm/s. The trend was the converse in the high concentration NaCl. In the 1500 mg/l treatment, tadpoles moved from an average of 44.7 m at 1.7 cm/s to 39.3 m at 1.6 cm/s (Table 1; Fig. 1).

Growth was highest during the first 28 days of the larval stage (mean ± SE = 0.03 ± 0.0009 cm/day), but decreased considerably afterwards (days 28–56: 0.006 ± 0.0005 cm/day). Growth rate from the beginning to the end of the experiment was similar among the different treatments (mixed model, $F_{3,85} = 1.74$, $P = 0.166$; clutch of origin as random factor: $F_{4,85} = 0.47$, $P = 0.759$; Fig. 3a). Survival was ≥88% in all treatments; mortality did not increase at increasing NaCl concentrations (generalized linear mixed model, $P > 0.9$) (Fig. 3b). Size had an overall significant effect on behavioral patterns: in all treatments, the larger tadpoles moved farther and faster (Table 1; Fig. 2).

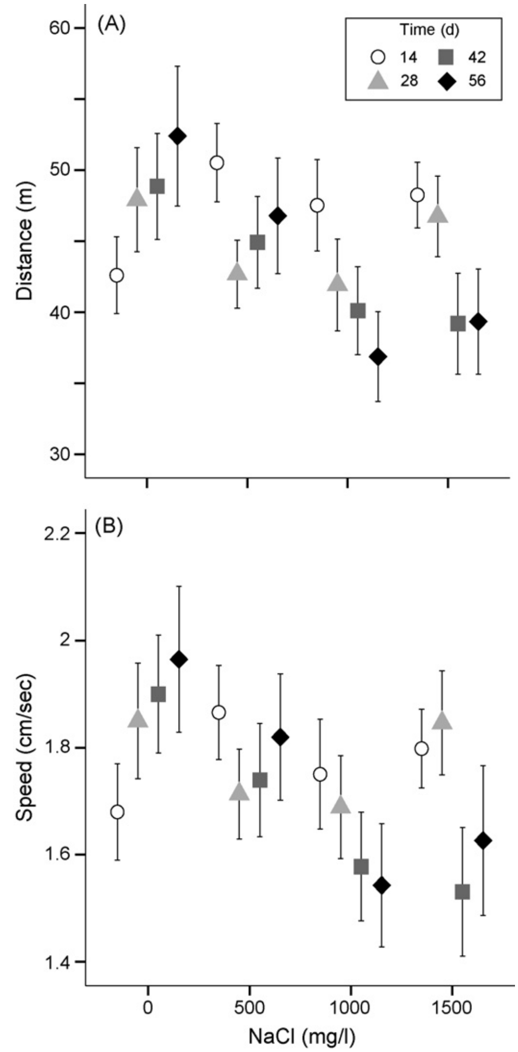

Fig. 1. Distance (A) and speed (B) of tadpoles as a function of NaCl concentration over time (mean ± SE values based on 1-h tracks, $n = 372$). See Tables 1 and 2 for statistical results.

4. Discussion

About one decade ago, it was recognised that behavioral studies can be extremely important components of conservation biology (Sutherland, 1998). However, the integration of behavioral and conservation studies has remained limited since then (Caro, 2007). The

Table 2
Post hoc models: effect of NaCl and size at each study period (time).

Time (day)	Factor	d.f.	Distance moved		Speed	
			F	*P*	*F*	*P*
14	NaCl	3, 84	0.169	0.917	0.209	0.890
	Size	1, 84	3.470	0.066	2.385	0.126
	Clutch	4, 84	0.822	0.515	1.392	0.244
28	NaCl	3, 84	1.772	0.159	1.688	0.176
	Size	1, 84	10.923	**0.001**	15.942	**0.0001**
	Clutch	4, 84	1.201	0.317	1.503	0.209
42	NaCl	3, 84	2.085	0.108	2.850	**0.042**
	Size	1, 84	0.860	0.356	1.255	0.266
	Clutch	4, 84	0.717	0.582	1.100	0.362
56	NaCl	3, 84	3.943	**0.011**	3.140	**0.030**
	Size	1, 84	4.283	**0.042**	6.823	**0.011**
	Clutch	4, 84	1.147	0.340	1.323	0.268

Significant values are highlighted in bold.

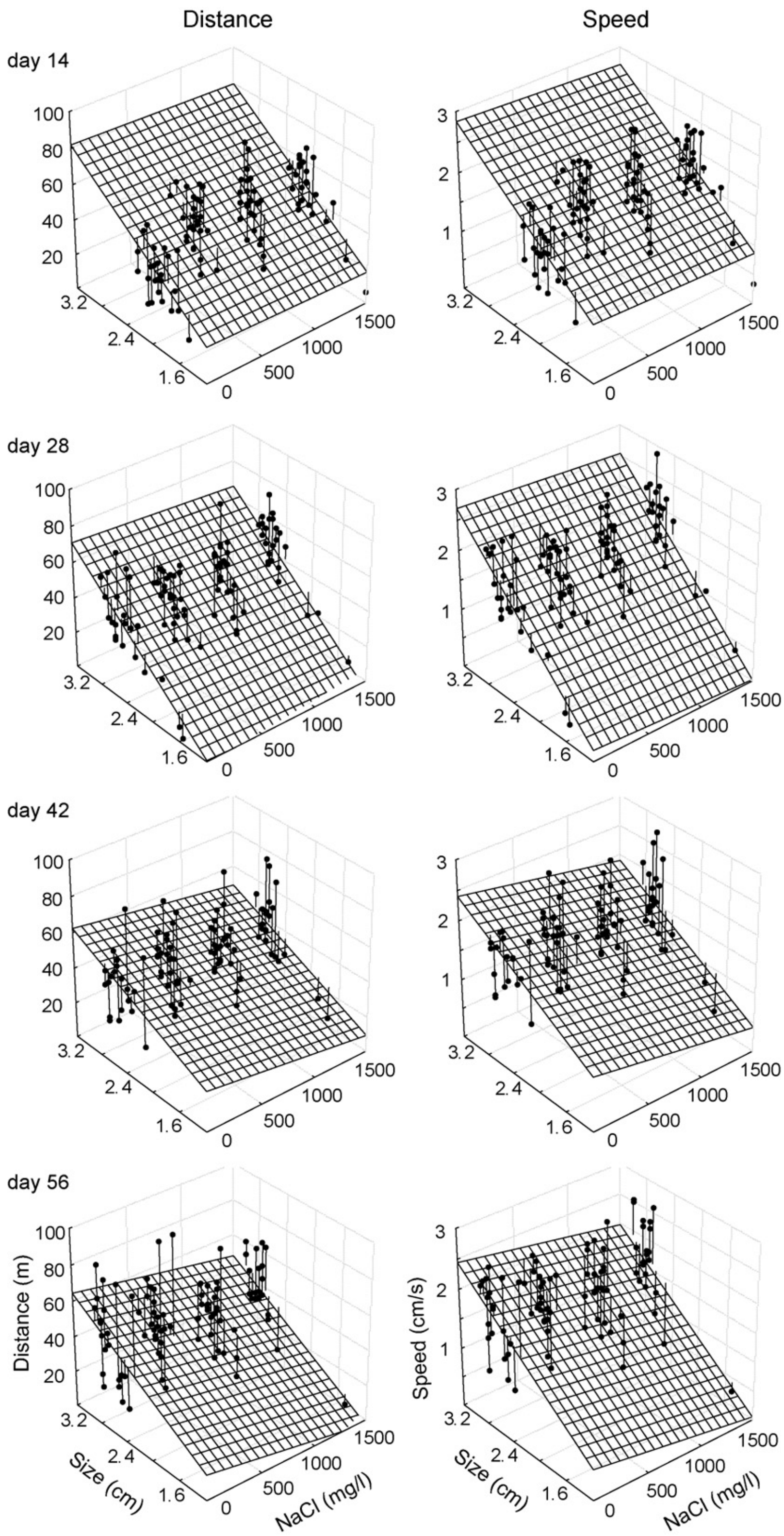

Fig. 2. Relative effect of size and NaCl concentration on behavioral patterns: distance and speed (mean ± SE values based on 1-h tracks, $n = 372$). See Tables 1 and 2 for statistical results.

use of new analytical tools and technological developments can help to better integrate behavioral and conservation science, and can easily be applied to less-studied taxa such as amphibians, reptiles, and insects (Ficetola et al., 2010). Behavioral ecotoxicology of organisms such as invertebrates, fish and amphibians is filling this gap in providing standardized tests to quantify the effects of pollutants on locomotor patterns (Saglio et al., 2001; Bayley, 2002; Mandrillon, 2007). However, video-tracking techniques, although used in a variety of behavioral studies, including on standard ecotoxicological tests of model species, such as the zebrafish (Eddins

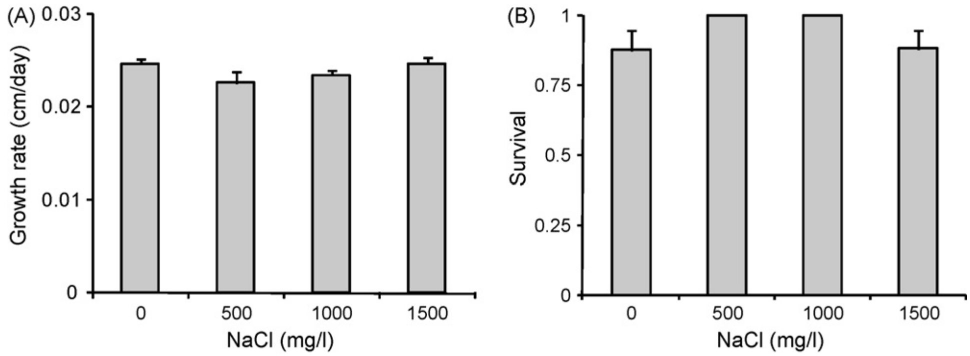

Fig. 3. Growth rate (A) and survival (B) of tadpoles as a function of NaCl concentration (mean ± SE values).

et al., 2010), are still not directly used in the field of conservation, and have never been applied to amphibians. Here, we applied newly developed video analysis software to the field of behavioral ecotoxicology for the study of a locally declining amphibian. We obtained quantitative support for negative effects of a pollutant on amphibian behavior despite the fact that the usual life-history traits (survival and growth) used to measure the impact of pollutants did not show any changes.

We found that road de-icing salt negatively affects the behavior of common frogs, but we did not detect an effect on survival or growth. This finding emphasizes the point that some impacts of pollutants on amphibians may be subtle and may not be detected by the most commonly measured responses (Birdsall et al., 1986). The analysis of video-tracked data showed that sodium chloride impairs locomotor patterns. At high salt concentrations, tadpoles moved at slower speed and on shorter distances than at low concentrations. Recent research has shown various effects of de-icing agents, particularly sodium chloride, on amphibian traits (for a review, see Karraker, 2008). Several species exhibited decreases in survival or changes in life-history traits, and in addition to their altered growth (Dougherty and Smith, 2006; Karraker et al., 2008), behavior was reported to be affected (Sanzo and Hecnar, 2006; Collins and Russell, 2009). The effect of salt on amphibians is species-specific as some coastal species are naturally adapted and tolerant to brackish and even saline environments, whereas the majority of amphibians are more continental and only adapted to freshwaters (Wells, 2007). In terms of growth and survival, tadpoles of the common frog, a typical freshwater species, is tolerant of the tested salt concentrations as no effects on growth or survival were observed in our study. Similar results were found on the survival of the embryonic and tadpole stages of another inland ranid species, *Rana* (*Lithobates*) *clamitans* (Karraker, 2007). In contrast, Viertel (1999) in a study on *R. temporaria* embryos, and other authors (Dougherty and Smith, 2006; Sanzo and Hecnar, 2006) on the tadpoles of other species, reported mortality at salinity within our range of tested values. This indicates that behavior may have been more affected if eggs rather tadpoles were contaminated. These differences among studies show the importance of using multiple life-history stages in ecotoxicological studies (Karraker et al., 2008), and the importance of targeting research directly on threatened and declining species rather than on laboratory models (Caro et al., 2005).

Although not globally threatened with extinction (Kuzmin et al., 2009), common frogs are locally declining because of a large number of factors (Beebee, 1996; Mandrillon, 2007; Piha et al., 2007; Elzanowski et al., 2009). As they experience salt concentrations in their ponds similar to those tested in the present study (Viertel, 1999; Kaushal et al., 2005), their lower performance might affect fitness. It is too premature to conclude on the exact impact of such alteration, but our experiment proves a sublethal effect: tadpoles exposed to de-icing salt had a slower speed and moved shorter distances than those in controls. In amphibians, low locomotor performance has been associated a decreased probability of escaping predators and may also limit foraging capabilities (Watkins, 1996; Teplitsky et al., 2005). However, a reduced activity may reduce detection by predators (Van Buskirk, 2001), but as wild control tadpoles are more active and swim faster, we may hypothesize that higher activity is adaptive. Further experimental work and mesocosm studies are particularly needed to understand in-depth the complex links between behavior and individual fitness in contaminated environments (Bayley, 2002).

Cumulative effects of contaminants are a well known process highlighting the need of longer-term experiments (Brunelli et al., 2009). Our analysis showed that effects on behavior can only been seen after long contamination exposures, as we observed effects only after more than 1 month of exposure. The natural developmental period of common frog tadpoles is longer than this period and starts usually at the end of the winter season (Beebee and Griffiths, 2000), i.e., during or shortly after the application of salts on the road (Karraker, 2008).

The data obtained by video-tracking software can give a tremendous amount of detailed spatial data, which allows one to compute clear patterns of locomotor behaviors such as distance and speed (Noldus et al., 2002; Eddins et al., 2010). This approach has advantages over standard visual methods in that it very accurately determines the positions of organisms and is able to process a large amount of data in a relatively short time period. For instance, the present study allowed us to investigate a large number of tadpoles at the same time (about 100 individuals video-recorded during 1 h in only 2 days of experiments for each time period). Quantitative ethology is thus a valuable tool to assess conservation concerns when other techniques cannot detect detrimental effects.

Acknowledgments

We are very grateful to Nancy Karreker and two anonymous reviewers for their constructive comments on the manuscript. The collecting permit was issued by the Ministère de la Région Wallonne. The study was carried out in an agreed laboratory, and the research project was accepted by the ethical commission of the university. M. Denoël and J. Delcourt are, respectively, Research Associate and Postdoctoral Researcher at the F.R.S. – Fonds National de la Recherche Scientifique. G.F. Ficetola was funded by a scholarship of the University of Milano-Bicocca. This research was supported by a F.R.F.C. grant 2.4.507.08.F of the F.R.S. and a F.S.R. (crédit classique) grant of the University of Liège.

References

APHA, 1985. Standard Methods for the Examination of Water and Wastewater, 16th edn. American Health Association, Washington, DC.

Bates, D., Maechler, M., 2009. lme4: linear mixed-effects models using S4 classes. R package version 0.999375-31, www.r-project.org.

Bayley, M., 2002. Basic behaviour: the use of animal locomotion in behavioural ecotoxicology. In: Dell'Omo, G. (Ed.), Behavioural Ecotoxicology. John Wiley & Sons Ltd, Chichester, UK, pp. 211–230.

Beebee, T.J.C., 1996. Ecology and Conservation of Amphibians. Chapman & Hall, London.

Beebee, T.J.C., Griffiths, R.A., 2000. Amphibians and Reptiles. Collins, London.

Birdsall, C.W., Grue, C.E., Anderson, A.A., 1986. Lead concentrations in bullfrog *Rana catesbeiana* and green frog *R. clamitans* tadpoles inhabiting highway drainages. Environ. Pollut. 40, 233–247.

Blomqvist, G., Johansson, E.-L., 1999. Airborne spreading and deposition of de-icing salt—a case study. Sci. Total Environ. 235, 161–168.

Boutilier, R.G., Stiffler, D.F., Toews, D.P., 1992. Exchange of respiratory gases, ions, and water in amphibious and aquatic amphibians. In: Feder, M.E., Burggren, W.W. (Eds.), Environmental Physiology of the Amphibians. The University of Chicago Press, Chicago, pp. 81–124.

Bridges, C.M., Semlitsch, R.D., 2005. Xenobiotics. In: Lannoo, M. (Ed.), Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley/Los Angeles, pp. 89–92.

Brunelli, E., Bernabò, I., Berg, C., Lundstedt-Enkel, K., Bonacci, A., Tripepi, S., 2009. Environmentally relevant concentrations of endosulfan impair development, metamorphosis and behaviour in *Bufo bufo* tadpoles. Aquat. Toxicol. 91, 135–142.

Carlile, W.R., 2006. Pesticide Selectivity, Health and the Environment. Cambridge University Press, Cambridge.

Caro, T., 2007. Behavior and conservation: a bridge too far? Trends Ecol. Evol. 22, 394–400.

Caro, T., Eadie, J., Sih, H., 2005. Use of substitute species in conservation biology. Conserv. Biol. 19, 1821–1826.

Chen, T.-H., Gross, J.A., Karasov, W.H., 2009. Chronic exposure to pentavalent arsenic of larval leopard frogs (*Rana pipiens*): bioaccumulation and reduced swimming performance. Ecotoxicology 18, 587–593.

Collins, S.J., Russell, R.W., 2009. Toxicity of road salt to Nova Scotia amphibians. Environ. Pollut. 157, 320–324.

Dougherty, C.K., Smith, G.F., 2006. Acute effects of road de-icers on the tadpoles of three anurans. Appl. Herpetol. 3, 87–93.

Duellman, W.E., Trueb, L., 1986. Biology of Amphibians, 2004 edn. The John Hopkins University Press, Baltimore, MD.

Eddins, D., Cerutti, D., Williams, P., Linney, E., Levin, E.D., 2010. Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. Neurotoxicol. Teratol. 32, 99–108.

Elzanowski, A., Ciesiolkiewicz, J., Kaczor, M., Radwanska, J., Urban, R., 2009. Amphibian road mortality in Europe: a meta-analysis with new data from Poland. Eur. J. Wildl. Res. 55, 33–43.

Environment Canada, 2001. Priority Substance List Assessment Report for Road Salts. Environment Canada, Ottawa, Canada.

Ficetola, G.F., De Bernardi, F., 2009. Offspring size and survival in the frog *Rana latastei*: from among-population to within-clutch variation. Biol. J. Linn. Soc. 97, 845–853.

Ficetola, G.F., Padoa-Schioppa, E., Wang, J.L., Garner, T.W.J., 2010. Polygyny, census and effective population size in the threatened frog, *Rana latastei*. Anim. Conserv. doi:10.1111/j.1469-1795.2009.00306.x.

Godwin, K.S., Hafner, S.D., Buff, M.F., 2003. Long-term trends in sodium and chloride in the Mowhawk river, New York: the effect of fifty years of road-salt application. Environ. Pollut. 124, 273–281.

Hautala, E.L., Rekilä, R., Tarhanen, J., Ruuskanen, J., 1995. Deposition of motor vehicle emissions and winter maintenance along roadside assessed by snow analyses. Environ. Pollut. 87, 45–49.

Hillyard, S.D., Viborg, A., Nagai, T., Hoff, K.V., 2007. Chemosensory function of salt and water transport by the amphibian skin. Comp. Biochem. Physiol. A 148, 44–54.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363.

Jacob, J.P., Kinet, T., 2007. La Grenouille rousse *Rana temporaria* (Linnaeus, 1758). In: Jacob, J.P., Percsy, C., De Wavrin, H., Graitson, E., Kinet, T., Denoël, M., Paquay, M., Percsy, N., Remacle, A. (Eds.), Amphibiens et Reptiles de Wallonie. Aves-Raînne & Région wallonne, Namur, pp. 180–193.

Kanen, A.S., Salierno, J.D., Brewer, S.K., 2005. Fish models in behavioral toxicology: automated techniques, updates and perspectives. In: Ostrander, G.K. (Ed.), Methods in Aquatic Toxicology, vol. 2. Lewis Publishers, Boca Raton, FL, pp. 559–590.

Karraker, N.E., 2007. Are embryonic and larval green frogs (*Rana clamitans*) insensitive to road de-icing salts? Herpetol. Conserv. Biol. 2, 35–41.

Karraker, N.E., 2008. Impacts of road deicing salts on amphibians and their habitats. In: Mitchell, J.C., Brown, R.E.J., Bartholomew, B. (Eds.), Urban Herpetology. Society for the Study of Amphibians and Reptiles, Salt Lake City, pp. 211–223.

Karraker, N.E., Gibbs, J.P., Vonesh, J.R., 2008. Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. Ecol. Appl. 18, 724–734.

Kaushal, S.S., Groffman, P.M., Likens, G.E., Belt, K.T., Stack, W.P., Kelly, V.R., Band, L.E., Fisher, G.T., 2005. Increased salinization of fresh water in the northeastern United States. Proc. Natl. Acad. Sci. U.S.A. 102, 13517–13520.

Kuzmin, S., Ishchenko, V., Tuniyev, B., Beebee, T., Andreone, F., Nyström, P., Anthony, B., Schmidt, B., Ogrodowczyk, A., Ogielska, M., Bosch, J., Miaud, C., Loman, J., Cogalniceanu, D., Kovács, T., Kiss, I., 2009. *Rana temporaria*. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2.

Laurila, A., Pakkasmaa, S., Merilä, J., 2006. Population divergence in growth rate and antipredator defences in *Rana arvalis*. Oecologia 147, 585–595.

Little, E.E., Finger, S.E., 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environ. Toxicol. Chem. 9, 13–19.

Mandrillon, A.L., 2007. Effet de substances indicatrices d'un risque de prédation sur le comportement, la morphologie et les traits d'histoire de vie de la Grenouille rousse (*Rana temporaria*) et du Crapaud commun (*Bufo bufo*). Modulation par un herbicide, l'Amitrole. University of Rennes.

Mandrillon, A.L., Saglio, P., 2007. Herbicide exposure affects the chemical recognition of a non native predator in common toad tadpoles (*Bufo bufo*). Chemoecology 17, 31–36.

Marsalek, J., 2003. Road salts in urban stormwater: an emerging issue in stormwater management in cold climates. Water Sci. Technol. 48, 61–70.

Noldus, L.P.J.J., Spink, A.J., Tegelenbosch, R.A.J., 2002. Computerised video tracking, movement analysis and behaviour recognition in insects. Comput. Electr. Agric. 35, 201–227.

Piha, H., Luoto, M., Piha, M., Merilä, J., 2007. Anuran abundance and persistence in agricultural landscapes during a climatic extreme. Global Change Biol. 13, 300–311.

Ramakrishna, D.M., Viraraghavan, T., 2005. Environmental impact of chemical deicers—a review. Water Air Soil Pollut. 166, 49–63.

Relyea, R.A., 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol. Appl. 15, 618–627.

Saglio, P., Olsén, K.H., Bretaud, S., 2001. Behavioral and olfactory responses to Prochloraz, Bentazone, and Nicosulfuron-contaminated flows in goldfish. Arch. Environ. Contam. Toxicol. 41, 192–200.

Sanzo, D., Hecnar, S.J., 2006. Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). Environ. Pollut. 140, 247–256.

Satterwaite, F.E., 1946. An approximate distribution of estimates of variance components. Biometrics 2, 110–114.

Snodgrass, J.W., Casey, R.E., Joseph, D., Simon, J.A., 2008. Microcosm investigations of stormwater pond sediment toxicity to embryonic and larval amphibians: variation in sensitivity among species. Environ. Pollut. 154, 291–297.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, D.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306, 1783–1786.

Sutherland, W.J., 1998. The importance of behavioural studies in conservation biology. Anim. Behav. 56, 801–809.

Teplitsky, C., Plenet, S., Léna, J.-P., Mermet, N., Malet, E., Joly, P., 2005. Escape behaviour and ultimate causes of specific induced defences in an anuran tadpole. J. Evol. Biol. 18, 180–190.

Turtle, S.L., 2000. Embryonic survivorship of the spotted salamander (*Ambystoma maculatum*) in roadside and woodland vernal pools in southeastern New Hampshire. J. Herpetol. 34, 60–67.

Van Buskirk, J., 2001. Specific induced responses to different predator species in anuran larvae. J. Evol. Biol. 14, 482–489.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, 4th edn. Springer, New York.

Viertel, B., 1999. Salt tolerance of *Rana temporaria*: spawning site selection and survival during embryonic development (Amphibia, Anura). Amphibia-Reptilia 20, 161–171.

Walker, C.H., Hopkin, S.P., Sibly, R.M., Peakall, D.B., 2006. Principles of Ecotoxicology. Taylor & Francis, Boca Raton, FL.

Watkins, T.B., 1996. Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. Physiol. Zool. 69, 154–167.

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