Making the dead talk: alarm cue-mediated antipredator behaviour and learning are enhanced when injured conspecifics experience high predation risk

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ESM1 Supplementary methods

Subjects’ collection and maintenance

The experiments took place in June 2015 in central Alberta, Canada. Six newly laid woodfrog egg clutches were placed into each of 5, 370-L plastic pools filled with well water. After hatching, tadpoles were fed alfalfa pellets and Tetramin flakes to supplement the algae already present in the pools. Experiments began two weeks after the tadpoles hatched (Gosner stage 25 [1]). Water used during all the procedures was obtained from a 1900-L pool filled with well water one month before the experiments. By adding plankton, algae, and aquatic plants from the pond, we ensured this water had odour characteristics similar to the ones tadpoles would experience in nature, but would not experience salamander odours. All procedure took place outdoors under natural weather conditions.

Simulated background predation risk

Thirty tadpoles (approx. length 9 - 11 mm), randomly chosen from among the 5 rearing pools, were placed into each of 24 pails, containing 3 L of water. Following previous work [2], we simulated high predation risk environments by injecting 20 mL of alarm cues (dilution: 3 donor tadpoles in 20mL) three times per day for 4 days in 12 pails. In the other 12 pails, we injected water instead of alarm cues to simulate a low-predation risk environment. Water injections, although innocuous per se, provide disturbance and uncertainty to tadpoles
experiencing the chemical and physical stimuli associated with the injections, and can elicit short-lived freezing behaviour. As such, we coined the treatment “low-risk”, as opposed to “no-risk”, to better reflect the reality of the manipulation.

Statistical analysis

Statistical analysis was made in R version 3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). All statistical tests were two tailed and significance threshold set at \( \alpha = 0.05 \) if not stated otherwise. As a response variable, we used the percentage of activity reduction between pre- and post- injection observation. After removing outliers (\( N=5 \) in experiment 1 and \( N=3 \) in experiment 2), data satisfied normality assumptions. The two-way ANOVA of experiment 1 tested the effect of subject tadpole risk regime (low-risk vs. high risk environment) and testing cue (alarm cue from high risk donors, alarm cues from low-risk donors, or water). The 1-way ANOVA of experiment 2 was fitted with conditioning cue (alarm cues from high-risk donors, alarm cues from low-risk donors or water) as fixed effect. For significant factors in the ANOVAs, we performed trend analysis and a post-hoc Student-Newman-Keuls test using the ‘SNK.test’ function of the R package ‘agicolae’.

References
