

Electronic Supplementary Material S1: Further detail on alarm, undisturbed, and disturbance cue preparation

Alarm cues

Alarm cues were prepared directly prior to each conditioning exposure by euthanizing 12 tadpoles via a blow to the head (using a mortar and pestle), emulsifying the bodies and mixing the result with 160 ml of filtered water, and filtering this solution through cotton gauze to remove any large pieces of tissue. This preparation yielded enough cues for eight injections (20 ml per injection containing ~1.5 tadpoles each). This concentration was similar to previous studies and known to elicit high-risk background phenotypes in tadpoles [1–3]. Injections involved slowly introducing 20 ml of either water (filtered water – low-risk treatment) or alarm cues (high-risk treatment) into the 1.5 l pails.

Undisturbed cues and disturbance cues

The two tadpole groups (8 low-risk and 8 high-risk) were briefly rinsed with filtered water and added to a 0.5-l plastic cup filled with 320 ml of filtered water (i.e. 0.025 tadpoles/ml, similar to 0.05 tadpoles/ml in Gonzalo et al. [3], who used a larger test tank volume[0.5 l vs. 3 l]). Tadpoles were left undisturbed for 1 h prior to cue collection. Similarly, eight tadpoles from the low-risk pails were also selected and added to a cup filled with 320 ml of filtered water. After the acclimation period, the cups were gently flushed with 0.5 l of filtered water, while ensuring the observer was out of sight of the tadpoles to minimize disturbance. Water was simultaneously added and removed via gravity-fed airline hoses, thus maintaining ~320 ml water volume during the water change. Low- and high-risk tadpoles were then left undisturbed for 10 min after the water change, upon which time we used the same airline hose system to extract 60 ml of undisturbed cue, exchanging it with 60 ml of fresh filtered water. Immediately following collection of undisturbed cues, we used two identical glass rods with black plastic ends to simulate a predator chase (1 min) on the two groups of tadpoles. To standardize disturbance, we moved the glass rod in both the low-risk and high-risk cups simultaneously, mirroring all movements and taking care to avoid physical contact with tadpoles. Both groups were then left alone for another minute to ensure tadpoles had enough time to produce disturbance cues prior to collection, which was done following the same methodology as previously described for the undisturbed cues [3]. All cups, airline tubes, and glass rods used in the chase simulation were subsequently washed thoroughly after the cue extraction. Each tadpole was used only once for cue production and subsequently removed from the experiment (they were not tested as receivers). The cues were produced as needed and used within 2 h of being produced.

References

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2. Lucon-Xiccato T, Chivers DP, Mitchell MD, Ferrari MCO. 2016 Making the dead talk: alarm cue-mediated antipredator behaviour and learning are enhanced when injured conspecifics experience high predation risk. *Biol. Lett.* **12**, 20160560. (doi:10.1098/rsbl.2016.0560)
3. Gonzalo A, López P, Martín J. 2010 Risk level of chemical cues determines retention of recognition of new predators in Iberian green frog tadpoles. *Behav. Ecol. Sociobiol.* **64**, 1117–1123. (doi:10.1007/s00265-010-0927-y)