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Chemical cues of conspecific predation elicit distinct behavioural responses in cannibalistic poison frog tadpoles

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Abstract

In cannibalistic species, conspecifics can be both predators and prey. As a result, conspecifics present a unique conflict at the intersection of predation, competition and nutritional resources in these species. To better understand how individuals respond to the complex information of conspecific chemical cues, we studied aggressive and cannibalistic tadpoles of the dyeing poison frog, *Dendrobates tinctorius*. We used a standardized open field test to compare behavioural responses to a positive cue (food), a negative cue (predator) and two conflicting cues (conspecific density and injured conspecifics). We specifically used chemical cues to understand how individuals respond in the absence of additional information that would disambiguate their status as conspecific predator versus prey. We found that the injured conspecific cue elicited a response distinct from either the food cue or the predator cue: tadpoles explored more relative to baseline and predator cues but spent less time moving compared to the food cue. We suggest that these patterns reflect cue-dependent behavioural strategies that maximize exploration while minimizing detection in the presence of conspecific cannibals. In addition to cue-specific changes in behaviour, we observed consistent differences in individuals' behaviour across environments and found that activity and exploratory behaviour were positively correlated across environments. Taken together, our results demonstrate that conspecific cues are interpreted as distinct from either food cues or predator cues in a cannibalistic species where they can represent both.

Keywords

Amphibian; behavioural plasticity; cannibalism; Dendrobatid; repeatability; tadpole

An essential challenge for all organisms is to detect and respond to environmental stimuli. To optimize responses for different situations, individuals must detect distinct cues and interpret their valence (Abrahams and Dill, 1989). Optimizing responses often depends on the ability to evaluate multiple, co-occurring and potentially conflicting stimuli at once.

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Author Contributions

L.L.S. and E.K.F. conceived of and designed the study. L.L.S. and S.S.O. collected data. L.L.S. analysed the data. L.L.S. wrote the manuscript with input from all authors.

Declaration of Interest

None.

For example, individuals must detect and respond to predator cues to minimize the risk of being eaten (Delia et al., 2014; Kishida & Nishimura, 2005; Maeno et al., 2018) and detect and respond to food cues to acquire nutrition (Veeranagoudar et al., 2004). Typically cues of danger and opportunity are distinct, but how do individuals respond to cues that carry conflicting information about these two fundamentally opposing things?

Cannibalism, defined as predator–prey interactions between conspecifics (Ferrari & Chivers, 2009), is a high-risk, high-reward behaviour, the benefits to those who win are great, but loss is lethal. Although cannibalistic behaviour may seem counterintuitive to species persistence, it can be a stable evolutionary strategy when the advantages to individuals in times of limited food availability are greater than the disadvantages during times of food abundance (Nishimura & Isoda, 2004). The benefits of cannibalism include resource acquisition and a reduction in intraspecific competition (Gabriel, 1985), while the costs of cannibalism include increased risk of injury and disease transmission and decreased inclusive fitness from cannibalism of family members (Pfennig et al., 1991). Particularly in the absence of additional cues that can disambiguate an individual's status as predator versus prey, conspecific chemical cues can simultaneously signal the extremes of predation threat versus food availability. Thus, individuals of cannibalistic species may uniquely optimize their responses to conspecific cues.

Dyeing poison frog, *Dendrobates tinctorius*, tadpoles exhibit cannibalistic behaviour in the wild (Rojas, 2014) and in the laboratory (Fischer et al., 2020; Fouilloux et al., 2022). Cannibalism in *D. tinctorius* tadpoles and related species likely evolved as a method of eliminating competition and as a secondary source of nutrition (Caldwell & De Araújo, 1998). Development and cannibalism are linked, as larger individuals are at lower risk of being eaten and are better able to eat conspecifics (Fouilloux et al., 2022; Petranka & Thomas, 1995; reviewed in Altig & Mcdiarmid, 1999). *Dendrobates tinctorius* tadpoles offer an opportunity to better understand how conspecific chemical cues are interpreted in cannibalistic species, and more generally how individuals respond to cues that carry conflicting information.

Although most species of tadpole are not cannibalistic (Polis & Myers, 1985), there is a rich literature on how tadpoles respond morphologically, physiologically and behaviourally to chemical cues (Hossie et al., 2010; 2017; Relyea, 2001). Commonly assayed behaviours include activity, exploration and space use, all of which can have consequences for resource acquisition and survival. For example, tadpoles increase activity and exploration in the presence of food cues, presumably to increase the likelihood of encounters with the food they have detected (Veeranagoudar et al., 2004). Conversely, tadpoles decrease activity in the presence of predators to minimize detection (Horat & Semlitsch, 1994; Steiner, 2007). Building on this existing literature documenting tadpole responses to cues of known valence, we were interested in how *D. tinctorius* tadpoles would respond to conspecific cues that could signal either finding prey or becoming prey. We specifically wanted to understand how individuals responded to conspecific cues in the absence of additional information (e.g. visual cues) that would signal their status as predator (e.g. bigger than a conspecific) or prey (e.g. smaller than a conspecific). The question of responses to conspecific cues is of broad interest in cannibalistic species and of specific ecological relevance is *D. tinctorius* as tadpoles of this species are naturally found in small water bodies of varying sizes and densities of conspecifics (Fouilloux et al., 2021).

The central aim of our study was to understand tadpole behavioural responses to conspecific cues. To this end, we compared *D. tinctorius* tadpole behaviour in open field tests with a neutral cue (plain water), a positive cue (food), a negative cue (predator) and two conflicting cues (conspecific density and injured conspecifics). The latter cues both indicated the presence of conspecifics, with the difference that the injured conspecific cue simulated a recent predation event. Open field tests, although not naturalistic, allowed us to present chemical cues and quantify and compare behaviour in a standardized way. We predicted that changes in movement and activity in response to conspecific cues would be distinct from responses to either food cues or predator cues. In other words, we predicted that tadpoles would uniquely respond to conspecific chemical cues.

We additionally assessed the potential for cue-specific behavioural strategies by characterizing individual variation in behaviour across cue types (i.e. behavioural repeatability across contexts; Bell et al., 2009). When individuals show context-specific behavioural strategies (behavioural plasticity), behaviour may be repeatable if individuals show consistent responses relative to others across environments, or not repeatable if individuals respond to differing degrees (Carlson & Langkilde, 2013). Thus, behavioural plasticity and repeatability can, but do not always, coexist. While behavioural plasticity is advantageous to immediate survival, repeatability is predicted to be beneficial to population persistence through variation in bet hedging (Sih et al., 2004). If there is individual variation in the spectrum of behaviour in a population (e.g. consistently more/less active and exploratory individuals), then this makes the group more diverse and robust to fluctuating environments.

Given alternative forces favouring repeatability and plasticity, we made four general predictions. (1) Behaviour is repeatable and plastic, and in this case, all individuals should alter their behaviour in a similar direction and to a similar magnitude, such that their relative ranks remain consistent. For example, all tadpoles might decrease movement in response to predation, but individuals most active at baseline should remain most active in the predator context (Trnka et al., 2018). (2) Behaviour is repeatable but not plastic, and in this case, individuals should behave consistently across environmental contexts, suggesting that individual ‘personalities’ may constrain plasticity (Biro & Stamps, 2008). (3) Behaviour is plastic but not repeatable, and in this case, individuals should alter their behaviour in response to environmental cues, but these shifts should not be predictable and should vary in direction and/or magnitude across individuals. This pattern may emerge when the necessity of quick behavioural changes supersedes individual consistency (Hofmann et al., 1999). (4) Behaviour is neither repeatable nor plastic. Importantly, the relationship between plasticity and repeatability may depend on the behavioural trait being measured, and jointly characterizing behavioural plasticity and repeatability may provide clues to the forces shaping behavioural variation within and among individuals. Taken together, our study took advantage of the unique biology of *D. tinctorius* tadpoles to examine behavioural responses to cues carrying conflicting information as well as individual behavioural consistency across environmental cues.

METHODS

Tadpole Housing and Care

We bred *D. tinctorius* in our captive frog colony (University of Illinois Urbana-Champaign, Urbana, IL, U.S.A.). In *D. tinctorius*, eggs are laid terrestrially and cared for by males. Upon hatching, fathers transport tadpoles piggyback to pools of water (Rojas & Andrius Pašukonis, 2019). In the laboratory, we remove tadpoles from these pools and rear them individually in mesh-bottomed plastic cups within a larger aquarium, following procedures standard in poison frog husbandry. Within each cup, we included a piece of sphagnum moss and a piece of tea leaf for vegetative cover, additional nutrition and antimicrobial properties. Thus, tadpoles are not in physical contact with one another but share a water source, such that chemical cues of conspecifics freely pass between individual containers during development analogous to the situation in the wild, where tadpoles are in murky pools of varying sizes and with varying densities of differently aged conspecifics, individuals have constant access to chemical cues but only intermittent access to visual, auditory and vibrational cues.

We kept the water temperature constant at 22.8 ± 1 °C. The rooms that housed the aquaria were under 12:12 h light:dark cycle. We fed tadpoles a combination of shrimp flakes and rabbit food pellets three times per week. We checked tadpole health daily and performed partial water changes and water quality checks (pH, salinity, temperature) weekly. Tadpoles were evenly and randomly chosen from three aquaria with a tadpole density of 12–20 individuals per aquarium in about 20 litres of reverse-osmosis (RO) water. Tadpoles included in this study ($N = 36$) were Gosner stages 25–27 with no noticeable external limb development. The average tadpole total body length was 26 mm (range 20–32 mm).

Behavioural Data Collection and Analysis

Each tadpole experienced six open field assays with 1 day between each test: a habituation test, a baseline test and four cue trials. Behavioural testing and analysis procedures were identical for all trials. Open field arenas consisted of 25 cm diameter white, plastic buckets with a camcorder (DVC model number HDV-604S) positioned overhead.

We transferred tadpoles from home aquaria to the open field arena using a spoon. We released tadpoles into the centre of the arena and recoded behaviour for 25 min. For all trials, we considered the first 5 min an acclimation period and excluded it from behavioural analyses. Because preliminary experiments revealed significantly lower activity levels between a tadpole’s first time in the arena and subsequent tests (Appendix, Table A1), we conducted an initial ‘habituation test’ in RO water that was not directly compared to subsequent tests. The second test, also conducted in RO water, was used as a baseline to which subsequent cue tests were compared. The open field tests for the four chemical cues (described below) followed the baseline test in a randomized order across individuals. We measured water temperature before the start of each trial via a multiparameter pocket tester instrument (APERA Instruments, model number AI316). We kept temperature (23.0–24.5 °C) and water volume (500 ml, depth ~15 mm) consistent across tests. This relatively shallow water depth is ecologically appropriate given the characteristics of pools where *D. tinctorius* are found in the wild (Fouilloux et al., 2021).

Following habituation and baseline tests, we exposed tadpoles to one of four chemical cues added to the RO water used for baseline: injured conspecific, conspecific density, food and predator (Fig. 1). To make the food cue, we soaked shrimp flakes in water for 30 min, strained out the food particles and diluted it to keep the colour of the water more consistent with other cues. This cue simulated food availability without actual possible consumption. To create the predator cue, we obtained dragonfly nymphs (genus Lubelluliidae) (Carolina Biological Supply, Burlington, NC, U.S.A., https://www.carolina.com/aquatic-insects/dragonfly-nymphs-living-pk-12/143526.pr, Item 143526) and reared them in individual cups with a shared water source ($N = 8$, in ~2500 ml of RO water). Dragonfly nymphs are known predators of tadpoles and have been observed to induce antipredator responses (Hossie et al., 2010; Kishida & Nishimura, 2005). An aliquot of 500 ml from the dragonfly's shared water source was used in open field assays without dilution. RO water in the dragonfly aquarium was replaced following removal to keep water levels consistent. We made the conspecific density cue by measuring out 500 ml of the tadpole rearing environment water (described above) without dilution. To make the 'injured' conspecific cue, we euthanized nine tadpoles using MS-222 (see also Ethics Note below), homogenized bodies using a tissue homogenizer (Biospec Products, Inc., Bartlesville, OK, U.S.A., model 1285) and strained out the particulates. To minimize contamination of the injured conspecific cue with MS-222, tadpoles were rinsed with deionized water following euthanasia and prior to grinding. Rinsing has been shown as an effective way to flush out MS-222 (Ramlochansingh et al., 2014); thus, we do not believe residual MS-222 had an effect on behaviour in this cue type. We added 10 ml of this solution to 490 ml of RO water for each injured conspecific trial.

We randomized the order in which each tadpole experienced the cues to control for order effects. Following each test, we returned tadpoles to their home aquarium. We measured total tadpole body length following the first and last behavioural trial. Body length did not significantly change across the first and last trials (10 days apart); therefore, we used the first measurement in statistical analyses (see below).

Videos were analysed using ToxTrac software (Rodriguez et al., 2018). ToxTrac tracks individuals and outputs various behavioural metrics of interest (Fig. 2a). Using ToxTrac, we quantified the proportion of time spent moving (referred to as mobility rate), the proportion of areas visited (referred to as exploratory rate), average speed (mm/s), total distance travelled (mm) and total time spent in the centre of the arena (seconds) for each tadpole. We calculated time spent in the centre of the arena as the sum of time in the middle two rings (Fig. 2c). We quantified multiple behavioural metrics to understand whether tadpoles changed how much they moved but also how they moved; that is, whether their behavioural strategy differed across contexts. For example, we expected that cues of predation would decrease overall movement but speculated that decreased movement could be correlated with or independent of space use.

Statistical Analyses

All statistical analyses were performed with R (version 2022.07.1 + 554; R Core Team, 2023) in RStudio (version 2022.12.0; RStudio Team, 2023). We tested for differences in open field behaviour (mobility rate, exploratory rate, total distance travelled, speed, time spent in centre) across cue types using type III ANOVAs in the package ‘lmerTest’ (Kuznetsova et al., 2017) followed by post hoc testing using the package ‘emmeans’ (Lenth, 2022). We fitted models for each behaviour with cue type and body length as fixed effects and individual identity (ID) as a random effect. We included day of test, temperature and the interaction between body length and cue type as fixed effects in our original models but removed them because they were nonsignificant and to prevent overfitting. Model residuals were assessed for normality via visual inspection of histograms and normal Q–Q plots (qqnorm) with a best-fit line (qqline), as well as Shapiro tests for normality. We log-transformed distance and speed (continuous variables) and arcsine-transformed exploratory rate and mobility rate (proportion data) to achieve normality. Time spent in centre proved problematic because some tadpoles never explored the centre, resulting in zeros in the data, but we found the model with square root transformation to be the best fit. We used Akaike’s information criterion (AIC) values to identify whether the models with transformed or untransformed data fit better. Exploratory rate and mobility rate models were better fit without transforming, but all other behavioural variables were better fit by transforming.

We analysed repeatability using the ‘rptR’ package (Stoffel et al., 2017). We tested for individual repeatability across all behavioural tests (baseline plus four cue tests). While this approach is distinct from testing for repeatability within the same assay, the approach is mathematically identical. Finally, we analysed correlations between all behaviours and body length on untransformed data across cue types and the average across all trials via Kendall’s rank order correlation coefficient in the ‘corrtest’ function (stats package version 4.1.3) and visualized them using the corrplot package (Wei & Simko, 2017).

Ethical Note

We designed all procedures to minimize distress. We monitored tadpoles for 8 weeks following the experiment and found no adverse effects on growth, behaviour or survivorship. Tadpoles sacrificed to produce the injured conspecific cue were euthanized quickly and humanely using an overdose of sodium bicarbonate buffered tricaine methane sulfonate (MS-222), an anaesthetic commonly used with fish and amphibians (Archard and Goldsmith, 2010; Topic Popovic et al., 2012). All animal care and experimental procedures were approved by the Animal Care and Use Committee of the University of Illinois at Champaign-Urbana (IACUC Protocol number 20147).

RESULTS

Tadpoles altered their mobility rate ($F_{4,140} = 5.89$, $P < 0.005$) and exploratory behaviour ($F_{4,140} = 3.02$, $P < 0.05$) across cue types (Table 1). Specifically, tadpoles spent more time moving in response to the food cue as compared to the baseline ($t_{136} = -3.240$, $P < 0.05$), injured conspecific cue ($t_{136} = -4.46$, $P < 0.001$) and predator cue ($t_{136} = 3.82$, $P < 0.005$) (Fig. 3a). Tadpoles explored more areas in the arena during the injured conspecific cue treatment compared to the baseline ($t_{136} = -3.0$, $P < 0.05$) and predator cue treatment ($t_{136} = 2.80$, $P < 0.05$) (Fig. 3b). Total distance travelled, average speed and time in the centre of the arena did not differ across cues (Table 1, Appendix, Fig. A1). Speed and exploration were correlated with body size such that larger tadpoles moved faster ($F_{1,\ 36.46} = 4.57$, $P < 0.05$) (Appendix, Fig. A2a) and explored marginally more ($F_{1,\ 36} = 3.79$, $P = 0.059$) (Appendix, Fig. A2b) than smaller tadpoles but did not differ in other behaviours (Table 1). Complete information for all post hoc comparisons is provided in the Appendix, Table A2.

Across all behavioural trials (i.e. cue types), exploratory rate, mobility rate, average speed and total distance travelled were strongly positively correlated with one another ($R > 0.3$, $P < 0.05$), but not with time spent in the centre nor with body length (the average across all trials is shown in Fig. 4, correlation plots by cue type are given in the Appendix, Fig. A3).

Exploratory rate ($R = 0.343$, CI = [0.18, 0.49], $P < 0.001$), distance travelled ($R = 0.322$, CI = [0.163, 0.478], $P < 0.001$) and speed ($R = 0.412$, CI = [0.0.243, 0.555], $P < 0.001$) were significantly repeatable across trials (Appendix, Fig. A4), while mobility rate and time spent in the centre were not (Table 1).

DISCUSSION

Many studies have shown that tadpoles can detect, distinguish and behaviourally, morphologically and physiologically respond to chemical cues of predators (Kishida & Nishimura, 2005; Lawler, 1989; Middlemis Maher et al., 2013; Relyea, 2001), food (Veeranagoudar et al., 2004) and conspecific alarm cues (Ferrari et al., 2007; Fraker et al., 2009). In cannibalistic tadpoles, conspecific chemical cues may function as all three. We compared behaviour in cannibalistic dyeing poison frog tadpoles across food, predator, conspecific density and injured conspecific chemical cues to ask how tadpoles respond to conspecific cues of mixed valence. We also evaluated behavioural repeatability across contexts to understand whether cue-specific responses coexist with consistent individual differences. We discuss the implications of our findings in an ecological context.

We found that the injured conspecific cue elicited a response distinct from either the food or the predator cue: tadpoles increased exploration compared to predator and baseline cues but did not increase movement compared to baseline as they did in response to the food cue. Tadpoles did not change the distance they moved or how fast they moved overall, as total distance travelled and average speed did not differ across different contexts. Rather, they changed how they moved: in the presence of injured conspecific chemical cues, tadpoles explored the arena in shorter bursts of activity interspersed with sessile periods. We interpret this movement pattern as a behavioural strategy that optimizes the balance of exploration to find food and/or shelter while minimizing the probability of detection by conspecifics when chemical cues signal cannibalism without additional information available on the relative size of the eater and the eaten.

In contrast to cues of injured conspecifics, the conspecific density cue did not elicit a unique behavioural response, suggesting that tadpoles differentiate between conspecifics generally and injured conspecifics specifically. A lack of response to conspecific density cues could be an artefact of this cue representing the tadpoles' rearing environment and thus tadpoles may have habituated to the cue, although tadpoles are typically found in groups of conspecifics and heterospecifics in the wild, making this scenario ecologically relevant (Fouilloux et al., 2021). Additionally, being reared in a high-density environment could have affected responses to the other cue types. Tadpoles may default to low movement in general to avoid detection, so we might see an increase in activity and exploration only in the presence of potential food (directly or via cannibalism). For example, in Balearic green toad, *Bufotes balearicus*, tadpoles, individuals reared in high-density environments decreased mobility rate in the presence of a predator significantly more than individuals reared alone (Guadin et al., 2021). Future experiments exploring the impact of conspecific density in the rearing environment on behaviour in poison frog tadpoles will further inform our interpretations of these data.

At present, we do not know whether *D. tinctorius* tadpoles produce a specific alarm pheromone, as known in fish (e.g. Brown et al., 2010; Wisenden et al., 2004) and some tadpoles (e.g. Fraker et al., 2009), or whether conspecifics respond to a chemical cue from injured conspecifics more generally (e.g. blood). In either case, responses to injured conspecifics are unique from those of conspecifics more generally and investigating whether tadpoles of *D. tinctorius* and other cannibalistic species emit an alarm pheromone is a topic for further research.

By using an open field assay, we were able to quantify behaviour in a standardized way across different cue types, and we found that, in addition to responding to cues, tadpoles exhibited individual consistency across contexts in exploratory rate, total distance travelled and average speed but not in mobility rate or time spent in the centre of the arena. The underlying assumption that behaviour is repeatable is often the basis for behavioural studies and comparisons, but it is rarely confirmed across multiple contexts (Bell et al., 2009; but see Carlson & Langkilde, 2013; Holveck & Riebel, 2007; Killen et al., 2016). In brief, we found both plasticity and repeatability across environmental contexts for some, but not all, behaviours.

Given complementary forces favouring repeatability and plasticity (Sih et al., 2004), we predicted four general categories for this relationship: behaviour could be repeatable and plastic, repeatable but not plastic, plastic but not repeatable, or neither plastic nor repeatable. In this study, we found all four relationships represented among the behaviours we measured. Exploratory rate was repeatable and consistently plastic, illustrating that flexibility and repeatability are not mutually exclusive. Speed and distance travelled were repeatable but not plastic, suggesting these behaviours may be more constrained by other factors, like body size (see below). Mobility rate was plastic but not repeatable, suggesting it may be advantageous for all individuals to strongly modulate this behaviour based on context and/or that unpredictable movement is most effective for predator evasion (Richardson et al., 2018). Finally, we found that time spent in the centre was not plastic or repeatable. This pattern suggests that behavioural changes in response to cues are small and noisy and that the degree of behavioural variation among individuals is equal to that within individuals. We suspect this final pattern is primarily a statistical artefact rather than a biological phenomenon (e.g. if individuals modify their behaviour at all, then they can be considered plastic, even if not significantly so); however, the outcome highlights a lack of consistency at the population level and then the individual level. The mix of relationships between plasticity and repeatability we identified may be a result of balance in the evolution of traits that are beneficial for the immediate survival of an individual with traits that support population persistence. Recognition of these patterns, individually and in concert, addresses our central question of behavioural responses to environmental cues and underscores the importance of confirming behavioural repeatability across contexts when drawing conclusions about the consequences of individual variation.

We found that, across environmental contexts, exploratory rate, mobility rate, speed and distance travelled were all positively correlated with one another. Correlations among these behaviours are interesting because, despite the differences in how plastic and repeatable the behaviours were, they were largely correlated. Additionally, these correlations were consistent across environmental contexts, implying that shared environmental, mechanistic and/or selective pressures keep the relationships stable (Garamszegi et al., 2013). In contrast, these behaviours were not correlated with time spent in the centre or body length. This suggests that time spent in the centre and the other behaviours are likely not mediated by the same mechanism (Garamszegi et al., 2013). The lack of correlation between body length and the behaviours we measured suggests that activity and exploration are not primarily driven by body size (but see further discussion below). However, we looked at a narrow range of developmental stages and body sizes, and it is possible that body size/behaviour correlations could change across ontogeny (Bell & Stamps, 2004; Urszán et al., 2015).

Although our study was not specifically designed to test for the effect of body size, we found that total body length predicted speed and exploration rate, with larger tadpoles moving significantly faster and exploring moderately more than smaller tadpoles. Due to our repeated measured design and because tadpoles did not change size significantly across the experiment period, these results do not alter the interpretation of behaviours across cues. *Dendrobates tinctorius* tails appear to grow allometrically with body size (Appendix, Fig. A5); thus, having a longer tail may generate more power and allow for faster swimming and/or more exploration (Arendt, 2010). Importantly, although body size predicted speed, body size was not correlated with other behaviours. This indicates that the effect of body size, although significant, was small overall. Larger tadpoles are better competitors (Fouilloux et al., 2022), possibly because speed and exploratory rate increase with body size. If so, assessing responses to cues across varying body sizes could reveal further behavioural responses specific to body size and/or developmental stage.

There were several unanticipated outcomes of our study. First, behaviour in the predator cue trial and baseline trial did not differ. This could mean that the predator cue was not strong enough to elicit a response unique from the baseline because of dilution or that tadpoles did not recognize the cue. Alternatively, if tadpoles have outgrown the risk of predation by dragonfly nymphs, they may detect but not respond to dragonfly cues (Crane & Ferrari, 2017). Finally, responses to dragonfly cues could depend on dragonfly diet. When designing our experiment we considered feeding the dragonfly nymphs a diet of tadpoles before the study, as this has been found to produce a strong aversion to predators (Hossie et al., 2017; Persons et al., 2001). We elected not to feed the dragonfly nymphs a diet of tadpoles prior to trials so as not to confound the direct predator cue with the tadpole alarm cue(s) that may have been in nymph excrement. Nevertheless, given that we found distinct behavioural responses to nymphs versus conspecific predators, further study comparing additional predators and/or predators that have recently consumed tadpoles would be of interest.

The second unanticipated outcome was that we did not see differences in time spent in the centre of the arena across cue types, and this behaviour was not repeatable. Spending relatively more time in the centre and away from arena walls is typically interpreted as boldness in open field assays (Burns, 2008; Hope et al., 2020; reviewed in Yuen et al., 2017). The lack of pattern in time spent in the centre in our study raises the question of whether this metric should be interpreted as boldness in our focal species. We conclude that tadpoles may not perceive risk/safety in the way typically assumed by this boldness metric, and the biological relevance of this measure should be interpreted with caution (Yuen et al., 2017).

Conclusion

Our study confirmed that signals that provide complex and/or mixed information elicit a unique response from those with a straightforward valence in cannibalistic tadpoles. Specifically, injured conspecific cues elicited a response unique from predator cues or food cues. Interestingly, tadpoles specifically changed their pattern of movement (maximizing exploration while minimizing time spent moving), suggesting a distinct behavioural strategy in the presence of conspecific cannibals. Additionally, we found variable relationships between behavioural plasticity and repeatability across behaviours. Overall, our findings highlight variation in the behavioural responses to cues carrying conflicting information and intraspecific variation in these behaviours.

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Appendix

Table A1

Repeatability results across acclimation and baseline tests

Behaviour	Repeatability			
	R	**SE**	**CI**	***P***
Exploratory rate	0.058	0.116	[0, 0.384]	0.395

Behaviour	**Repeatability**			
	R	**SE**	**CI**	***P***
Mobility rate	0.207	0.150	[0, 0.525]	0.121
Speed	0.158	0.136	[0, 0.445]	0.193
Distance	0.089	0.124	[0, 0.405]	0.326
Time in centre	0.090	0.121	[0, 0.396]	0.324

CI: confidence interval. Behaviours were not repeatable across acclimation and baseline tests; therefore, we did not compare subsequent trials directly to acclimation trials.

Table A2

Results of post hoc analyses of behavioural ANOVAs used to identify differences in mobility rate and exploratory rate across cue types

Cues compared	**Mobility rate**		**Exploratory rate**	
	t_{136}	***P***	t_{136}	***P***
Baseline – Injured	1.218	0.7409	−2.998	<0.05
Baseline – Density	−0.614	0.9727	−1.803	0.376
Baseline – Food	−3.240	<0.05	−1.428	0.6111
Baseline – Predator	0.582,	0.9775	−0.202	0.9996
Injured – Density	−1.832	0.3595	1.195	0.7544
Injured – Food	−4.459	<0.001	1.570	0.5193
Injured – Predator	−0.636	0.9689	2.795	<0.05
Density – Food	−2.627	0.0712	0.375	0.9957
Density – Predator	1.196	0.7536	1.600	0.4997
Food – Predator	3.822	<0.005	1.225	0.7367

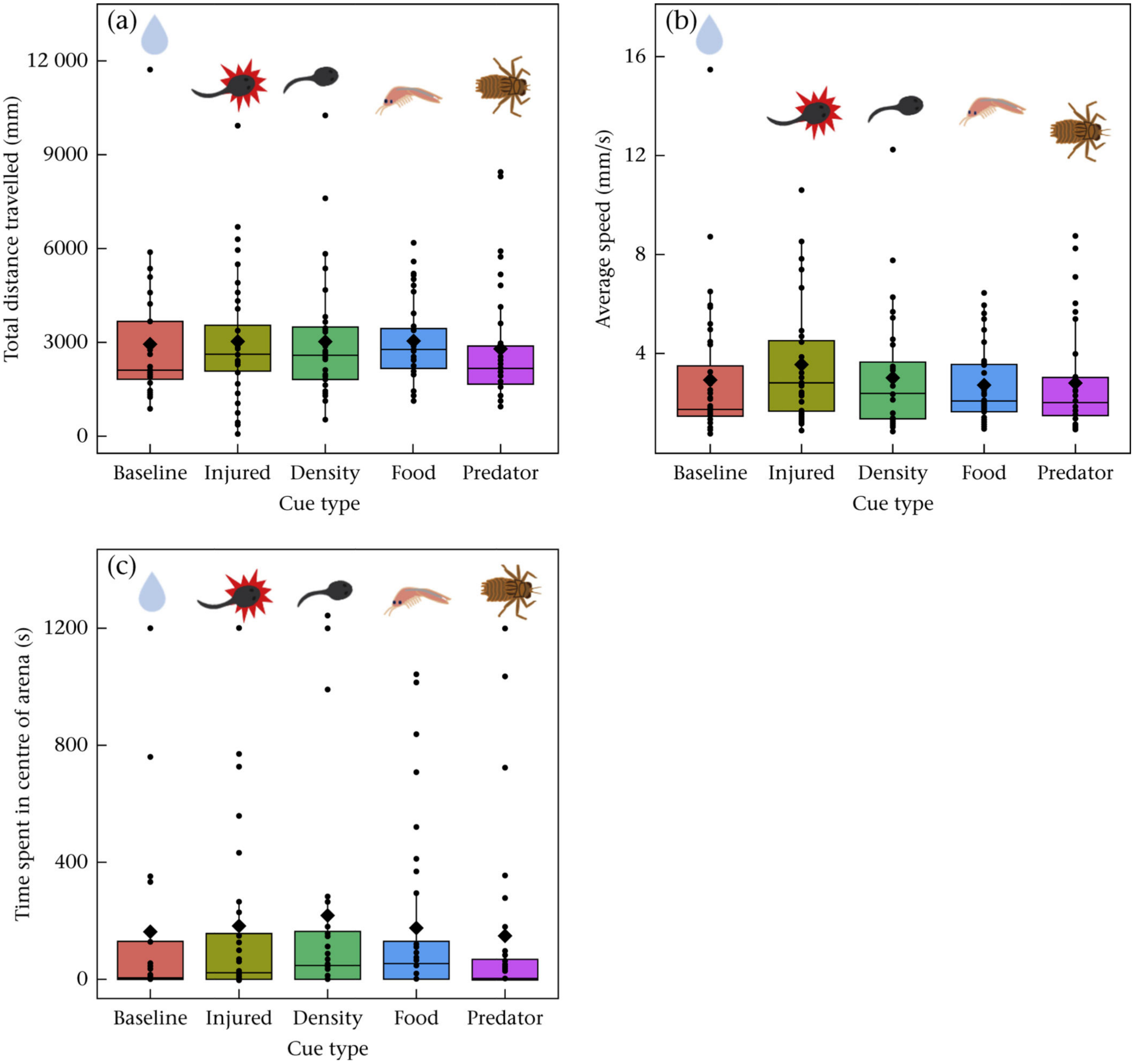

Figure A1.

(a) Total distance travelled, (b) average speed and (c) time spent in the centre of the arena in response to the baseline treatment and four chemical cue treatments (injured conspecific, high conspecific density, food, predator).

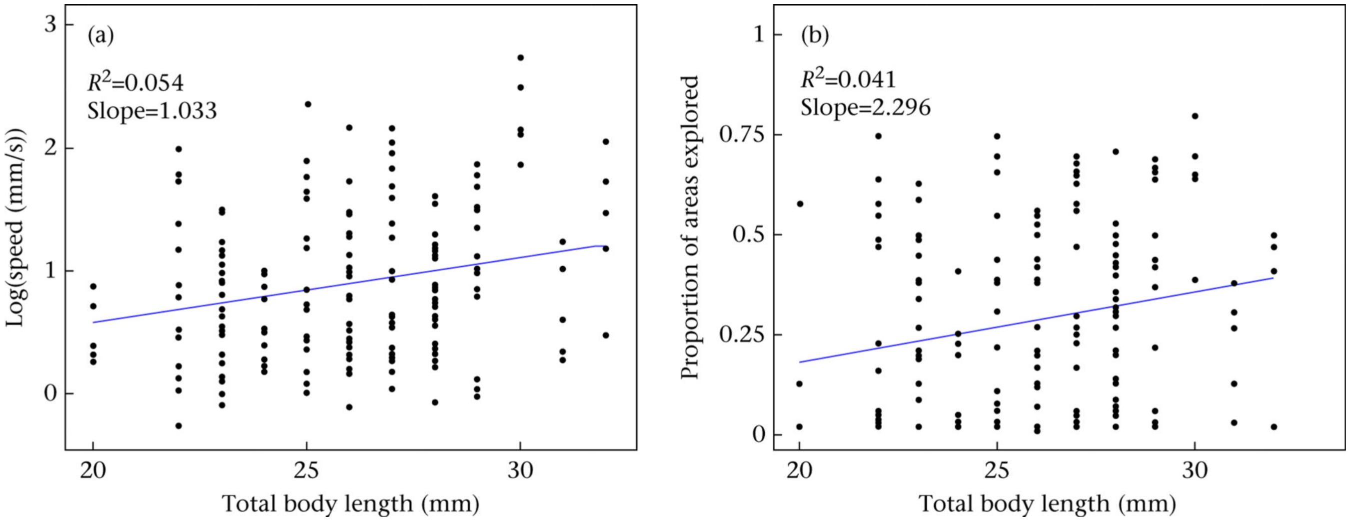

Figure A2.

Relation between body size and (a) average speed and (b) exploratory behaviour.

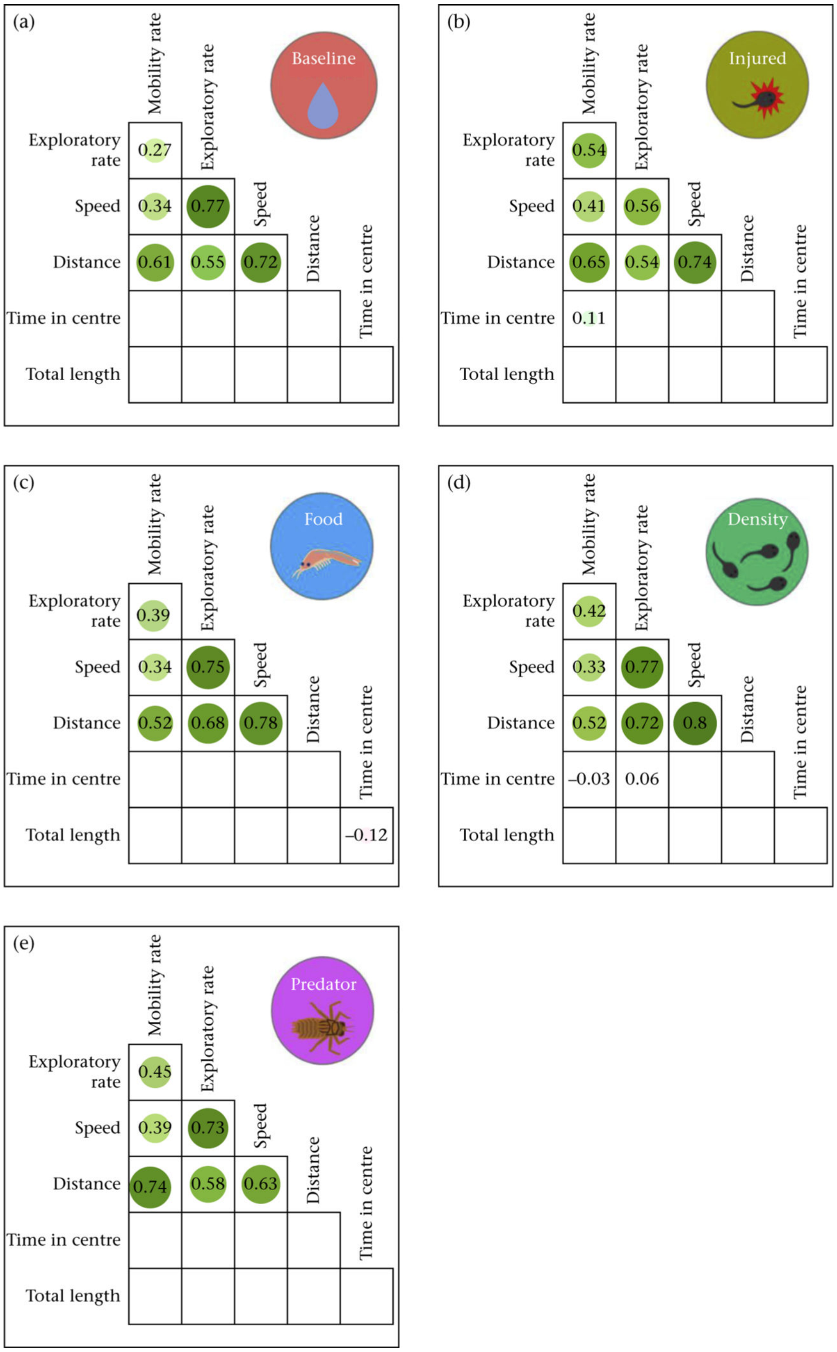

Figure A3.

Correlation (*R*) of behaviours in (a) baseline, (b) injured conspecific, (c) food, (d) high conspecific density and (e) predator treatments. Significant correlation values ($P < 0.05$) are illustrated in corresponding squares, with darker green representing strong positive correlations.

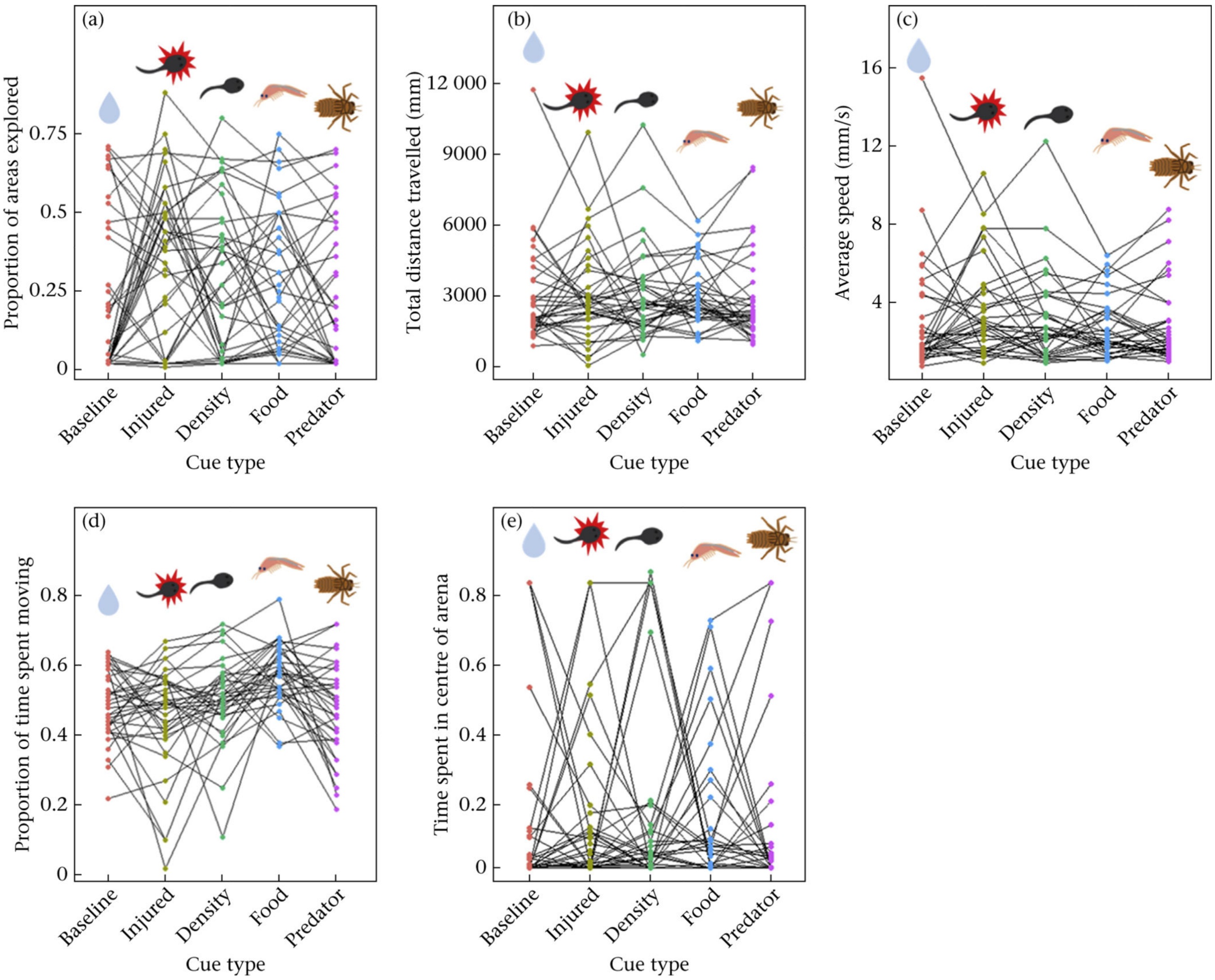

Figure A4.

Repeatability of (a) exploratory rate, (b) total distance travelled, (c) average speed, (d) mobility rate and (e) time spent in the centre of the arena based on cue type (baseline, injured conspecific, high conspecific density, food, predator).

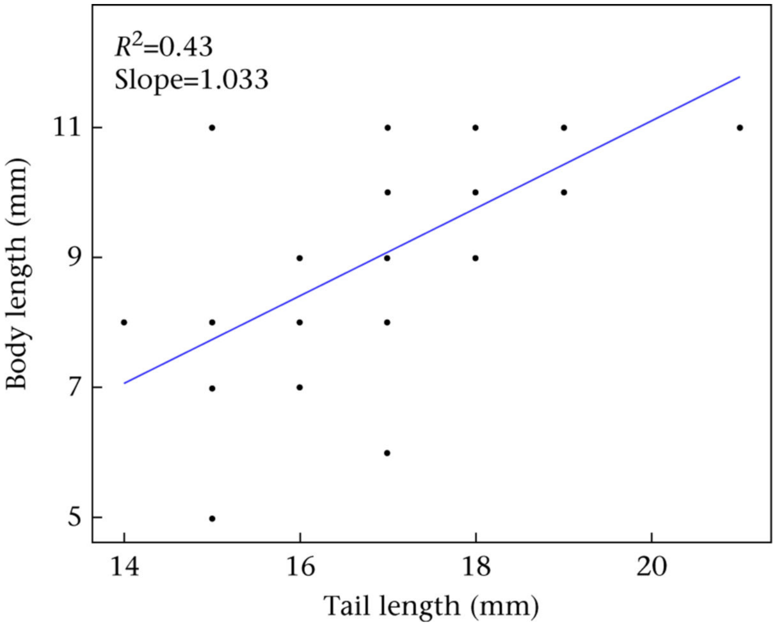

Figure A5.

Relation between tadpole tail length and body length.

Data Availability

All raw data and analysis code are available on Dryad (https://10.5061/dryad.76hdr7t33).

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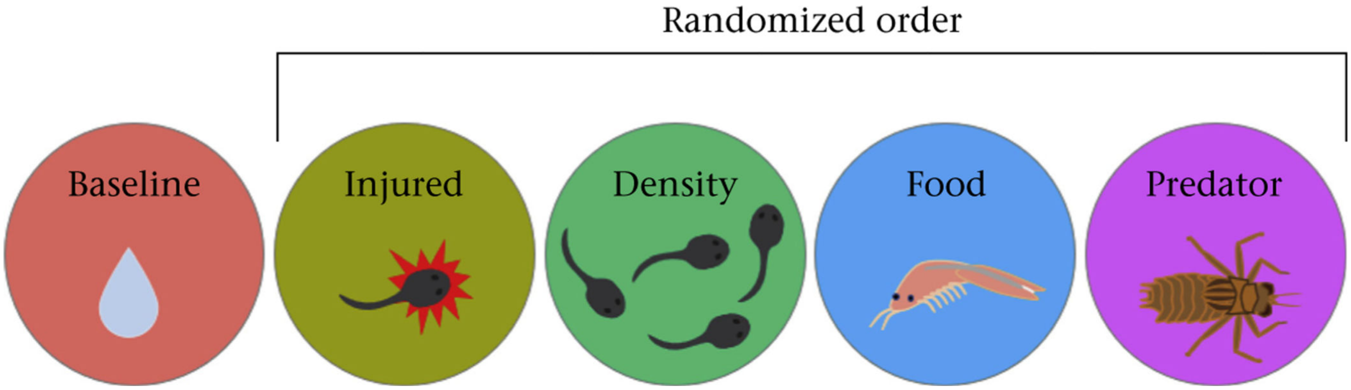

Figure 1.

Individuals experienced five open field tests: a baseline test followed by four cue tests in a randomized order (injured conspecific cue, high conspecific density cue, food cue, predator cue). We conducted behavioural trials with 1 day between each test.

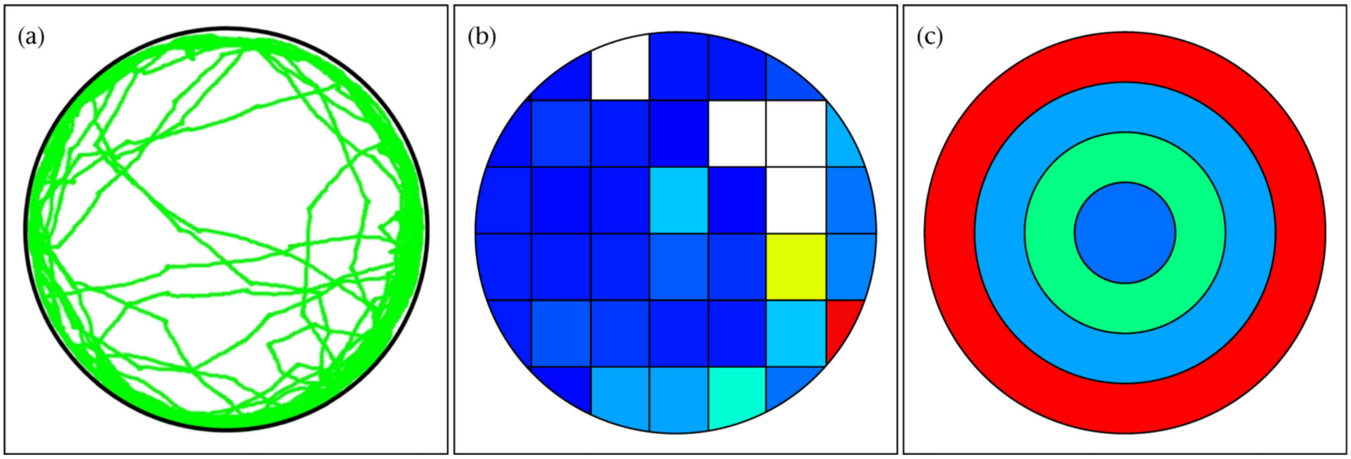

Figure 2.

ToxTrac software was used to quantify tadpole activity levels and spatial use in open field trials. This is an example of an individual in a baseline treatment trial with outputs showing (a) the pathway of the tadpole depicted in green, which was used to quantify total distance travelled, (b) an exploration heatmap, with more explored areas in red and lesser explored areas in blue, and (c) a spatial use heatmap of the centre versus the edges of the arena, with more time spent in the area shown in red and less time spent in the area shown in blue (the two inner rings were added together to calculate time spent in the centre of the arena).

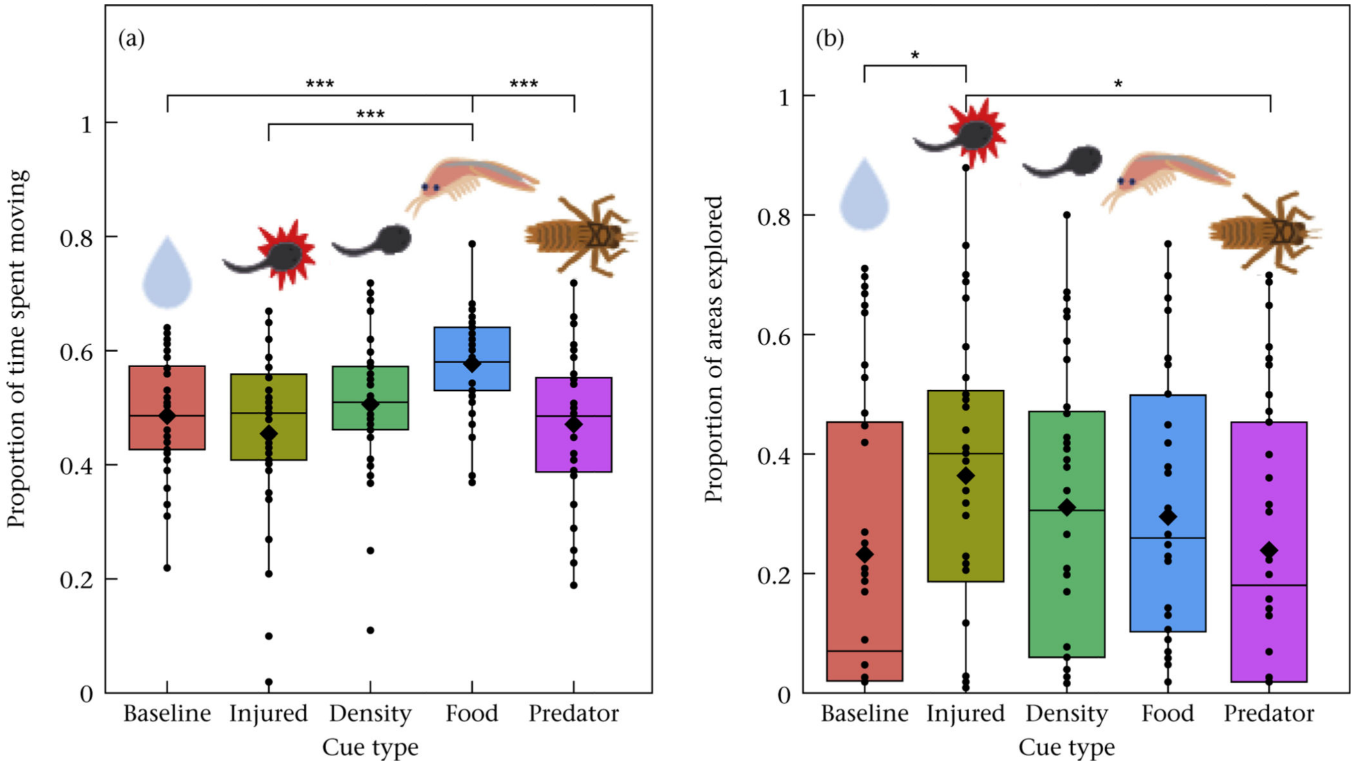

Figure 3.

Proportion of (a) time spent moving (mobility rate) and (b) areas explored (exploratory behaviour) in response to the baseline treatment and four chemical cue treatments (injured conspecific, high conspecific density, food, predator). Points indicate individual tadpoles and black diamonds indicate group means.

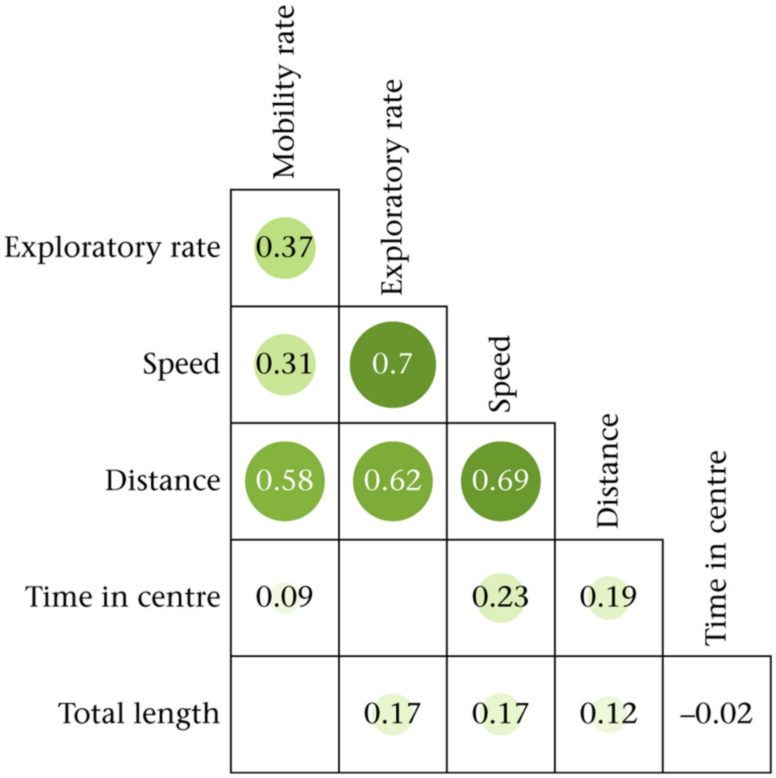

Figure 4.

Correlation (R) of behaviours averaged across all trials. Significant correlations ($P < 0.05$) are illustrated in corresponding squares, with darker green representing strong positive correlations. Correlation plots by cue type are shown in the Appendix, Fig. A3.

Table 1

Summary of statistical analyses

Behaviour	**Effect of cue type**			**Effect of body length**			**Repeatability**		
	F	***df***	***P***	***F***	***df***	***P***	***R***	**CI**	***P***
Exploratory rate	3.02	4, 140	**<0.05**	0.379	1,36	**0.059**	0.34	[0.18, 0.49]	**<0.001**
Mobility rate	5.89	4, 140	**<0.005**	0.31	1, 34.7	0.58	0.05	[0, 0.18]	0.23
Time centre (transformed)	0.70	4, 140	0.60	1.84	1, 34.8	0.18	0.08	[0, 0.22]	0.090
Distance travelled (transformed)	0.75	4, 140	0.51	2.62	1, 35.8	0.11	0.32	[0.16, 0.48]	**<0.001**
Speed (transformed)	1.76	4, 140	0.14	4.57	1, 36.5	**<0.05**	0.41	[0.0.24, 0.56]	**<0.001**

CI: confidence interval. Shown are ANOVA results, with cue type and body length as fixed effects and individual ID as a random effect, and results of repeatability across contexts. Significant *P* values (<0.05) are shown in bold.