Social learning of predators by tadpoles: does food restriction alter the efficacy of tutors as information sources?

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Prey animals often need to learn the identity of unknown predators, and not surprisingly, nearby conspecs provide a rich source of information about both the identity and risk level posed by unknown predators. Individuals that learn from watching conspecs that show a weak response to a predator often learn that the predator is a mild threat, while those that observe conspecs that show a strong response to the predator, learn that the predator is a high-level threat. This means that any factor that influences the intensity of the antipredator response of the tutor can potentially influence the efficacy of information transfer to the observer. We know that food resources influence the activity levels of prey, making them more or less conspicuous to nearby conspecs. We also know that prey often ignore risk if they have restricted access to food resources. This means that food resources have the potential to dramatically change social learning dynamics. In the present study, we found that tadpoles fed restricted diets had much higher activity levels than those fed ad libitum food resources. Their high activity made them more conspicuous to nearby conspecs, causing them to be much more efficient as tutors. Our work highlights the dynamic nature of social learning, as information transfer is likely to change considerably from place to place and year to year as resources change through space and time.

The field of behavioural ecology is full of examples of ways in which animals adjust when, where and how they forage and reproduce in response to spatial and temporal variation in predation risk (Ferrari, Sih, & Chivers, 2009; Lima & Dill, 1990; Sih, 2005). For prey to make these dynamic adjustments in their behaviour, they need to distinguish predators from nonpredators (Mitchell, McCormick, Ferrari, & Chivers, 2011), and often they acquire this information from nearby conspecs through the process of social learning (Brown & Laland, 2003; Crane & Ferrari, 2013; Griffin, 2004). Social learning of predators, the process of acquiring information about predators by observing the actions of other individuals, has received considerable attention (reviewed in: Crane & Ferrari, 2013; Griffin, 2004).

For the sake of clarity, it is important to consider some terminology used in the social learning literature. The individual that acts as the information source is typically referred to as the tutor, while the individual acquiring the information is the observer. We need to be aware that there is little evidence that social learning of risk involves the tutor doing anything specific to ‘teach’ the observer something. Rather, the observers gains information by paying attention to the intensity of the antipredator responses of nearby individuals (Crane & Ferrari, 2013). Early work on social learning of risk focused on asking the simple question of whether a given species was able to learn the identity of a particular predator. However, in the past decade we have seen a number of studies that have examined subtle differences in social learning. Many studies ask how a change in tutor behaviour translates into differences in learning in observers. Several studies have shown that observers that watch tutors respond strongly to unknown predators learn that those predators are highly dangerous, while observers that watch tutors that respond weakly to unknown predators learn that those predators are a mild threat (Ferrari, Trowell, Brown, & Chivers, 2005; Griffin & Evans, 2003; Vieth, Curio, & Ernst, 1980). Other experiments have manipulated the tutors-to-observers ratio to ask whether this influences the intensity of learned responses (Ferrari & Chivers, 2008; Hirvonen, Ranta, Rita, & Peuhkuri, 1999), while others have asked whether relatives are better tutors than nonrelatives (Kavaliers, Colwell, & Choleris, 2005), or whether adults act as better tutors than juveniles (Karplus, Katzenstein, & Goren, 2006). Our current work is similar in that we address whether differences in tutor behaviour translate into differential learning in observers.

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Crane and Ferrari’s (2013) recent review highlighted that social learning of predators is widespread in the animal kingdom, with birds, mammals and schooling fishes receiving the most attention. This is not surprising as these taxa are known to form tight social aggregations and often have highly coordinated escape behaviours. What about other taxa? Do other taxa have the ability to socially learn predators? Tadpoles represent a group of animals that often show large aggregations but clearly lack any form of coordinated antipredator behaviours (Blausstein & O’Hara, 1986). In the first examination of social learning in tadpoles, Ferrari, Messier, and Chivers (2007) found that woodfrogs (Lithobates sylvatica) show very consistent social learning. Individuals that are naïve to predatory tiger salamanders (Ambystoma tigrinum) do not show antipredator responses to salamander odour. However, when tadpoles are raised with salamanders, they quickly learn salamander as a threat and will show dramatic reductions in activity (the typical predator responses to salamander odour). Other individuals that are naïve to the predator learn the predator odour when they are paired with an experienced conspecific (tutor) that is showing a reduction in activity. This social learning occurs very quickly, with information acquisition occurring in a single trial. The only other species of amphibian that is known to learn predator identities socially is the boreal chorus frog, Pseudacris maculata (Ferrari & Chivers, 2008). They learn the identity of predatory salamanders by observing the antipredator response of nearby woodfrog tadpoles.

Woodfrog tadpoles often occur in temporary ponds and consequently encounter considerable spatial and temporal variation in food resources (Relyea, 2002). We hypothesize that these differences in food resources could alter the behaviour of tutor tadpoles and inadvertently change the social learning dynamics. It is well established that animals that have restricted food resources tend to ignore risk in order to feed or seek additional food patches (Chivers, Putfiltz, & Blaustein, 2000; Lima, 1998a; Lima & Dill, 1990). In such cases, tutors on restricted diets could ignore risk and continue swimming when presented with predator cues. This lack of a reduction in activity would make them less reliable as tutors. In sharp contrast, if food restriction increases searching, then these tutors may be more active and more noticeable to observers prior to exposure to predator cues. An abrupt change in their behaviour (i.e. a reduction in activity) could be more noticeable to nearby conspecifics, hence making them more efficient tutors (Ferrari & Chivers, 2008). Here, we conditioned naïve tadpoles to recognize the odour of a salamander and used them as tutors to test for social learning. We then manipulated the food level of the tutors prior to the social learning trial to test whether observers would learn different information from the tutors fed different diets. We found that food restriction dramatically increased social learning. Consequently, to further investigate the mechanisms of information transfer, we followed this up by explicitly testing whether food restriction increased the activity level of tadpoles.

**METHODS**

**Water and Test Species**

- One week prior to starting the experiment, we filled a 1900-litre tub with well water and seeded it with aquatic plants (sedges, slough grasses) and plankton from a local pond. This procedure ensured that the water that would be used in our experiments contained natural pond odours but lacked any cues from potential predators. This water was used for all experiments.

- We collected five newly laid clutches of woodfrog eggs (each containing several hundred eggs) from our test pond and placed them in a plastic pool filled with well water and left the pool to float on the pond. Floating the pool on the pond served to equalize temperature and sun exposure between the pond and the pool. The pool contained sedges and algae to provide the tadpoles with a food source when they hatched. After hatching, we supplemented the pools with alfalfa pellets and Tetramin fish flakes. The experiment started 3 weeks later using Gosner stage 25 tadpoles (Gosner, 1960). Our previous work had established that tadpoles from this pond do not show antipredator responses to salamander odour unless they are conditioned to recognize them (Chivers & Ferrari, in press; Ferrari & Chivers, 2009; Ferrari, Vrtelová, Brown, & Chivers, 2012).

**Predator Odours**

- We prepared the predator odours from six adult tiger salamanders (range 11.9–14.1 cm snout–vent length) that were fed a diet of earthworms for 2 weeks prior to odour collection. Odours were obtained by soaking individual salamanders in 1.5 litres of well water for 24 h and then freezing the water until needed. We randomly selected which salamander odour to use for each trial.

**Experiment 1: Does Food Restriction Influence the Efficacy of Tutor Tadpoles in Social Learning?**

Our experiment followed the basic methodology of Ferrari, Messier, and Chivers (2007) and Ferrari and Chivers (2008) to test for social learning. The experiment consisted of a three-step process. First, we conditioned groups of tutors to recognize salamander odour as a threat and had another group undergo a pseudoconditioning protocol. In the second phase, these naïve and experienced tutors were then paired with naïve observer tadpoles and exposed to predator odour. Observers paired with naïve tutors should not learn to recognize the predator as dangerous, whereas those paired with experienced tutors should learn that the salamander is dangerous. We manipulated the diet of the tutors prior to pairing them with tutors to determine whether this influenced the learned recognition of the tadpoles. The third step tested observer tadpoles for learned recognition of the salamander cues. Here, we exposed observer tadpoles from phase 2 to salamander odour or a control of pond water. Our experiment can be summarized as a 2 × 2 × 2 fully factorial design, where we had two types of tutors ( naïve and experienced) that were maintained under two food regimes (food restricted or not) and observers were later exposed to two types of cues (salamander odour or water).

**Phase 1: conditioning tutors to recognize salamanders**

- Tadpoles were conditioned (or pseudoconditioned) in groups to recognize the salamander as a predator. We removed tadpoles from the holding pool and placed 20 tadpoles into each of 10 plastic pails filled with 3 litres of well water and fed them with alfalfa pellets and Tetramin flakes. The following day, tadpoles in five of the pails were exposed to 30 ml of salamander odour paired with tadpole alarm cues (true conditioning). Alarm cues were prepared by crushing three tadpoles (15–20 mm total length) in a mortar and pestle and then adding 10 ml of pond water. The solution was filtered through cotton gauze prior to being used. We did not anaesthetize the tadpoles prior to crushing them because we did not want to contaminate the alarm cue solution with anaesthetics. However, the entire body of the tadpoles were completely crushed into a paste by the mortar and pestle in a split second and hence represent a humane method of euthanasia. In the other five pails, we added 30 ml of salamander odour paired with 10 ml of well water (pseudoconditioning). Tadpoles exposed to salamander odour paired with alarm cues should learn to recognize the salamander as a threat and hence act as experienced tutors. In contrast,
tadpoles exposed to water paired with salamander odour should not learn the salamander as a threat and should act as naïve tutors. After 2 h, the tadpoles were removed from the pails and placed in individual cups. All cups had a thin layer of biofilm and algae on the sides, providing the tadpoles with some food. Woodfrog tadpoles are coprophagous, and, consequently, should not be highly stressed by relatively short periods of food restriction. Half of the tutor cups were supplemented with alfalfa pellets and Tetramin flakes. Tadpoles remained under these food conditions for 36 h, at which time phase 2 began.

**Phase 2: conditioning observers to recognize salamander odour**

In this phase, we placed single naïve or experienced tutors from phase 1 into clean plastic cups (containing 500 ml of water) along with a naïve observer. After a 2 h acclimation period, we exposed each pair to 5 ml of salamander odour and left them for 2 h. Afterwards, the pair was separated and the observer was placed into a new cup containing fresh well water for testing (phase 3) the following day. We could always differentiate the observers because they were larger (mean ± SD total length of observers = 22.4 ± 2.1 mm) than the tutors (16.2 ± 1.4 mm). All tadpoles were at Gosner stage 25 (Gosner, 1960) at the time of testing. Time constraints precluded us from observing the behaviour of the pair during the conditioning phase.

**Phase 3: learned recognition of salamander odour**

In this phase, we exposed the observers from phase 2 to either 5 ml of salamander odour or 5 ml of well water and recorded their antipredator responses. We predicted that observers from phase 2 that were previously paired with experienced tutors would respond to the salamander odour, while those that were previously paired with a naïve tutor would not. If a tutor’s recent diet influences its response to predator odour, we expected that it might also influence the learned response of the observers. Tadpoles respond to known predators by reducing activity (Chivers & Mirza, 2001; Ferrari, Messier, & Chivers, 2008), and it is this reduction in activity that conspecific observers use to learn to recognize a predator. In a previous study, we found that the antipredator responses of observer tadpoles increased with the proportion of experienced tutors (Ferrari & Chivers, 2008), suggesting that this learned response may increase with the proportion of tutors observed ‘freezing’ in response to a predator. Whether or not a restricted diet in the tutors influences learning should depend on the relative magnitude of the reduction in activity of the tutors. Many prey animals on restricted diets tend to ignore risk in order to feed or seek additional food patches (Brown & Chivers, 2006; Lima, 1998a; Stankovich & Blumstein, 2005). If tutors on restricted diets ignore risk and continue to swim (presumably looking for food), they may not be able to act as reliable tutors. On the other hand, if food restriction increases searching, then these tutors may be more active and hence may be able to reduce their activity more than those that are not on a restricted diet. This could make them more efficient tutors.

We used a well-established testing protocol to quantify the responses of tadpoles to predator odours (Ferrari, Manek, & Chivers, 2010). Individual tadpoles were placed into 0.5-litre plastic cups and allowed to acclimatise for 1 h. We then observed the behaviour of each tadpole for 4 min prior to stimulus injection and 4 min after stimulus injection. The injection consisted of introducing 5 ml of salamander odour or well water into the cup using a syringe. We recorded the number of times the tadpole crossed the medial line of the cup during both observation periods. We considered that the tadpole crossed the line when the entire body of the tadpole crossed the line. A reduction in activity is a typical antipredator response displayed by most animals and is a standard bioassay for tadpole antipredator responses (Chivers & Mirza, 2001; Ferrari, Brown, Bortolotti, & Chivers, 2010). All tests were done blind with respect to the treatments. We conducted 17–24 replicates per treatment, with individual tadpoles being used only once.

**Statistical analysis**

The line-cross data were computed to obtain a proportion of change in activity from the pre-stimulus baseline ((post-stimulus – pre-stimulus)/pre-stimulus). The data were analysed in a three-way ANOVA, which tested for the main effects and interactions among three factors: tutor experience (naïve versus experienced), tutor diet (restricted versus ad libitum) and test cue (water versus predator odour). In addition, we added ‘pail’ as a random factor (making it a four-way ANOVA), to ensure that the conditioning pail from which the tutors were drawn did not influence the results.

**Experiment 2: Does Food Restriction Influence the Activity Level of Tadpoles?**

Given that we observed dramatic differences in the ability of tutors fed different diets to act as information sources in social learning, we conducted a follow-up experiment to examine the baseline activity of tadpoles under different feeding conditions. We used the same protocol as in experiment 1 to produce tadpoles fed different diets. Eighty tadpoles were placed individually into 0.5-litre plastic cups that contained a thin layer of biofilm and algae on the sides, providing the tadpoles with a restricted diet. Half of the cups were then supplemented with alfalfa pellets and Tetramin flakes. After 36 h, all tadpoles were transferred to clean cups, and 1 h later we began to record tadpole activity. We scanned each cup at 2 min intervals for 20 min. We then repeated the scanning procedure 2 h later. Given that tutors on a restricted diet appear to facilitate much stronger learning in observers, we predicted that the tutors fed a restricted diet would be more active than those fed an ad libitum diet and thus might also show a greater reduction in activity.

**Statistical analysis**

We converted the binomial outcome of each activity scan (moving or not moving) into a proportion of scans where the tadpole was moving (number of scans where the tadpole was moving/total number of scanning events (10)). These data were analysed using a two-way repeated measures ANOVA, where tutor diet (restricted versus ad libitum), trial (trial 1 versus trial 2) and their possible interaction were investigated.

**RESULTS**

**Experiment 1**

The four-way ANOVA revealed a significant interaction between tutor experience and tutor diet ($F_{1,401} = 9.8, P = 0.035$) and between tutor experience and cue ($F_{1,401} = 39.4, P = 0.003$; Fig. 1). In addition, none of the factors containing ‘pail’ significantly affected the behavioural response of the tadpoles (pail: $P = 0.9$; pail × experience: $P = 0.9$; pail × diet: $P = 0.7$; pail × cue: $P = 0.4$; pail × experience × diet: $P = 0.7$; pail × experience × cue: $P = 0.7$; pail × diet × cue: $P = 0.3$; pail × experience × diet × cue: $P = 0.5$), thus ‘pail’ was removed from further analysis.

To investigate the interactions, we examined the responses of observers to each cue separately. Observers exposed to water were not affected by tutor experience ($F_{1,85} = 0.6, P = 0.4$), tutor diet ($F_{1,85} = 0.1, P = 0.8$) or the interaction between the two factors ($F_{1,85} = 1.1, P = 0.3$).
When observers were exposed to the predator odour, however, their response was affected by tutor experience ($F_{1,77} = 26.0$, $P < 0.001$) and, to a lesser extent, by the interaction between tutor experience and tutor diet ($F_{1,77} = 3.2$, $P = 0.07$). This pattern emerged because observers that learned from naïve tutors did not respond to predator odour, regardless of their tutors’ diet ($F_{1,42} = 0.1$, $P = 0.9$). In contrast, tadpoles that learned from experienced tutors responded to the predator odour with antipredator behaviour, but the intensity of their response differed with tutor diet: observers learning from tutors on a restricted diet showed a significantly higher intensity of response than observers learning from satiated tutors ($F_{1,35} = 9.4$, $P = 0.004$).

Experiment 2

The two-way RM ANOVA revealed a significant effect of tutor diet ($F_{1,77} = 41.4$, $P < 0.001$), but no effect of trial ($F_{1,77} = 0.2$, $P = 0.6$) and no hunger level × trial interaction ($F_{1,77} = 0.7$, $P = 0.4$) on tadpole activity (Fig. 2). Here, tadpoles on a restricted diet showed increased activity compared with those fed ad libitum.

**DISCUSSION**

Our study demonstrates that tadpoles can learn the identity of an unknown predator by observing the antipredator response of conspecifics responding to the predator. We found that social learning occurred through a single conditioning trial, consistent with our previous work with tadpoles (Ferrari & Chivers, 2008; Ferrari, Messier, & Chivers, 2007). More interestingly, we found that observers showed differential learning of the predator depending on tutor diet. Observers that learned from tutors that were food restricted showed much stronger learned responses. What could explain this difference? Did the tutors fed different diets do something different to ’teach’ the observers something about how strong of a response to display to the predator? This seems very unlikely as there is little evidence that social learning of risk involves anything other than observers paying attention to the intensity of the antipredator response of nearby individuals (Crane & Ferrari, 2013). Prey learn that a predator is high risk if they observe strong responses from tutors, and they learn that a predator is low risk if they observe tutors showing weak responses.

Behavioural ecology theory predicts that individuals on a restricted diet often ignore risk in order to eat and/or search for additional food patches (Lima, 1998b). If food-restricted tutors ignore risk, then they should act as less efficient tutors. However, this is exactly the opposite of what we observed. Observers learned to respond appropriately to the predator when their tutors were food restricted. This means that food-restricted tutors must have been showing stronger antipredator responses. However, we must remember that a stronger response for a tadpole is simply a greater reduction in activity. If tadpoles under restricted diets spend more time actively searching for food, then a reduction in their activity would be much more noticeable to nearby conspecifics. Time constraints precluded us from observing the activity of the tadpoles during the conditioning trials; consequently, we conducted a

![Figure 1](image1.png)

**Figure 1.** Mean (±SE) proportion of change in activity for tadpoles exposed to salamander odour (black bars) or well water (white bars). Tadpoles were previously paired with either naïve or experienced tutors that were fed ad libitum or had a restricted diet.

![Figure 2](image2.png)

**Figure 2.** Mean (±SE) proportion of time that tadpoles on different diets spent moving.
second experiment where we tested whether there were any differences in baseline activity of tadpoles fed different diets. Tadpoles on a restricted diet were indeed much more active and hence could easily have shown a greater reduction in activity, explaining why food restriction resulted in more efficient tutors.

Prey animals, such as tadpoles, that learn to recognize predators often remember high-risk predators for longer than they do low-risk predators (Chivers & Ferrari, in press; Ferrari, Brown, et al., 2010). In our system this would mean that the memory of salamander odour would be greater for tadpoles that learned from tutors on restricted diets. Longer retention of the predator’s identity under conditions of food restriction is likely adaptive as food resources are a good predictor of growth rate, and lower growth rates translate into higher vulnerability for tadpoles.

Our results add to a rapidly developing body of work that identifies the factors that lead to differential transmission of information in a social learning context. Our work is particularly interesting because it suggests that social learning is indirectly linked to resource level, implying that social learning dynamics probably change considerably from place to place and year to year as resources change through space and time.

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