Cultural learning of predator recognition in mixed-species assemblages of frogs: the effect of tutor-to-observer ratio

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Animals that live in social groups have the opportunity to acquire information about foraging opportunities, mates and predators. Traditionally, social learning has been studied in birds and mammals, but few tests have been conducted on less social taxa such as amphibians. Moreover, few studies have considered cross-species learning among members of mixed-species assemblages. We examined social learning in tadpoles of the boreal chorus frog, *Pseudacris maculata*, and found that they do not display a fright response to the odour of predacious tiger salamanders, *Ambystoma tigrinum*, without prior experience with salamanders, but they can learn to recognize the salamanders when they are paired with predator-experienced woodfrog, *Rana sylvatica*, tadpoles. Moreover, the efficacy of learning is enhanced when the ratio of tutors-to-observers increases. Social learning has far-reaching implications for survival of individuals in mixed-species assemblages.

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Many animals living in groups have the ability to learn novel information or behaviours by observing nearby conspecifics. For instance, naïve birds can learn to locate rewarding foraging areas by watching experienced conspecifics (Ward & Zahavi 1973; reviewed by Galef & Giraldeau 2001). Social learning has also been shown in the context of reproduction. For example, young female guppies, *Poecilia reticulata*, choose mates according to the choice of older, more experienced conspecific females (Dugatkin & Godin 1992). Social learning of predator recognition has been shown in a variety of taxa. Much of the early work in this field concentrated on birds (Curio et al. 1978; Vieth et al. 1980; Curio 1988). For example, zebra finches, *Taeniopygia guttata*, and European blackbirds, *Turdus merula*, learn to mob a novel bird by observing the mobbing response of conspecifics. The conditioned response towards the novel bird is transmitted along a chain of at least six individuals (Curio et al. 1978). Likewise, Herzog & Hopf (1984) showed that juvenile squirrel monkeys, *Saimiri sciureus*, learn to avoid stuffed toy animals (snakes or tigers) when alarm calls are given in association with the stuffed toy. Griffin & Evans (2003) also found that naïve tammar wallabies, *Macropus eugenii*, could learn to recognize a model fox as a threat by observing a conspecific displaying a fearful response to the model fox. The same phenomenon has been shown in fishes (Mathis et al. 1996; Ferrari et al. 2005).

Social learning in a variety of contexts appears to be widespread among mammals, birds and fishes (Griffin 2004). However, the degree to which social learning influences behaviour in other taxa appears somewhat limited (Griffin 2004); however, this limitation may be due to a lack of research on those taxa. For example, only one study has considered social learning of predator recognition by larval amphibians (Ferrari et al. 2007a). Tadpoles are known to aggregate. Aggregations, however, have been more generally considered in the context of foraging, where moving aggregations stir up the bottom, thereby suspending particles of food (Duellman & Trueb 1994). Despite the apparent lack of complex social organization, Ferrari et al. (2007a) showed that larval woodfrogs, *Rana sylvatica*, could learn to recognize the odour of a novel predatory salamander (tiger salamander, *Ambystoma tigrinum*) when paired with an experienced conspecific. This...
work raised the question of the importance of social learning in amphibians and other taxa that are traditionally considered less social than most birds and mammals.

Several studies have examined factors affecting the transmission of social information regarding predation risk. For example, Vilhunen et al. (2005) tested the effect of tutor-to-observer ratio on the transmission of recognition of pikeperch, Sander lucioperca, by Arctic charr, Salvelinus alpinus. With a greater ratio of tutors to observers, one could predict an increase in the transmission of information due to the increase in opportunities for the observers to learn. Contrary to predictions, Vilhunen et al. found that an increase in the number of tutors reduced the ability of naive charr to learn the novel predator. They argued that groups with a large number of knowledgeable individuals reduced the intensity of response of the tutors, due to the dilution effect. The results of Ferrari et al. (2005) suggest reduced the intensity of response of the tutors, due to the groups with a large number of knowledgeable individuals to learn. Contrary to predictions, Vilhunen et al. found an increase in the intensity of the learned response of the observer responding to the learned cue.

Behavioural ecologists typically consider social learning as transmitting information from knowledgeable tutors to naive conspecifics. However, animals may also learn by observing the behaviour of other species. Only two studies have considered this in the context of social learning of predator recognition. Vieth et al. (1980) showed that European blackbirds could learn to recognize a stimulus as dangerous when exposure to the stimulus was paired with a taped heterospecific chorus of mobbing calls from chaffinches, Fringilla coelebs, great tits, Parus major, and nuthatches, Sitta europaea. Mathis et al. (1996) documented that brook stickleback, Culaea inconstans, could learn to recognize the odour of an unknown predatory pike, Esox lucius, by observing the response of pike-experienced fathead minnows. Cross-species cultural learning deserves more attention, both in the context of learned recognition of risk and learned recognition of food.

The purpose of our experiment was two-fold: (1) to test whether cross-species cultural transmission of predator recognition occurs in larval amphibians (i.e. whether chorus frog, Pseudacris maculata, tadpoles could learn to recognize novel tiger salamanders from woodfrog tadpoles) and (2) to test whether the tutor-to-observer ratio affects learning efficacy. We conducted the experiment in three phases: (1) we obtained salamander-naive and salamander-experienced woodfrog tutors, (2) we paired experienced and naive tutors with naive chorus frog observers and exposed them to salamander odour and (3) we subsequently tested the chorus frog observers for a response to salamander odour or a water control. To test for the effect of tutor-to-observer ratio, we paired either two woodfrog tutors with five chorus frog observers, or alternatively five woodfrog tutors with two chorus frog observers. The ability of prey to learn to recognize novel predators has far-reaching implications for individual survival.

**METHODS**

**Water, Predators and Test Subjects**

Five weeks before the experiment, we filled a 1900-litre tub with well water and left it outdoors. The tub was enriched with aquatic plants (sedges: Carex spp, slough grass, horsetail: Equisetum spp.), zooplankton and phytoplankton from a local pond using a fine-mesh dip net. Our use of a fine-mesh dip net ensured that the water (hereafter well water) contained a full array of algae and plankton but no salamander cues.

We caught two tiger salamanders (snout–vent length: 18 cm) from a pond on the University of Saskatchewan campus, Saskatoon, in April 2007, using Gee’s Improved minnow traps (cylindrical wire cages, 43 cm long, 22 cm diameter with inverted cone entrances at each end; Tackle Factory, Fillmore, NY, U.S.A.). We kept the salamanders in a 30-litre plastic tub filled with 15 litres of well water and fed them earthworms.

We collected four woodfrog egg clutches and 20 boreal chorus frog egg clutches, all of which were laid within a 24 h period, on 27 April 2007 from the same pond in central Alberta. Our field research for the past 4 years has shown that no salamanders were present in this pond, even though they are present in this region of Alberta. Moreover, Ferrari et al. (2007a) found that woodfrog tadpoles from this population do not display fright responses to salamander cues without prior experience with them. No studies have considered whether chorus frogs similarly show no antipredator responses to salamander predators in the absence of experience. We held the tadpoles of the two species separately in two pools (60 cm diameter) containing pond water and aquatic plants until 2 weeks posthatching. The pools were positioned on the pond to equalize the temperature of the pool water with the pond water. After hatching, each tadpole received about two pellets of rabbit food (Essex Top Crop Sales Ltd, ON, Canada) per day to supplement the algae already present in the pools.

**Experimental Protocol**

**Training of woodfrog tutors**

Following the methodology of Ferrari et al. (2007a), we placed two groups of approximately 400 woodfrog tadpoles into two tubs (56 × 42 cm) containing 47 litres of well water and rabbit chow. We added a tiger salamander to one of the tubs and left the two tubs undisturbed for 3 days. We did not quantify the predation rate on tadpoles in the tub. However, we observed the salamander feeding on tadpoles numerous times and the tadpoles avoiding the area of the tub containing the salamander. After 72 h, we removed the salamander and replaced the water in both tubs with fresh well water. Tadpoles from the tub containing the salamander were considered salamander-experienced tutors and tadpoles from the tub without the salamander were considered salamander-naive tutors.
Conditioning of chorus frog observers  
We placed groups of either two or five salamander-naive chorus frog observers in 3.7-litre plastic pails containing 3 litres of well water and added either experienced or naive woodfrog tutors to obtain a total of seven tadpoles per pail. Owing to the size difference between the tadpoles of the two species, we could always identify the woodfrog tadpoles from the chorus frog tadpoles (mean ± SD total length of woodfrog tutors: 1.60 ± 0.07 cm; total length of chorus frogs: 1.37 ± 0.01 cm). We let the tadpoles acclimate for 5 h. We then injected 20 ml of salamander odour in each pail and performed a 100% water change 2 h after the injection of the stimulus in the pails. The salamander odour was obtained by placing a salamander in a plastic tub (56 × 42 cm) containing 15 litres of well water for 3 days. The soaking tub water was used as salamander odour. The salamander used for odour collection was maintained on an earthworm diet and was never fed tadpoles. This ensured that the response of the tadpoles to salamander odour was not confounded by cues emanating from the predator’s diet (reviewed by Chivers & Mirza 2001a).

Testing of chorus frog observers  
The following day, we placed two chorus frog tadpoles from each bucket in individual 0.5-litre plastic cups filled with well water and left them to acclimate for 1 h. We then exposed one of the two chorus frog observers to 5 ml of salamander odour while exposing the other tadpole to 5 ml of well water. We recorded their behavioural responses using the methodology described below.

Behavioural Assay  
Numerous studies have established that frog tadpoles decrease activity when exposed to predation risk (e.g. Hokit & Blaustein 1995; Kiesecker & Blaustein 1997; Chivers & Mirza 2001b). To quantify activity, a diameter line was drawn on the bottom of our testing cups and the number of line crosses was counted. A line cross occurred when the entire body of the tadpole crossed over the line. Our testing protocol consisted of quantifying line crosses for 4 min before and 4 min after the injection of the stimulus (5 ml of either salamander odour or well water) in the cup. The stimulus was injected gently on the side of the cup to minimize disturbance. We recorded the antipredator behaviour of 126 tadpoles in a 2 × 2 × 2 design testing the effect of tutor experience (naive versus experienced), tutor-to-observer ratio (2 to 5 versus 5 to 2) and cue (water versus salamander odour).

At the end of testing all animals were released at their original capture sites.

Statistical Analysis  
We analysed the change in number of line crosses from the prestimulus baseline using parametric tests, as the data was normally distributed and homoscedastic. The effects of tutor experience, tutor number and cue were analysed using a mixed-model ANOVA (which included ‘pail’ as a random factor), followed by post hoc tests. The alpha was set to 0.012 using a Bonferroni correction factor for type I errors.

RESULTS  
The results of the ANOVA are presented in Table 1. The three-way interaction prevented us from concluding on the main effects of the factors, but subsequent post hoc comparisons revealed that, contrary to the responses to water, the responses of chorus frog tadpoles to salamander odour were determined by the experience of the tutors and also the number of tutors involved. Chorus frog tadpoles that were paired with experienced tutors responded to salamander odour with a stronger intensity than did tadpoles that were paired with naive tutors (two naive versus two experienced: \( P < 0.001 \); five naive versus five experienced: \( P < 0.001 \)). Although the number of tutors did not affect the intensity of the tadpoles’ response to salamander odour when tutors were naive (\( P = 0.994 \)), it did affect the intensity of responses of tadpoles that were paired with experienced tutors. Indeed, tadpoles that were paired with five experienced tutors showed a greater response intensity to salamander odour than did tadpoles that were paired with only two experienced tutors (\( P = 0.003 \); Fig. 1).

DISCUSSION  
Our results provide clear evidence that salamander-naive chorus frog tadpoles do not show antipredator behaviour to salamander odour without prior experience, but they can learn to recognize the cues as a threat when paired with salamander-experienced woodfrog tadpoles. These results raise the possibility that cultural learning in amphibians is indeed widespread. This is only the second species of amphibians for which cultural learning of predator recognition has been considered (see also Ferrari et al. 2007a). Further work should test for social learning of predators in this and other taxa that have traditionally been considered much less social. Even more interesting, researchers should examine cross-species cultural learning. Our experiment clearly shows that chorus frogs that naturally co-occur in the same pond and that share similar

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predators with woodfrogs can learn to recognize predators through social learning from each other. This is the first documentation of this phenomenon in amphibians. Many groups of animals show mixed-species aggregations (e.g. mammals, birds, amphibians, fishes). Information transfer regarding predation risk may be a prime factor leading to the evolution of multispecies assemblages.

In our experiment, we documented that chorus frog tadpoles’ response intensity was higher when tadpoles were paired with five experienced tutors than when they were paired with only two experienced tutors, suggesting that naive observers’ opportunities to learn increased with the number of experienced tutors, resulting in better information transmission. Alternatively, individual tadpoles might use an averaging process to adjust the intensity of their response to a given threat. For example, in our study, each observer was faced either with one nonresponding conspecific and five responding heterospecifics, or with four nonresponding conspecifics and two responding heterospecifics. Whether responses of conspecific chorus frogs and heterospecific woodfrogs are equally reliable from the perspective of a naive chorus frog is unknown, but such reliability could be adaptive because these species share the same predators (salamanders, diving beetles, larval dragonflies, etc.).

An unexplored aspect of social learning in amphibians is the exact mode of transmission of the information. In fishes, for example, it has been established that the transmission of the information could be purely visual because observers can learn from watching tutors in an adjacent tank (Ferrari et al. 2005). Likewise, cultural learning of predator recognition by birds is based on the sight of conspecifics mobbing an unknown predator. In larval amphibians, however, visual or mechanical transmissions are both probable mechanisms for learning. If the transmission is based on mechanical stimuli, then we would predict that the higher the number of tutors, the better the rate of transmission. It is important to realize that tadpoles’ antipredator response is to reduce activity. Consequently, the decrease in mechanical disturbance that is associated with the antipredator response of the tutors will be linked to the ratio of active versus nonactive tadpoles. Moreover, in our case, woodfrog tadpoles were larger than the chorus frog tadpoles, and it is likely that larger individuals create more disturbances, and thus induced a higher decrease in disturbance following the injection of the cues.

Social learning is categorized into several types of cognitive mechanisms, ranging from stimulus enhancement (increase in attention of an individual for an object because another individual pays attention to this object) to imitation (an individual imitates the exact behaviour of another individual to achieve a desired goal). The social learning process occurring in this experiment is probably observational conditioning. Observational conditioning, often associated with social learning of predator recognition, is a form of Pavlovian conditioning in which the response of the demonstrator acts as an unconditioned stimulus that elicits a matching response on the part of the observer (Emery & Clayton 2005). However, more testing is needed to ascertain this hypothesis.

Predation is an important selective force, acting on the morphology, behaviour and life history of prey species (Lima & Dill 1990; Chivers et al., in press). Prey often face a trade-off between predator avoidance and fitness-related activities such as foraging, territorial defence and reproduction. An obvious prerequisite for prey to respond appropriately to predators is to be able to recognize their predators as a threat (Mathis et al. 1996; Ferrari et al. 2007b). Social learning provides an effective mechanism to acquire such recognition. Our research opens the possibility that social learning is widespread in taxa that have been traditionally considered less social and also that prey guild members in mixed-species assemblages can learn from each other.

Studying learned predator recognition is of particular importance in amphibian species. One of the reasons put forwards for the global decline of amphibian populations is their inability to cope with introduced competitive or predatory species of fish and amphibians (Gamradt & Kats 1996; Blaustein & Kiesecker 2002; Blaustein & Bancroft 2007). More work is needed to investigate whether amphibians have similar predator-learning abilities as other vertebrates. In particular, researchers should focus on the limitations (both spatial and temporal) of learned predator recognition in amphibians, which could partly explain why some species seem to be particularly vulnerable following the introduction of new predators.

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