Generalization of predators and nonpredators by juvenile rainbow trout: learning what is and is not a threat

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Learned recognition of novel predators allows prey to respond to ecologically relevant threats. Prey could minimize the costs associated with learning the identity of both predators and nonpredators by making educated guesses on the identity of a novel species based on their similarities with known predators and nonpredators, a process known as generalization. Here, we tested whether juvenile rainbow trout, *Oncorhynchus mykiss*, have the ability to generalize information from a known predator (experiment 1) or a known harmless species (experiment 2) to closely related but novel species. In experiment 1, we taught juvenile trout to recognize a predatory pumpkinseed sunfish, *Lepomis gibbosus*, by pairing pumpkinseed odour with conspecific alarm cues or a distilled water control. We then tested the trout for a response to pumpkinseeds and to novel longear sunfish, *Lepomis megalotis* (same genus as pumpkinseed), rock bass, *Ambloplites rupestris* (same family as pumpkinseed) or yellow perch, *Perca flavescens* (different family). Trout showed strong learned recognition of pumpkinseed and longear sunfish odour and a weak learned response to rock bass odour but no recognition of yellow perch. In experiment 2, we used latent inhibition to teach juvenile trout that pumpkinseeds were harmless. During subsequent predator learning trials, trout did not learn to recognize pumpkinseed or longear sunfish odour as potential threats, but they did learn that rock bass and yellow perch were threatening. Taken together, these results demonstrate that juvenile rainbow trout can generalize learned recognition of both predator and nonpredator odours based on the phylogenetic relatedness of predators.

**Keywords:**
acquired predator recognition, alarm cue, generalization, latent inhibition, rainbow trout, threat-sensitive trade-off
To balance the conflicting demands of predator avoidance and other fitness-related activities such as foraging, territorial defence and mating, prey organisms require accurate information regarding local threats (Helfman 1989; Lima & Dill 1990). However, uncertainty in quality and/or reliability of information may limit the ability of prey to make appropriate behavioural decisions (Sih 1992; Lima 1998). Thus, in response to this uncertainty, individuals capable of acquiring information about their environment should be better able to deal with variable risks (Dall et al. 2005). Within the context of threat-sensitive predator avoidance, the use of personal (direct interactions) and/or social information (Dall et al. 2005) could allow prey to minimize the likelihood of inappropriate responses.

Learning to recognize realistic threats would increase the probability of survival during encounters with potential predators (Mirza & Chivers 2000; Darwish et al. 2005). One highly effective means to recognize predators is through Pavlovian conditioning, whereby prey are exposed to the cues of a novel predator combined with cues of injured conspecifics (Brown 2003; Brown & Chivers 2005). Such learning has been shown in a diverse range of prey, including both invertebrate and vertebrate aquatic species (reviewed in Ferrari et al. 2010). Analogous learning systems have been demonstrated in birds (Curio et al. 1978) and mammals (McLean et al. 1996; Griffin et al. 2001). However, such learning is costly, because prey must survive the initial conditioning event (i.e. encounter with the predator; Ferrari et al. 2007). In addition, selection favouring learning abilities may represent a fitness cost due to increased physiological costs associated with increased energetic investments into neural and/or sensory structures.
underlying learning and memory (Mery & Kawecki 2003, 2005). The ability to
generalize learned information, such as predator cues, is argued to reduce these costs
(Griffin et al. 2001; Ferrari et al. 2007). Phylogenetically related predators may share
predatory tactics and/or preferred diets (Olson et al. 1995; Ferrari et al. 2007; Barco et al.
2010). For example, several sympatric species of centrarchids show a high degree of
similarity in ontogeny, foraging tactics and diet preferences (Scott & Crossman 1973;
Brown 1984). As such, prey at risk of predation from one species may also be at risk of
predation from closely related species. Thus, generalizing recognition of potential
predators may allow prey to increase their likelihood of survival without compromising

Learning to recognize nonpredators should allow prey to avoid wasting time and
energy responding to irrelevant cues (Brown & Chivers 2005; Ferrari et al. 2007;
Stankowich & Coss 2007). Latent inhibition is a form of associative learning in which
novel stimuli are repeatedly encountered in the absence of either a positive or a negative
outcome (Allen et al. 2002; Rankin et al. 2009). As a result of this repeated exposure,
individuals may be inhibited from forming ecologically relevant associations (Drezner-
Levy et al. 2009), and this inhibition has been shown to shape learned foraging
preferences (Chandra et al. 2010) and mate choice (Akins 1994). In the case of
chemically mediated learning to recognize predators, pre-exposure to predator odour in
the absence of a reinforcing risk stimulus is known to reduce the likelihood that prey will
acquire recognition of this species as predatory. Such inhibition has been demonstrated in
several taxa, including virile crayfish, *Orconectes virilis* (Acquistapace et al. 2003),
fathead minnows, *Pimephales promelas* (Ferrari & Chivers 2006) and woodfrogs, *Rana sylvatica* (Ferrari & Chivers 2009). For example, when exposed to a novel predator odour for 2 h/day for 3 days, crayfish were unable to learn to associate the predator cue with a real predation threat. Likewise, fathead minnows were unable to learn to recognize brook charr, *Salvelinus fontinalis*, as a predation threat when the minnows had been pre-exposed to charr odour.

Whether prey can further refine their predator recognition learning by generalizing inhibited information, however, remains unknown. Generalizing learned information is argued to be beneficial, as it would minimize the costs associated with having to learn specific cues of similar predators. We can also argue that generalizing inhibited information would be beneficial. Presumably, if prey can learn that a particular heterospecific is not a predation threat, as a result of repeated exposure with no reinforcement, they would benefit by generalizing this inhibited information to phylogenetically related species.

However, the question of whether generalization of non-predator recognition should be as widespread as generalization of predator recognition requires us to do a quick cost–benefit analysis of these responses. When encountering a novel species, the individual must decide whether it poses a threat. When the novel species is reminiscent of a known predator, the prey may decide to deal with their uncertainty by generalizing its knowledge and treating this novel species as a threat. If the prey is correct, it survives. If the novel species was not a predator, the prey wasted time and energy that could have
otherwise been allocated to foraging or courting. Thus, generalizing predator recognition may allow prey to survive their first encounter with a novel predator at best, or it may result in missed opportunities at worse. Similarly, when the novel species is reminiscent of a non-predator, the prey may decide to generalize their responses from known non-predators. In this case, if the novel species was indeed harmless, the prey made the right decision. If the novel species was not harmless, the prey not only failed to respond to threat, but it labelled it as a nonthreatening cue, which will prevent the prey from learning to recognize this species as a predator during subsequent encounters. This is a direct consequence of the proximate mechanism behind the labelling of non-risky species: latent inhibition. When an individual encounters a stimulus for the first time, the stimulus is neither negative nor positive, but rather neutral, at least in the absence of any sensory bias. Learned predator recognition allows prey to label unknown, neutral stimuli as threatening. Conversely, latent inhibition allows prey to label unknown, neutral stimuli as harmless. However, once a stimulus is labelled as harmless, subsequent associations with a known threat (unconditioned stimulus) will reduce or prevent learning (Acquistapace et al. 2003; Ferrari & Chivers 2006). Hence, generalizing non-predator recognition will quicken the labelling of a novel species as harmless at best, or it may delay the recognition of a predator at worse, which may result in an increase in predation risk to prey. Because of the asymmetry between the potential costs of errors between generalization of predators and non-predators, we predict that generalization of predators may be more widespread than generalization of non-predators.
The purpose of the current study was two-fold. First, we tested the hypothesis that juvenile rainbow trout, *Oncorhynchus mykiss*, can generalize acquired predator recognition from one predator species to the cues of phylogenetically related predators. We conditioned trout to recognize the odour of a pumpkinseed sunfish, *Lepomis gibbosus*, and then tested them for their recognition of pumpkinseed or the odour of longear sunfish, *Lepomis megalotis*, rock bass, *Ambloplites rupestris*, and yellow perch, *Perca flavescens*. All four predator species are perciform fishes. Pumpkinseed, longear sunfish and rock bass belong to the family Centrarchidae, with pumpkinseed and longear sunfish (same genus as pumpkinseed, thus congeneric) representing the most phylogenetically similar predators, and with the rock bass a more distantly related centrarchid (i.e. confamilial). The yellow perch represented the most distant predator, belonging to the family Percidae. If rainbow trout are capable of generalizing learned predator recognition, we predicted the strongest learned response to pumpkinseed and the congeneric longear sunfish and the weakest (or absent) response to the more distantly related yellow perch.

The second goal of this study was to test the hypothesis that juvenile rainbow trout can also generalize information about nonpredators. We pre-exposed juvenile trout to pumpkinseed odour (latent inhibition) or a distilled water control and conditioned them to recognize each of the four predator odours. If pre-exposure to pumpkinseed odour resulted in latent inhibition, we predicted a lack of a learned response to pumpkinseed odour. If this inhibition was generalized, we predicted that there would also be an
absence of a learned response to the phylogenetically related predators, but not towards the more distantly related predators.

METHODS

Test Fish and Stimulus Collection

Juvenile rainbow trout were obtained from a commercial supplier (Pisciculture des Arpents Verts, Ste Edwidge-de-Clifton, Quebec, Canada) and transported to the laboratory. Given that they were hatchery-reared trout, they would have had no prior experience with predators and thus were considered naïve. Rainbow trout do, however, commonly co-occur with each of the predators used (Scott & Crossman 1973). Prior to testing, trout were housed in 390-litre recirculating holding tanks (~18 °C, pH ~7.0) under a 14:10 h light:dark cycle and fed ad libitum daily with commercial trout chow (Corey Mills, Fredericton, NB, Canada).

Predator odour donors (adult pumpkinseed, longear sunfish, rock bass and yellow perch), two of each species, were collected from Canal Lachine, Montreal, Quebec. We chose these predators since they are sympatric and share similar life histories. Predators were held in similar tanks (separate holding tanks for each species) as described for trout and fed a diet of brine shrimp and commercial cichlid pellets daily. All predators (12.5–16.1 cm standard length) were held for at least 14 days prior to use as stimulus donors to ensure that any prey fish in the diet had been evacuated.
Trout alarm cues were generated using standard protocols (Brown & Smith 1997; Brown et al. 2006; Kim et al. 2011). We generated trout alarm cues from 23 juvenile trout (mean ± SD fork length = 5.01 ± 0.52 cm). Donor trout were killed via cervical dislocation (in accordance with Concordia University Animal Research Ethics Committee protocol number AREC-2008-BROW) and skin fillets were removed from either side of the donors and immediately placed into 100 ml of chilled distilled water. Skin fillets were then homogenized using a hand-held tissue homogenizer, filtered through polyester filter floss, and diluted to the desired volume with the addition of distilled water. We collected a total of 155.8 cm² of skin (in a final volume of 1558 ml of distilled water). This concentration of skin extract is known to elicit an antipredator response in juvenile trout under laboratory conditions (Brown & Smith 1998; Smith et al. 2008). Chemical alarm cues were frozen in 20 ml aliquots at -20 °C until needed. As a control, we also froze 20 ml samples of distilled water.

To collect predator odours from each of the four species, we placed individual predators (two of each species) into 37-litre glass aquaria (filled with 15 litres of dechlorinated tap water) for 3 days. The donor tanks contained a gravel substrate (~18 °C, pH ~7.0) and were aerated, but not filtered. Donors were not fed during the stimulus collection period. Following this period, we returned the predators to their holding tanks. The tank water (predator cue) for the two donors of each species was combined to generate a single cue per predator type. Predator odours were filtered and frozen in 50 ml aliquots at -20 °C until needed (Leduc et al. 2004; Smith et al. 2008). The predator
odours from each donor were combined to ensure a consistent cue concentration for each
predator species (Ferrari et al. 2007, 2009).

Experiment 1: Generalization of Predator Recognition

Conditioning phase

Juvenile trout were transferred to individual test tanks (one trout per test tank) and
allowed a 24 h acclimation period prior to testing. Test tanks consisted of a series of 10
37-litre glass aquaria (18 °C, pH ~ 7.0), filled with 32 litres of dechlorinated tap water,
equipped with a gravel substrate and a single airstone fixed to the back wall of the tank.
In addition, we attached a 2 m length of airline to the back wall that terminated
immediately above the airstone to allow for the introduction of experimental cues without
disturbing test fish. Immediately prior to conditioning, we withdrew and discarded 60 ml
of tank water through the stimulus tube to remove any stagnant water. We then withdrew
and retained an additional 60 ml of water. We injected 10 ml of pumpkinseed odour,
immediately followed by either 10 ml of trout alarm cue (conditioned trout) or 10 ml of
distilled water (pseudoconditioned trout). Trout remained in the conditioning tank for
approximately 60 min and were then transferred to an identical test tank for recognition
testing. Previous studies have used similar protocols to demonstrate chemically mediated
learning in a variety of prey fishes (Brown & Smith 1998; Brown 2003).

Recognition trials
We conducted recognition trials 24 h after the conditioning phase. Trials consisted of a 5 min prestimulus and a 5 min poststimulus introduction period. Prior to the prestimulus observation, we withdrew 60 ml of tank water, as described above. Immediately following the prestimulus observation period, we introduced 10 ml of one of five stimuli. We exposed trout to odour of pumpkinseed (the reference predator), longear sunfish (congeneric predator), rock bass (confamilial predator), yellow perch (unrelated predator) or an additional control of distilled water. We fed test fish approximately 30 min prior to testing. Sufficient food was added to ensure that there were food particles remaining during the behavioural observations, allowing us to quantify foraging without presenting additional food (Vavrek & Brown 2009). A feeding attempt was defined as a pecking movement towards a food particle, either on the substrate or in the water column. A reduction in both time moving and foraging attempts is indicative of an acute antipredator response in juvenile rainbow trout (Smith et al. 2008). We conducted a total of 12 replicates per treatment combination ($N = 120$). Mean ± SD standard length of test fish was 3.43 ± 0.31 cm at time of testing. In all cases, the observer was blind to the treatments (previous conditioning and recognition test stimuli) and the order of treatments was randomized.

Statistical analysis

We calculated the change in foraging attempts and time spent moving (poststimulus – prestimulus values) and used these difference scores as dependent variables in our analyses. We tested the effects of initial conditioning stimulus (alarm cue versus distilled water) and predator cues (and the additional distilled water control) on the
change in foraging attempts and time spent moving using univariate GLM ANOVAs (PASW Statistics, Version 18, 2009; IBM, Armonk, NY, U.S.A.). Post hoc comparisons between conditioned versus pseudoconditioned trout exposed to the same predator cue during recognition trials were made using independent samples t tests. We employed a Bonferroni correction to account for increasing type I error rates. The data met the requirements (normality and homeoscedasticity) for parametric analysis.

Experiment 2: Generalization of Nonpredator Recognition

Pre-exposure and conditioning phase

Juvenile trout were placed individually into a series of 20 15-litre plastic containers. Each container was equipped with a single airstone and a gravel substrate. We repeated the pre-exposure procedures in nine blocks to generate sufficient replicates for all treatment combinations (see below). The containers were positioned along a pair of flow-through stream channels (1.92 x 0.76 m, water depth ~20 cm) to ensure stable temperature. Individual containers were visually and chemically isolated from each other. We introduced 10 ml of either pumpkinseed odour or distilled water, twice daily for 3 consecutive days (at ~ 0900 and ~ 1400 hours). Approximately 30 min following the introduction of the cue into the container, we conducted a 100% water change with tank water from a separate system to ensure that no chemical cues remained and there was no incidental exposure of the control containers with pumpkinseed odour. As a result, trout were pre-exposed to pumpkinseed or distilled water for a maximum of 60 min/day. Containers receiving pumpkinseed odour and distilled water preconditioning were
randomly distributed across both stream channels. Three hours following the final exposure, trout were transferred to individual test tanks, as described above, and allowed 24 h acclimation prior to conditioning.

The conditioning phase was conducted as described above for experiment 1, with the exception that individual trout were conditioned (or pseudoconditioned) to recognize one of four predator odours (pumpkinseed, longear sunfish, rock bass and yellow perch). One hour after conditioning, individual trout were moved to identical testing tanks for recognition testing the next day.

**Recognition trials**

Recognition trials followed the same general protocol as described above for experiment 1, except individual trout were only tested for the recognition of the predator odour to which they were conditioned. We also omitted the additional distilled water control stimulus. We conducted a total of 10 replicates (12 replications for the pumpkinseed) for each treatment combination (2 levels of preconditioning x 2 levels of conditioning x 4 levels of predator odour) for a total of 168 replicates. Mean ± SD standard length of test fish size was 3.29 ± 0.34 cm at time of testing. All observations were made blind to the treatments and the order of treatments was randomized.

**Statistical analysis**

As in experiment 1, we calculated the change in foraging attempts and time spent moving, and used these difference scores as dependant variables. Data were tested in two
ways. Initially, we tested for the overall effects of preconditioning (pumpkinseed odour versus distilled water), conditioning stimulus (alarm cue versus distilled water) and four predator odours using univariate GLM ANOVAs (PASW Statistics version 18, 2009). Given the significant interactions between the preconditioning and conditioning stimuli treatments (see below), we conducted a second analysis for trout preconditioned with pumpkinseed odour and trout preconditioned with distilled water separately. In this second level analysis, we tested the effects of conditioning stimulus (alarm cue versus distilled water and predator odour on the change in foraging attempts and time spent moving using univariate GLMs. We conducted post hoc comparisons between conditioned versus pseudoconditioned trout exposed to the same predator cue during recognition trials using independent samples *t* tests to explore significant two-way interaction terms found in this second analysis. We employed a Bonferroni correction to account for increasing type I error rates. The data met the requirements (normality and homeoscedasticity) for parametric analysis.

**RESULTS**

*Experiment 1: Generalization of Predator Recognition*

The results of our overall univariate ANOVA revealed significant main effects for conditioning stimulus (*F*.<sub>1, 110</sub> = 9.12, *P* = 0.003) and predator odour (*F*.<sub>4, 110</sub> = 2.56, *P* = 0.042) for the change in foraging attempts. Moreover, we found a significant interaction between the two main effects for the change in foraging attempts (*F*.<sub>4, 110</sub> = 2.85, *P* =
Likewise, we found significant main effects of conditioning stimulus ($F_{1,110} = 7.73, P = 0.006$) and predator odour ($F_{4,110} = 5.83, P < 0.001$) on the change in time spent moving. However, we found no significant interaction between the main effects for time spent moving ($F_{4,110} = 1.65, P = 0.17$).

Post hoc analyses revealed that trout conditioned with alarm cue + pumpkinseed odour showed a significant reduction in foraging attempts when exposed to pumpkinseed ($t_{22} = -2.84, P < 0.05$) or longear sunfish ($t_{22} = -2.67, P < 0.05$) odour, relative to pseudoconditioned controls tested with the same predator odour (Fig. 1a). There was no difference in the change in foraging attempts for conditioned versus pseudoconditioned trout when exposed to rock bass ($t_{22} = 0.31, P = 0.76$) or perch ($t_{22} = -0.21 P = 0.83$) odour or to the distilled water control ($t_{22} = -0.69, P = 0.49$; Fig. 1a). Likewise, when compared to the pseudoconditioned trout, conditioned trout showed a significant reduction in time spent moving (Fig. 1b) only when exposed to pumpkinseed ($t_{22} = -2.54, P < 0.05$) or longear sunfish ($t_{22} = -2.60, P < 0.05$; Fig. 1b) odour. We found no difference between conditioned and pseudoconditioned trout exposed to rock bass odour ($t_{22} = -1.44, P = 0.16$), yellow perch odour ($t_{22} = 0.31, P = 0.74$) or the distilled water control ($t_{22} = -0.08, P = 0.94$; Fig. 1b).

**Experiment 2: Generalization of Nonpredator Recognition**
For the change in foraging attempts, the results of our initial overall univariate ANOVA revealed a significant effect of predator odour ($F_{3, 152} = 3.25, P = 0.024$) and conditioning stimulus ($F_{1, 152} = 35.22, P < 0.001$). While we found no significant main effect of preconditioning stimulus ($F_{1, 152} = 1.69, P = 0.19$), we did find a significant interaction between the preconditioning stimulus (pumpkinseed odour versus distilled water) and the conditioning stimulus ($F_{1, 152} = 4.04, P = 0.046$). We found no significant preconditioning*predator odour ($F_{3, 152} = 1.04, P = 0.38$), predator odour*conditioning stimulus ($F_{3, 152} = 0.72, P = 0.54$) or three-way interactions ($F_{3, 152} = 2.16, P = 0.09$).

For the change in time spent moving, we found significant effects of preconditioning stimulus ($F_{1, 152} = 6.68, P = 0.011$) and conditioning stimulus ($F_{1, 152} = 31.14, P < 0.001$). We found no significant main effect of predator odour ($F_{3, 152} = 1.84, P = 0.14$). Similar to the change in foraging attempts, we found a significant preconditioning stimulus*predator odour interaction ($F_{1, 152} = 4.59, P = 0.034$). As above, we found no other significant interaction terms (preconditioning*predator odour, $F_{3, 152} = 2.08, P = 0.11$; predator odour*conditioning stimulus, $F_{3, 152} = 0.89, P = 0.44$; three-way interaction, $F_{3, 152} = 2.2, P = 0.08$).

Based on significant interaction between the effects preconditioning treatment and conditioning stimulus (see above), we further compared the change in foraging attempts and time spent moving for trout preconditioned with distilled water and trout preconditioned with pumpkinseed odour separately. Trout initially preconditioned with distilled water alone showed a significant effect of conditioning stimulus for both the
change in foraging attempts ($F_{2,76} = 25.34, P < 0.001$; Fig. 2a) and time spent moving
($F_{2,76} = 28.46, P < 0.001$; Fig. 2b). However, we found no effect of predator odour on
either the change in foraging attempts ($F_{3,76} = 0.51, P = 0.67$; Fig. 2a) or time spent
moving ($F_{3,76} = 0.11, P = 0.95$; Fig. 2b). Likewise, there was no significant interaction
between the main effects for trout preconditioned with water (change in foraging
attempts: $F_{3,76} = 0.29, P = 0.83$; change in time moving: $F_{3,76} = 0.26, P = 0.86$; Fig. 2a
and Fig. 2b, respectively). Overall, trout preconditioned with distilled water did not
appear to be inhibited from learning any of the predator odours (Fig. 2a, b).

However, when considering trout preconditioned with pumpkinseed odour alone,
we found significant main effects of conditioning stimulus (foraging attempts: $F_{1,76} =
10.21, P = 0.002$; time moving: $F_{1,76} = 6.21, P = 0.015$) and predator odour (foraging
attempts: $F_{3,76} = 4.83, P = 0.004$, Fig. 2c; time moving: $F_{3,76} = 3.98, P = 0.011$, Fig. 2d).
More importantly, we found a significant interaction between conditioning stimulus and
 predator odour for both the change in foraging attempts ($F_{3,76} = 3.33, P = 0.024$; Fig. 2c)
and time spent moving ($F_{3,76} = 2.90, P = 0.036$; Fig. 2d). Post hoc tests revealed that
following preconditioning exposure to pumpkinseed odour, there was no significant
difference between conditioned and pseudoconditioned trout exposed to pumpkinseed
(foraging attempts: $t_{22} = 0.40, P = 0.69$; time spent moving: $t_{22} = -0.28, P = 0.79$) or
longear sunfish odour (foraging attempts: $t_{18} = -0.49, P = 0.63$; time spent moving: $t_{18} =
0.81, P = 0.43$; Fig. 2c, d) during the recognition trials. However, preconditioning with
pumpkinseed odour had no effect on the ability of trout to learn to recognize rock bass
(foraging attempts: $t_{18} = -3.21, P < 0.05$; time spent moving: $t_{18} = -2.65, P < 0.05$) or
yellow perch (foraging attempts: $t_{18} = -2.67, P < 0.05$; time spent moving: $t_{18} = -4.38, P < 0.05$; Fig. 2c, d) odours as predation threats.

DISCUSSION

We found support for our hypothesis that juvenile rainbow trout that are conditioned to recognize the chemical cues of pumpkinseeds generalize this recognition to the cues of closely related predators. We found a significant learned response to pumpkinseed and longear sunfish odours but not to the odour of the confamilial rock bass or the more distantly related yellow perch. These results are consistent with previous studies showing generalization of chemically mediated predator recognition in fathead minnows (Ferrari et al. 2007), woodfrog tadpoles (Ferrari et al. 2009) and freshwater snails (*Physella virgata*, Langerhans & Dewitt 2002).

Ferrari et al. (2007) demonstrated a graded response with respect to the phylogenetic relatedness among predators. Fathead minnows showed a strong response to the conditioned predator cue and a progressively weaker response to the cues of congeneric and confamilial predators. Our results, however, do not indicate a gradation in the intensity of antipredator responses displayed to the known predator (pumpkinseed) compared with the novel species. Trout showed similar response intensities to pumpkinseed and the congeneric longear sunfish, but no response to the confamilial rock bass. This pattern may result from different methods in which the 5 min observation period did not allow us to capture the subtleties in antipredator waning (Zhao et al. 2006).
Alternatively, trout may be using cues that prevent them from discriminating between two congeneric species, namely pumpkinseed and longear sunfish.

Our second experiment demonstrates that pre-exposure to a predator cue (pumpkinseed odour) twice per day for 3 days results in the inability to learn the recognition of pumpkinseed as a predation threat, via the mechanism of latent inhibition. These results are consistent with previous work with crayfish (Acquistapace et al. 2003), fathead minnows (Ferrari & Chivers 2006) and woodfrog tadpoles (Ferrari & Chivers 2009), but differ from a number of studies with aquatic invertebrates that demonstrate enhanced predator avoidance in response to predator cues alone (Gyssels & Stoks 2006; Dalesman et al. 2007a; Gall & Brodie 2009). Previous studies have also demonstrated latent inhibition of ecologically relevant learning including foraging cues (Loy et al. 2006; Chandra et al. 2010) and mate preferences (Crawford et al. 1993). Repeated exposure to the visual cues of novel predators, however, appears not to result in latent inhibition (Curio et al. 1978; Mineka & Cook 1986).

More importantly, the results of our second experiment support our second hypothesis that the recognition of a nonpredator can also be generalized. Juvenile trout, which were inhibited from learning pumpkinseed odour, showed no evidence of learned recognition when conditioned to recognize the odour of a congeneric predator (longear sunfish). However, pre-exposure to pumpkinseed odour did not impair the ability of trout to learn the odour of rock bass or yellow perch, demonstrating that the inhibition of learning was not generalized towards more distantly related predators. In effect, juvenile
rainbow trout appear able to generalize the recognition of predator cues and the recognition of non-predator cues. This is the first demonstration that non-predator recognition can be generalized. Combined, the ability to generalize the learned recognition of predator and non-predator cues should increase the likelihood of prey responding only to ecologically relevant predation threats.

Recently, Dalesman et al. (2007b) demonstrated that the freshwater gastropod *Lymnaea stagnalis* shows increased predator avoidance responses to heterospecific gastropod alarm cues and that the strength of this response decreases with increasing phylogenetic distance. However, the response to heterospecifics only occurred if *L. stagnalis* were sympatric with the donor species; allopatric heterospecifics were largely ignored regardless of phylogenetic relatedness. The reported effect of sympatry probably did not result from direct experience (i.e. learning to recognize heterospecific alarm cues; Pollock et al. 2003), since tests were conducted on laboratory-reared F1 *L. stagnalis*. This suggests a strong interaction between phylogenetic relatedness and the possibility for ecological interactions. It is unknown to what extent a similar phylogeny–sympatry interaction might be present in our current results. All trout used in the current study were hatchery reared, and hence had no direct experience with predators. However, rainbow trout do commonly occur in sympatry with all the predators tested here (Scott & Crossman 1973). If such an interaction were operating within the context of generalized learning of predator recognition, we might expect even more fine-scale discrimination among predator types.
The ability to assess local predation threats is critical for prey attempting to balance the conflicting pressures of successful detection and avoidance of predators and a suite of other fitness-related behavioural activities such as foraging, mating and/or territorial defence. These threat-sensitive trade-offs presumably would allow prey to adjust the overall intensity of predator avoidance behaviours according to the level of perceived risk (Helfman 1989; Brown et al. 2006; Gonzalo et al. 2009). Previous work has shown that prey acquire recognition of novel predator cues proportional to the intensity of perceived risk during the initial conditioning (Ferrari et al. 2005, 2006). For example, fathead minnows showed a stronger learned response to a novel predator odour when it was initially paired with a high- versus low-risk cue (Ferrari et al. 2005).

Moreover, minnows show threat-sensitive generalization of learned predator odours. Ferrari et al. (2008) exposed fathead minnows to the odour of brown trout paired with a high- versus low-risk cue and found that minnows only generalized the learned predator response if they had been conditioned with a high-risk cue. Our current results build on this notion of threat-sensitive learning, showing that prey show a sophisticated ability to learn and recognize both risky and nonrisky cues. The next logical study should examine threat-sensitive generalization of nonpredators. The ability to generalize known predators and known nonpredators should allow prey to continuously update their assessment of local predation threats (Brown & Chivers 2005) and to better balance the conflicting needs of predator avoidance and other behavioural activities in the face of variable predation threats.
The reliance on learned (versus ‘innate’) predator recognition should be favoured under conditions of spatially and/or temporally variable predation risk or when there is a diverse predator guild (Brown & Chivers 2005; Ferrari et al. 2007). Learning is adaptive in the sense that it allows prey to acquire context-appropriate responses to variable predation threats (Brown & Chivers 2005; Dall et al. 2005; Ferrari et al. 2007). However, learning is not without risk. Any initial learning opportunity would involve exposure to a potentially high-risk predation event, and thus may be considered costly. Generalization of learned predator information would allow prey to respond to acquired cues without the increased cost associated with direct learning (Ferrari et al. 2007). The ability to generalize predator recognition would be particularly beneficial for prey exposed to unpredictable, yet intense, predation threats because it would limit the time prey are actually exposed to a real threat. Likewise, generalizing what is not an actual predation threat would also reduce time and energy otherwise spent on directly assessing threats. Thus, we would predict generalization of predator and non-predator cues under conditions of variable predation risks (Ferrari et al. 2007, 2008), the same conditions that are thought to favour learned predator recognition (Brown & Chivers 2005).

Despite the evidence showing remarkably sophisticated learned predator recognition (Brown 2003; Brown & Chivers 2005), there is a wealth of examples of both aquatic vertebrate and invertebrate prey that show an innate avoidance of novel predators. For example, both Arctic charr, Salvelinus alpinus (Vilhunen & Hirvonen 2003) and chinook salmon, Oncorhynchus tshawytscha (Berejikian et al. 2003) reared under hatchery conditions (hence no previous experience with predators) showed predictable
antipredator responses when exposed to predator cues. Perhaps more interesting are the growing number of studies showing a complex innate predator recognition in aquatic invertebrates. For example, the freshwater gastropod *L. stagnalis* shows a strong response to the odour of a predatory fish (tench, *Tinca tinca*), but no response to ground conspecifics (relative to a control; Dalesman et al. 2007a). However, the response to tench odour paired with conspecific odours resulted in a significantly stronger predator avoidance response (Dalesman et al. 2007a). Similar ‘additive’ responses have been shown for damselfly larvae (*Ischnura elegans*; Gyssels & Stoks 2006) and caddisfly larvae (*Hesperophylax occidentalis*; Gall & Brodie 2009). Together, these studies suggest that in systems where the costs associated with failing to respond to intense predation pressure are high, selection may favour an innate (i.e. fixed) response to novel predator cues (Brown & Chivers 2005).

Our current results suggest that generalization is based on chemical similarities among phylogenetically related predators. Presumably, related predator species would produce chemosensory cues more similar to each other than would more distantly related predators. We might also expect predators sharing similar diets, regardless of phylogenetic relatedness, to produce cues that are readily generalized by prey. Dietary cues are known to allow for the recognition of novel predators (Mathis & Smith 1993; Chivers & Mirza 2001). It is likely that a combination of chemical similarities among related predators plus common diets (especially among sympatric predators) would allow for sufficient information to allow generalization of learned predator recognition. Future
research is required to identify the specific compounds or classes of compounds that allow for differential recognition of predators.

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References


Figure 1. Mean ± SE change in foraging attempts (a) and time spent moving (b) during the recognition phase for trout initially conditioned with pumpkinseed odour + trout alarm cue (dark bars) or pseudoconditioned with pumpkinseed odour + distilled water (open bars) and tested for the recognition of odour of pumpkinseed (PS), longear sunfish (LE), rock bass (RB), yellow perch (YP) or distilled water (DW). N = 12 for each treatment combination. *Denotes a significant difference (P < 0.05) between conditioned and pseudoconditioned trout for each predator odour.

Figure 2. Mean ± SE change in foraging attempts (a, c) and time spent moving (b, d) during the recognition phase for trout conditioned with one of four predator odours + trout alarm cue (shaded bars; PS = pumpkinseed, LE = longear sunfish, RB = rock bass, YP = yellow perch) or pseudoconditioned with one of four predator odours + distilled water (open bars). Trout were initially pre-exposed, twice per day for 3 days with distilled water (a, b) or pumpkinseed odour (c, d). N = 10 for each treatment combination, except for trout tested for recognition of pumpkinseed, where N = 12. *Denotes a significant difference (P < 0.05) between conditioned and pseudoconditioned trout for each predator odour.
Mean change in foraging attempts

Mean change in time moving (sec)

 Predator odour

PS  LE  RB  YP  DW

NS  NS  NS  NS  NS

*  *  *  *  *

0  25  50  75

-100  -75  -50  -25  0

-5  -3  -1  1  3
Mean change in foraging attempts

Mean change in time moving (sec)

Pre-exposed to distilled water

Pre-exposed to pumpkinseed odour

Predator odour

PS  LE  RB  YP

NS  NS  *  *

Predator odour

NS  NS  *