

1 Generalization of predators and nonpredators by juvenile rainbow trout: learning what is
2 and is not a threat
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26

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

47

48 *Keywords:*

49 acquired predator recognition, alarm cue, generalization, latent inhibition, rainbow trout,
50 threat-sensitive trade-off

51

52 To balance the conflicting demands of predator avoidance and other fitness-
53 related activities such as foraging, territorial defence and mating, prey organisms require
54 accurate information regarding local threats (Helfman 1989; Lima & Dill 1990).
55 However, uncertainty in quality and/or reliability of information may limit the ability of
56 prey to make appropriate behavioural decisions (Sih 1992; Lima 1998). Thus, in response
57 to this uncertainty, individuals capable of acquiring information about their environment
58 should be better able to deal with variable risks (Dall et al. 2005). Within the context of
59 threat-sensitive predator avoidance, the use of personal (direct interactions) and/or social
60 information (Dall et al. 2005) could allow prey to minimize the likelihood of
61 inappropriate responses.

62

63 Learning to recognize realistic threats would increase the probability of survival
64 during encounters with potential predators (Mirza & Chivers 2000; Darwish et al. 2005).
65 One highly effective means to recognize predators is through Pavlovian conditioning,
66 whereby prey are exposed to the cues of a novel predator combined with cues of injured
67 conspecifics (Brown 2003; Brown & Chivers 2005). Such learning has been shown in a
68 diverse range of prey, including both invertebrate and vertebrate aquatic species
69 (reviewed in Ferrari et al. 2010). Analogous learning systems have been demonstrated in
70 birds (Curio et al. 1978) and mammals (McLean et al. 1996; Griffin et al. 2001).
71 However, such learning is costly, because prey must survive the initial conditioning event
72 (i.e. encounter with the predator; Ferrari et al. 2007). In addition, selection favouring
73 learning abilities may represent a fitness cost due to increased physiological costs
74 associated with increased energetic investments into neural and/or sensory structures

75 underlying learning and memory (Mery & Kawecki 2003, 2005). The ability to
76 generalize learned information, such as predator cues, is argued to reduce these costs
77 (Griffin et al. 2001; Ferrari et al. 2007). Phylogenetically related predators may share
78 predatory tactics and/or preferred diets (Olson et al. 1995; Ferrari et al. 2007; Barco et al.
79 2010). For example, several sympatric species of centrarchids show a high degree of
80 similarity in ontogeny, foraging tactics and diet preferences (Scott & Crossman 1973;
81 Brown 1984). As such, prey at risk of predation from one species may also be at risk of
82 predation from closely related species. Thus, generalizing recognition of potential
83 predators may allow prey to increase their likelihood of survival without compromising
84 threat-sensitive trade-offs (Ferrari et al. 2007, 2008, 2009).

85

86 Learning to recognize nonpredators should allow prey to avoid wasting time and
87 energy responding to irrelevant cues (Brown & Chivers 2005; Ferrari et al. 2007;
88 Stankowich & Coss 2007). Latent inhibition is a form of associative learning in which
89 novel stimuli are repeatedly encountered in the absence of either a positive or a negative
90 outcome (Allen et al. 2002; Rankin et al. 2009). As a result of this repeated exposure,
91 individuals may be inhibited from forming ecologically relevant associations (Drezner-
92 Levy et al. 2009), and this inhibition has been shown to shape learned foraging
93 preferences (Chandra et al. 2010) and mate choice (Akins 1994). In the case of
94 chemically mediated learning to recognize predators, pre-exposure to predator odour in
95 the absence of a reinforcing risk stimulus is known to reduce the likelihood that prey will
96 acquire recognition of this species as predatory. Such inhibition has been demonstrated in
97 several taxa, including virile crayfish, *Orconectes virilis* (Acquistapace et al. 2003),

98 fathead minnows, *Pimephales promelas* (Ferrari & Chivers 2006) and woodfrogs, *Rana*
99 *sylvatica* (Ferrari & Chivers 2009). For example, when exposed to a novel predator odour
100 for 2 h/day for 3 days, crayfish were unable to learn to associate the predator cue with a
101 real predation threat. Likewise, fathead minnows were unable to learn to recognize brook
102 charr, *Salvelinus fontinalis*, as a predation threat when the minnows had been pre-
103 exposed to charr odour.

104

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

113

114 However, the question of whether generalization of nonpredator recognition
115 should be as widespread as generalization of predator recognition requires us to do a
116 quick cost–benefit analysis of these responses. When encountering a novel species, the
117 individual must decide whether it poses a threat. When the novel species is reminiscent of
118 a known predator, the prey may decide to deal with their uncertainty by generalizing its
119 knowledge and treating this novel species as a threat. If the prey is correct, it survives. If
120 the novel species was not a predator, the prey wasted time and energy that could have

121 otherwise been allocated to foraging or courting. Thus, generalizing predator recognition
122 may allow prey to survive their first encounter with a novel predator at best, or it may
123 result in missed opportunities at worse. Similarly, when the novel species is reminiscent
124 of a nonpredator, the prey may decide to generalize their responses from known
125 nonpredators. In this case, if the novel species was indeed harmless, the prey made the
126 right decision. If the novel species was not harmless, the prey not only failed to respond
127 to threat, but it labelled it as a nonthreatening cue, which will prevent the prey from
128 learning to recognize this species as a predator during subsequent encounters. This is a
129 direct consequence of the proximate mechanism behind the labelling of nonrisky species:
130 latent inhibition. When an individual encounters a stimulus for the first time, the stimulus
131 is neither negative nor positive, but rather neutral, at least in the absence of any sensory
132 bias. Learned predator recognition allows prey to label unknown, neutral stimuli as
133 threatening. Conversely, latent inhibition allows prey to label unknown, neutral stimuli as
134 harmless. However, once a stimulus is labelled as harmless, subsequent associations with
135 a known threat (unconditioned stimulus) will reduce or prevent learning (Acquistapace et
136 al. 2003; Ferrari & Chivers 2006). Hence, generalizing nonpredator recognition will
137 quicken the labelling of a novel species as harmless at best, or it may delay the
138 recognition of a predator at worse, which may result in an increase in predation risk to
139 prey. Because of the asymmetry between the potential costs of errors between
140 generalization of predators and nonpredators, we predict that generalization of predators
141 may be more widespread than generalization of nonpredators.

142

143 The purpose of the current study was two-fold. First, we tested the hypothesis that
144 juvenile rainbow trout, *Oncorhynchus mykiss*, can generalize acquired predator
145 recognition from one predator species to the cues of phylogenetically related predators.
146 We conditioned trout to recognize the odour of a pumpkinseed sunfish, *Lepomis*
147 *gibbosus*, and then tested them for their recognition of pumpkinseed or the odour of
148 longear sunfish, *Lepomis megalotis*, rock bass, *Ambloplites rupestris*, and yellow perch,
149 *Perca flavescens*. All four predator species are perciform fishes. Pumpkinseed, longear
150 sunfish and rock bass belong to the family Centrarchidae, with pumpkinseed and longear
151 sunfish (same genus as pumpkinseed, thus congeneric) representing the most
152 phylogenetically similar predators, and with the rock bass a more distantly related
153 centrarchid (i.e. confamilial). The yellow perch represented the most distant predator,
154 belonging to the family Percidae. If rainbow trout are capable of generalizing learned
155 predator recognition, we predicted the strongest learned response to pumpkinseed and the
156 congeneric longear sunfish and the weakest (or absent) response to the more distantly
157 related yellow perch.

158

159 The second goal of this study was to test the hypothesis that juvenile rainbow
160 trout can also generalize information about nonpredators. We pre-exposed juvenile trout
161 to pumpkinseed odour (latent inhibition) or a distilled water control and conditioned them
162 to recognize each of the four predator odours. If pre-exposure to pumpkinseed odour
163 resulted in latent inhibition, we predicted a lack of a learned response to pumpkinseed
164 odour. If this inhibition was generalized, we predicted that there would also be an

165 absence of a learned response to the phylogenetically related predators, but not towards
166 the more distantly related predators

167

168 **METHODS**

169

170 *Test Fish and Stimulus Collection*

171

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

180

181 Predator odour donors (adult pumpkinseed, longear sunfish, rock bass and yellow
182 perch), two of each species, were collected from Canal Lachine, Montreal, Quebec. We
183 chose these predators since they are sympatric and share similar life histories. Predators
184 were held in similar tanks (separate holding tanks for each species) as described for trout
185 and fed a diet of brine shrimp and commercial cichlid pellets daily. All predators (12.5–
186 16.1 cm standard length) were held for at least 14 days prior to use as stimulus donors to
187 ensure that any prey fish in the diet had been evacuated.

188

189 Trout alarm cues were generated using standard protocols (Brown & Smith 1997;
190 Brown et al. 2006; Kim et al. 2011). We generated trout alarm cues from 23 juvenile
191 trout (mean \pm SD fork length = 5.01 ± 0.52 cm). Donor trout were killed via cervical
192 dislocation (in accordance with Concordia University Animal Research Ethics
193 Committee protocol number AREC-2008-BROW) and skin fillets were removed from
194 either side of the donors and immediately placed into 100 ml of chilled distilled water.
195 Skin fillets were then homogenized using a hand-held tissue homogenizer, filtered
196 through polyester filter floss, and diluted to the desired volume with the addition of
197 distilled water. We collected a total of 155.8 cm^2 of skin (in a final volume of 1558 ml of
198 distilled water). This concentration of skin extract is known to elicit an antipredator
199 response in juvenile trout under laboratory conditions (Brown & Smith 1998; Smith et al.
200 2008). Chemical alarm cues were frozen in 20 ml aliquots at $-20 \text{ }^\circ\text{C}$ until needed. As a
201 control, we also froze 20 ml samples of distilled water.

202

203 To collect predator odours from each of the four species, we placed individual
204 predators (two of each species) into 37-litre glass aquaria (filled with 15 litres of
205 dechlorinated tap water) for 3 days. The donor tanks contained a gravel substrate (~ 18
206 $^\circ\text{C}$, pH ~ 7.0) and were aerated, but not filtered. Donors were not fed during the stimulus
207 collection period. Following this period, we returned the predators to their holding tanks.
208 The tank water (predator cue) for the two donors of each species was combined to
209 generate a single cue per predator type. Predator odours were filtered and frozen in 50 ml
210 aliquots at $-20 \text{ }^\circ\text{C}$ until needed (Leduc et al. 2004; Smith et al. 2008). The predator

211 odours from each donor were combined to ensure a consistent cue concentration for each
212 predator species (Ferrari et al. 2007, 2009).

213

214 *Experiment 1: Generalization of Predator Recognition*

215

216 *Conditioning phase*

217 Juvenile trout were transferred to individual test tanks (one trout per test tank) and
218 allowed a 24 h acclimation period prior to testing. Test tanks consisted of a series of 10
219 37-litre glass aquaria (18 °C, pH ~ 7.0), filled with 32 litres of dechlorinated tap water,
220 equipped with a gravel substrate and a single airstone fixed to the back wall of the tank.
221 In addition, we attached a 2 m length of airline to the back wall that terminated
222 immediately above the airstone to allow for the introduction of experimental cues without
223 disturbing test fish. Immediately prior to conditioning, we withdrew and discarded 60 ml
224 of tank water through the stimulus tube to remove any stagnant water. We then withdrew
225 and retained an additional 60 ml of water. We injected 10 ml of pumpkinseed odour,
226 immediately followed by either 10 ml of trout alarm cue (conditioned trout) or 10 ml of
227 distilled water (pseudoconditioned trout). Trout remained in the conditioning tank for
228 approximately 60 min and were then transferred to an identical test tank for recognition
229 testing. Previous studies have used similar protocols to demonstrate chemically mediated
230 learning in a variety of prey fishes (Brown & Smith 1998; Brown 2003).

231

232 *Recognition trials*

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

250

251 *Statistical analysis*

252 We calculated the change in foraging attempts and time spent moving
253 (poststimulus – prestimulus values) and used these difference scores as dependent
254 variables in our analyses. We tested the effects of initial conditioning stimulus (alarm cue
255 versus distilled water) and predator cues (and the additional distilled water control) on the

256 change in foraging attempts and time spent moving using univariate GLM ANOVAs
257 (PASW Statistics, Version 18, 2009; IBM, Armonk, NY, U.S.A.). Post hoc comparisons
258 between conditioned versus pseudoconditioned trout exposed to the same predator cue
259 during recognition trials were made using independent samples *t* tests. We employed a
260 Bonferroni correction to account for increasing type I error rates. The data met the
261 requirements (normality and homoscedasticity) for parametric analysis.

262

263 *Experiment 2: Generalization of Nonpredator Recognition*

264

265 *Pre-exposure and conditioning phase*

266 Juvenile trout were placed individually into a series of 20 15-litre plastic
267 containers. Each container was equipped with a single airstone and a gravel substrate. We
268 repeated the pre-exposure procedures in nine blocks to generate sufficient replicates for
269 all treatment combinations (see below). The containers were positioned along a pair of
270 flow-through stream channels (1.92 x 0.76 m, water depth ~20 cm) to ensure stable
271 temperature. Individual containers were visually and chemically isolated from each other.
272 We introduced 10 ml of either pumpkinseed odour or distilled water, twice daily for 3
273 consecutive days (at ~ 0900 and ~ 1400 hours). Approximately 30 min following the
274 introduction of the cue into the container, we conducted a 100% water change with tank
275 water from a separate system to ensure that no chemical cues remained and there was no
276 incidental exposure of the control containers with pumpkinseed odour. As a result, trout
277 were pre-exposed to pumpkinseed or distilled water for a maximum of 60 min/day.
278 Containers receiving pumpkinseed odour and distilled water preconditioning were

279 randomly distributed across both stream channels. Three hours following the final
280 exposure, trout were transferred to individual test tanks, as described above, and allowed
281 24 h acclimation prior to conditioning.

282

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

288

289 *Recognition trials*

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

298

299 *Statistical analysis*

300 As in experiment 1, we calculated the change in foraging attempts and time spent
301 moving, and used these difference scores as dependant variables. Data were tested in two

302 ways. Initially, we tested for the overall effects of preconditioning (pumpkinseed odour
303 versus distilled water), conditioning stimulus (alarm cue versus distilled water) and four
304 predator odours using univariate GLM ANOVAs (PASW Statistics version 18, 2009).
305 Given the significant interactions between the preconditioning and conditioning stimuli
306 treatments (see below), we conducted a second analysis for trout preconditioned with
307 pumpkinseed odour and trout preconditioned with distilled water separately. In this
308 second level analysis, we tested the effects of conditioning stimulus (alarm cue versus
309 distilled water and predator odour on the change in foraging attempts and time spent
310 moving using univariate GLMs. We conducted post hoc comparisons between
311 conditioned versus pseudoconditioned trout exposed to the same predator cue during
312 recognition trials using independent samples *t* tests to explore significant two-way
313 interaction terms found in this second analysis. We employed a Bonferroni correction to
314 account for increasing type I error rates. The data met the requirements (normality and
315 homoscedasticity) for parametric analysis.

316

317 **RESULTS**

318

319 *Experiment 1: Generalization of Predator Recognition*

320

321 The results of our overall univariate ANOVA revealed significant main effects for
322 conditioning stimulus ($F_{1,110} = 9.12, P = 0.003$) and predator odour ($F_{4,110} = 2.56, P =$
323 0.042) for the change in foraging attempts. Moreover, we found a significant interaction
324 between the two main effects for the change in foraging attempts ($F_{4,110} = 2.85, P =$

325 0.027). Likewise, we found significant main effects of conditioning stimulus ($F_{1,110} =$
326 7.73, $P = 0.006$) and predator odour ($F_{4,110} = 5.83$, $P < 0.001$) on the change in time spent
327 moving. However, we found no significant interaction between the main effects for time
328 spent moving ($F_{4,110} = 1.65$, $P = 0.17$).

329

330 Post hoc analyses revealed that trout conditioned with alarm cue + pumpkinseed
331 odour showed a significant reduction in foraging attempts when exposed to pumpkinseed
332 ($t_{22} = -2.84$, $P < 0.05$) or longear sunfish ($t_{22} = -2.67$, $P < 0.05$) odour, relative to
333 pseudoconditioned controls tested with the same predator odour (Fig. 1a). There was no
334 difference in the change in foraging attempts for conditioned versus pseudoconditioned
335 trout when exposed to rock bass ($t_{22} = 0.31$, $P = 0.76$) or perch ($t_{22} = -0.21$, $P = 0.83$)
336 odour or to the distilled water control ($t_{22} = -0.69$, $P = 0.49$; Fig. 1a). Likewise, when
337 compared to the pseudoconditioned trout, conditioned trout showed a significant
338 reduction in time spent moving (Fig. 1b) only when exposed to pumpkinseed ($t_{22} = -2.54$,
339 $P < 0.05$) or longear sunfish ($t_{22} = -2.60$, $P < 0.05$; Fig. 1b) odour. We found no
340 difference between conditioned and pseudoconditioned trout exposed to rock bass odour
341 ($t_{22} = -1.44$, $P = 0.16$), yellow perch odour ($t_{22} = 0.31$, $P = 0.74$) or the distilled water
342 control ($t_{22} = -0.08$, $P = 0.94$; Fig. 1b).

343

344 *Experiment 2: Generalization of Nonpredator Recognition*

345

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

354

355 For the change in time spent moving, we found significant effects of
356 preconditioning stimulus ($F_{1, 152} = 6.68, P = 0.011$) and conditioning stimulus ($F_{1, 152} =$
357 $31.14, P < 0.001$). We found no significant main effect of predator odour ($F_{3, 152} = 1.84,$
358 $P = 0.14$). Similar to the change in foraging attempts, we found a significant
359 preconditioning stimulus*predator odour interaction ($F_{1, 152} = 4.59, P = 0.034$). As above,
360 we found no other significant interaction terms (preconditioning*predator odour, $F_{3, 152} =$
361 $2.08, P = 0.11$; predator odour*conditioning stimulus, $F_{3, 152} = 0.89, P = 0.44$; three-way
362 interaction, $F_{3, 152} = 2.2, P = 0.08$).

363

364 Based on significant interaction between the effects preconditioning treatment and
365 conditioning stimulus (see above), we further compared the change in foraging attempts
366 and time spent moving for trout preconditioned with distilled water and trout
367 preconditioned with pumpkinseed odour separately. Trout initially preconditioned with
368 distilled water alone showed a significant effect of conditioning stimulus for both the

369 change in foraging attempts ($F_{2,76} = 25.34, P < 0.001$; Fig. 2a) and time spent moving
370 ($F_{2,76} = 28.46, P < 0.001$; Fig. 2b). However, we found no effect of predator odour on
371 either the change in foraging attempts ($F_{3,76} = 0.51, P = 0.67$; Fig. 2a) or time spent
372 moving ($F_{3,76} = 0.11, P = 0.95$; Fig. 2b). Likewise, there was no significant interaction
373 between the main effects for trout preconditioned with water (change in foraging
374 attempts: $F_{3,76} = 0.29, P = 0.83$; change in time moving: $F_{3,76} = 0.26, P = 0.86$; Fig. 2a
375 and Fig. 2b, respectively). Overall, trout preconditioned with distilled water did not
376 appear to be inhibited from learning any of the predator odours (Fig. 2a, b).

377

378 However, when considering trout preconditioned with pumpkinseed odour alone,
379 we found significant main effects of conditioning stimulus (foraging attempts: $F_{1,76} =$
380 $10.21, P = 0.002$; time moving: $F_{1,76} = 6.21, P = 0.015$) and predator odour (foraging
381 attempts: $F_{3,76} = 4.83, P = 0.004$, Fig. 2c; time moving: $F_{3,76} = 3.98, P = 0.011$, Fig. 2d).
382 More importantly, we found a significant interaction between conditioning stimulus and
383 predator odour for both the change in foraging attempts ($F_{3,76} = 3.33, P = 0.024$; Fig. 2c)
384 and time spent moving ($F_{3,76} = 2.90, P = 0.036$; Fig. 2d). Post hoc tests revealed that
385 following preconditioning exposure to pumpkinseed odour, there was no significant
386 difference between conditioned and pseudoconditioned trout exposed to pumpkinseed
387 (foraging attempts: $t_{22} = 0.40, P = 0.69$; time spent moving: $t_{22} = -0.28, P = 0.79$) or
388 longear sunfish odour (foraging attempts: $t_{18} = -0.49, P = 0.63$; time spent moving: $t_{18} =$
389 $0.81, P = 0.43$; Fig. 2c, d) during the recognition trials. However, preconditioning with
390 pumpkinseed odour had no effect on the ability of trout to learn to recognize rock bass
391 (foraging attempts: $t_{18} = -3.21, P < 0.05$; time spent moving: $t_{18} = -2.65, P < 0.05$) or

392 yellow perch (foraging attempts: $t_{18} = -2.67$, $P < 0.05$; time spent moving: $t_{18} = -4.38$, $P <$
393 0.05 ; Fig. 2c, d) odours as predation threats.

394

395 **DISCUSSION**

396

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

405

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

415 Alternatively, trout may be using cues that prevent them from discriminating between
416 two congeneric species, namely pumpkinseed and longear sunfish.

417

418 Our second experiment demonstrates that pre-exposure to a predator cue
419 (pumpkinseed odour) twice per day for 3 days results in the inability to learn the
420 recognition of pumpkinseed as a predation threat, via the mechanism of latent inhibition.
421 These results are consistent with previous work with crayfish (Acquistapace et al. 2003),
422 fathead minnows (Ferrari & Chivers 2006) and woodfrog tadpoles (Ferrari & Chivers
423 2009), but differ from a number of studies with aquatic invertebrates that demonstrate
424 enhanced predator avoidance in response to predator cues alone (Gyssels & Stoks 2006;
425 Dalesman et al. 2007a; Gall & Brodie 2009). Previous studies have also demonstrated
426 latent inhibition of ecologically relevant learning including foraging cues (Loy et al.
427 2006; Chandra et al. 2010) and mate preferences (Crawford et al. 1993). Repeated
428 exposure to the visual cues of novel predators, however, appears not to result in latent
429 inhibition (Curio et al. 1978; Mineka & Cook 1986).

430

431 More importantly, the results of our second experiment support our second
432 hypothesis that the recognition of a nonpredator can also be generalized. Juvenile trout,
433 which were inhibited from learning pumpkinseed odour, showed no evidence of learned
434 recognition when conditioned to recognize the odour of a congeneric predator (longear
435 sunfish). However, pre-exposure to pumpkinseed odour did not impair the ability of trout
436 to learn the odour of rock bass or yellow perch, demonstrating that the inhibition of
437 learning was not generalized towards more distantly related predators. In effect, juvenile

438 rainbow trout appear able to generalize the recognition of predator cues and the
439 recognition of nonpredator cues. This is the first demonstration that nonpredator
440 recognition can be generalized. Combined, the ability to generalize the learned
441 recognition of predator and nonpredator cues should increase the likelihood of prey
442 responding only to ecologically relevant predation threats.

443

444 Recently, Dalesman et al. (2007b) demonstrated that the freshwater gastropod
445 *Lymnaea stagnalis* shows increased predator avoidance responses to heterospecific
446 gastropod alarm cues and that the strength of this response decreases with increasing
447 phylogenetic distance. However, the response to heterospecifics only occurred if *L.*
448 *stagnalis* were sympatric with the donor species; allopatric heterospecifics were largely
449 ignored regardless of phylogenetic relatedness. The reported effect of sympatry probably
450 did not result from direct experience (i.e. learning to recognize heterospecific alarm cues;
451 Pollock et al. 2003), since tests were conducted on laboratory-reared F1 *L. stagnalis*. This
452 suggests a strong interaction between phylogenetic relatedness and the possibility for
453 ecological interactions. It is unknown to what extent a similar phylogeny–sympatry
454 interaction might be present in our current results. All trout used in the current study were
455 hatchery reared, and hence had no direct experience with predators. However, rainbow
456 trout do commonly occur in sympatry with all the predators tested here (Scott &
457 Crossman 1973). If such an interaction were operating within the context of generalized
458 learning of predator recognition, we might expect even more fine-scale discrimination
459 among predator types.

460

461 The ability to assess local predation threats is critical for prey attempting to
462 balance the conflicting pressures of successful detection and avoidance of predators and a
463 suite of other fitness-related behavioural activities such as foraging, mating and/or
464 territorial defence. These threat-sensitive trade-offs presumably would allow prey to
465 adjust the overall intensity of predator avoidance behaviours according to the level of
466 perceived risk (Helfman 1989; Brown et al. 2006; Gonzalo et al. 2009). Previous work
467 has shown that prey acquire recognition of novel predator cues proportional to the
468 intensity of perceived risk during the initial conditioning (Ferrari et al. 2005, 2006). For
469 example, fathead minnows showed a stronger learned response to a novel predator odour
470 when it was initially paired with a high- versus low-risk cue (Ferrari et al. 2005).
471 Moreover, minnows show threat-sensitive generalization of learned predator odours.
472 Ferrari et al. (2008) exposed fathead minnows to the odour of brown trout paired with a
473 high- versus low-risk cue and found that minnows only generalized the learned predator
474 response if they had been conditioned with a high-risk cue. Our current results build on
475 this notion of threat-sensitive learning, showing that prey show a sophisticated ability to
476 learn and recognize both risky and nonrisky cues. The next logical study should examine
477 threat-sensitive generalization of nonpredators. The ability to generalize known predators
478 and known nonpredators should allow prey to continuously update their assessment of
479 local predation threats (Brown & Chivers 2005) and to better balance the conflicting
480 needs of predator avoidance and other behavioural activities in the face of variable
481 predation threats.
482

483 The reliance on learned (versus ‘innate’) predator recognition should be favoured
484 under conditions of spatially and/or temporally variable predation risk or when there is a
485 diverse predator guild (Brown & Chivers 2005; Ferrari et al. 2007). Learning is adaptive
486 in the sense that it allows prey to acquire context-appropriate responses to variable
487 predation threats (Brown & Chivers 2005; Dall et al. 2005; Ferrari et al. 2007). However,
488 learning is not without risk. Any initial learning opportunity would involve exposure to a
489 potentially high-risk predation event, and thus may be considered costly. Generalization
490 of learned predator information would allow prey to respond to acquired cues without the
491 increased cost associated with direct learning (Ferrari et al. 2007). The ability to
492 generalize predator recognition would be particularly beneficial for prey exposed to
493 unpredictable, yet intense, predation threats because it would limit the time prey are
494 actually exposed to a real threat. Likewise, generalizing what is not an actual predation
495 threat would also reduce time and energy otherwise spent on directly assessing threats.
496 Thus, we would predict generalization of predator and nonpredator cues under conditions
497 of variable predation risks (Ferrari et al. 2007, 2008), the same conditions that are
498 thought to favour learned predator recognition (Brown & Chivers 2005).

499

500 Despite the evidence showing remarkably sophisticated learned predator
501 recognition (Brown 2003; Brown & Chivers 2005), there is a wealth of examples of both
502 aquatic vertebrate and invertebrate prey that show an innate avoidance of novel predators.
503 For example, both Arctic charr, *Salvelinus alpinus* (Vilhunen & Hirvonen 2003) and
504 chinook salmon, *Oncorhynchus tshawytscha* (Berejikian et al. 2003) reared under
505 hatchery conditions (hence no previous experience with predators) showed predictable

506 antipredator responses when exposed to predator cues. Perhaps more interesting are the
507 growing number of studies showing a complex innate predator recognition in aquatic
508 invertebrates. For example, the freshwater gastropod *L. stagnalis* shows a strong response
509 to the odour of a predatory fish (tench, *Tinca tinca*), but no response to ground
510 conspecifics (relative to a control; Dalesman et al. 2007a). However, the response to
511 tench odour paired with conspecific odours resulted in a significantly stronger predator
512 avoidance response (Dalesman et al. 2007a). Similar ‘additive’ responses have been
513 shown for damselfly larvae (*Ischnura elegans*; Gyssels & Stoks 2006) and caddisfly
514 larvae (*Hesperophylax occidentalis*; Gall & Brodie 2009). Together, these studies suggest
515 that in systems where the costs associated with failing to respond to intense predation
516 pressure are high, selection may favour an innate (i.e. fixed) response to novel predator
517 cues (Brown & Chivers 2005).

518

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

528 research is required to identify the specific compounds or classes of compounds that
529 allow for differential recognition of predators.

530

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532

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540

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- 697

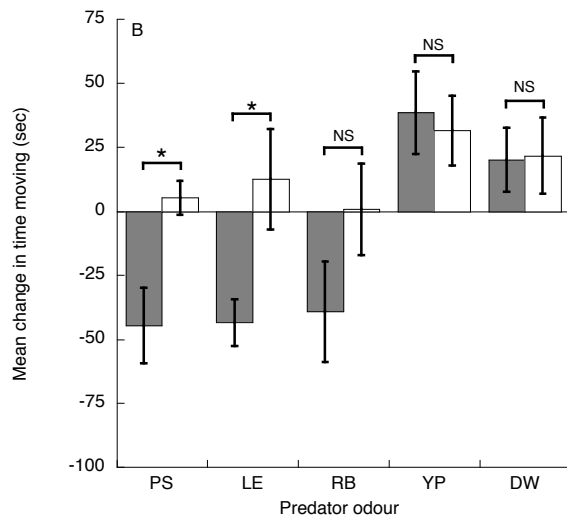
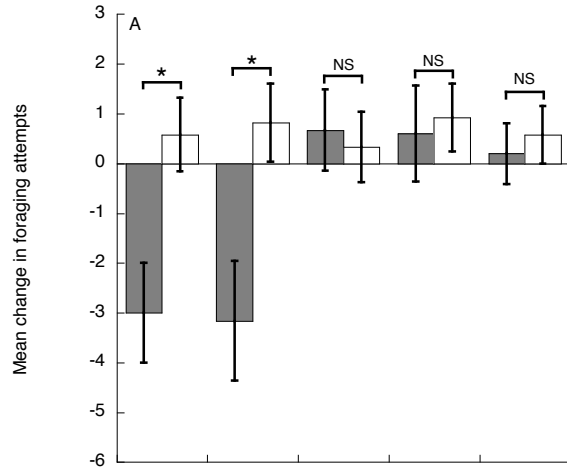
698 **Figure 1.** Mean \pm SE change in foraging attempts (a) and time spent moving (b) during
699 the recognition phase for trout initially conditioned with pumpkinseed odour + trout
700 alarm cue (dark bars) or pseudoconditioned with pumpkinseed odour + distilled water
701 (open bars) and tested for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

705

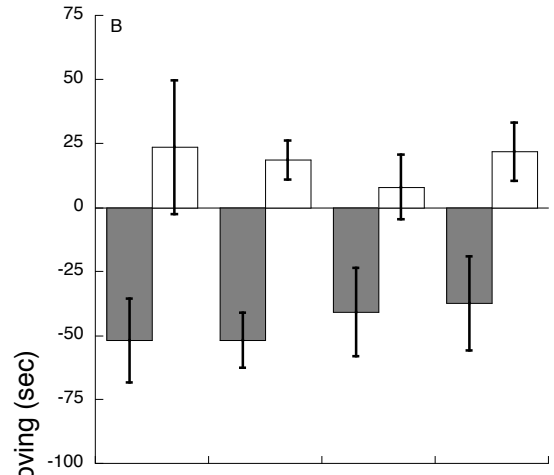
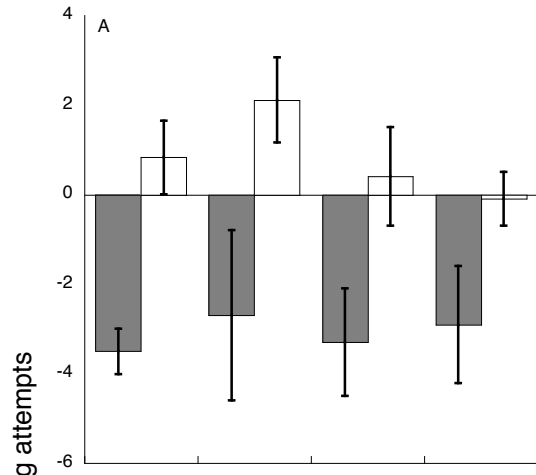
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707 **Figure 2.** Mean \pm SE change in foraging attempts (a, c) and time spent moving (b, d)
708 during the recognition phase for trout conditioned with one of four predator odours +
709 trout alarm cue (shaded bars; PS = pumpkinseed, LE = longear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

716



Pre-exposed to distilled water



Pre-exposed to pumpkinseed odour

