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

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

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 flavenscens*. 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 ± 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 °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   °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 °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 ± 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 homeoscedasticity) 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 ± 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   homoeoscedasticity) 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 pumkinseed 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 hetrospecifics 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 tshwaytscha* (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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540

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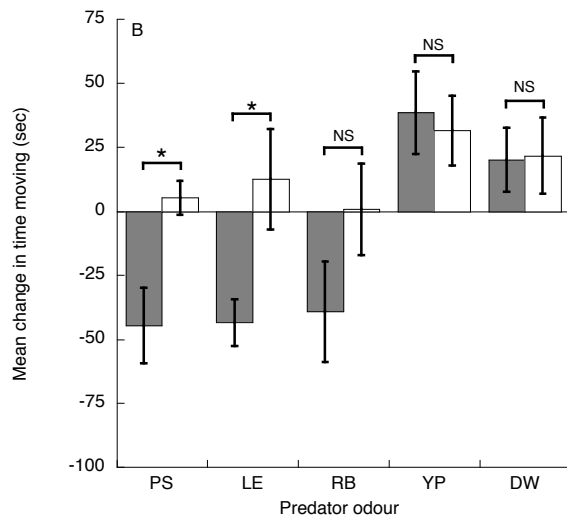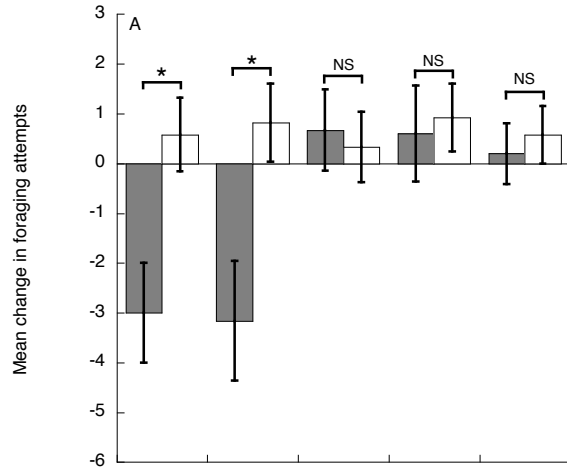696   odours by a prey fish. *Behaviour*, **143**, 1103-1121.

697

698   **Figure 1.** Mean ± 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

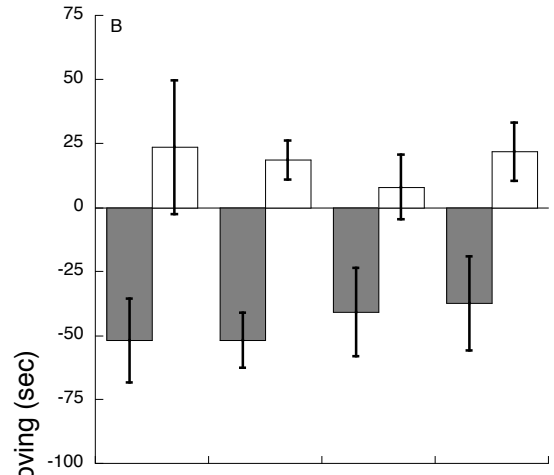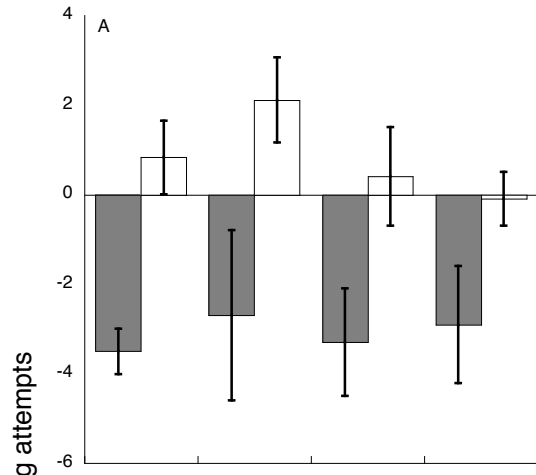704   and pseudoconditioned trout for each predator odour

705

706

707   **Figure 2.** Mean ± 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