1   Growth rate and retention of learned predator cues by juvenile rainbow trout: faster

2   growing fish forget sooner.

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17

18 **Abstract**

19   Under conditions of spatial and/or temporal variability in predation risk, prey

20 organisms often rely on acquired predator recognition to balance the trade-offs between

21 energy intake and risk avoidance. The question of 'for how long' should prey retain this

22 learned information is poorly understood. Here, we test the hypothesis that the growth

23 rate experienced by prey should influence the length of the 'memory window'. In a

24 series of laboratory experiments, we manipulated growth rate of juvenile rainbow trout

25 and conditioned them to recognize a novel predator cue. We subsequently tested for

26 learned recognition either 24 hours or 8 days post-conditioning. Our results suggest that

27 trout with high versus low growth rates did not differ in their response to learned predator

28 cues when tested 24 hours post-conditioning. However, trout on a high growth rate

29 exhibited no response to the predator cues after 8 days (i.e., did not retain the recognition

30 of the predator odour), whereas trout on a lower growth rate retained a strong recognition

31 of the predator. Trout that differed in their growth rate only after conditioning did not

32 differ in their patterns of retention, demonstrating growth rate after learning does not

33 influence retention. Trout of different initial sizes fed a similar diet (% body mass.day^{-1})

34 showed no difference in retention of the predator cue. Together, these data suggest that

35 growth rate at the time of conditioning determines the 'memory window' of trout. The

36 implications for threat-sensitive predator avoidance models are described.

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39

40   **Introduction**

41   The ability of prey to recognize potential predators is key to individual survival.

42   However, simply responding to any local threat may not represent an optimal strategy

43   (Lima and Dill 1990), as predation pressure is known to be spatially and temporally

44   variable (Griffin 2004; Lima and Steury 2005; Ferrari et al. 2009). A wide variety of

45   taxonomically diverse prey species rely, therefore, on associative learning (acquired

46   predator recognition) to assess the risk associated with potential predators (Brown 2003;

47   Griffin 2004). Learned, versus 'innate', predator recognition allows prey to make

48   dynamic adjustments to predation threats, and to balance the conflicting pressures of

49   predator avoidance and energy intake (i.e., threat-sensitive learning; Ferrari et al. 2005;

50   Ferrari and Chivers 2006; Gonzalo et al. 2010). Within aquatic ecosystems, such predator

51   recognition learning is often facilitated through the pairing of damage released chemical

52   alarm cues (Chivers and Smith 1998) with the sight or smell of a novel predator (Brown

53   2003). Acquired predator recognition has been shown to increase probability of survival

54   during staged encounters with live predators (Mirza and Chivers 2000; Darwish et al.

55   2005; Eiben and Persons 2007; Shier and Owings 2007).

56   Given that predation is indeed variable, a relevant, yet poorly understood question

57   is how long should prey exhibit (i.e., retain) an overt response to acquired information?

58   Following a single pairing of an alarm cue and a novel predator odour, hatchery reared

59   rainbow trout (*Oncorhynchus mykiss*) retain an overt learned response for up to 21 days

60   (Brown and Smith 1998) though the response begins to decline rapidly after 10 days

61   (Mirza and Chivers 2000). Likewise, Iberian green frog tadpoles (*Pelophylax perezi*)

62   retain a learned response for up to nine days following a single conditioning event

63   (Gonzalo et al. 2009). Presumably, prey should only respond to learned predator cues as

64   long as they represent an actual threat (Kotler 1992; Gonzalo et al. 2009).

65   Within the context of foraging decisions, several models have addressed the issue

66   of retention of learned responses (McNamara and Houston 1989; Mangel 1990; Hirvonen

67   et al. 1999). These models generally predict that there should exist a 'memory retrieval'

68   window (Shettleworth and Plowright 1992; Grubb and Pravosudov 1994; Dunlap et al.

69   2009) that allows for a flexible response pattern. Under relatively constant environmental

70   conditions, information regarding foraging decisions should be retained for a longer

71   period (i.e., remain within the memory window), whereas under highly variable

72   environmental conditions, older learned foraging information would be of lower value,

73   and hence be 'forgotten' (i.e., fall outside this window) more quickly (Kotler 1992;

74   Kraemer and Golding 1997; Kerr and Feldman 2003). Thus, such models predict that

75   learned information should only be retained as long as it is relevant (Pravosudov and

76   Clayton 2002; Brydges et al. 2008). Learned information that is no longer relevant is

77   forgotten (i.e., no longer capable of eliciting a behavioural response).

78   Recently, Ferrari et al. (2010) have developed an analogous model for the learned

79   response to predation threats. They suggest that a suite of extrinsic (i.e., predator

80   community diversity, predator encounter rates) and intrinsic (i.e., morphological

81   adaptations to predators, life history stage) factors should influence the duration of an

82   individual prey's memory window. One such factor is growth. As prey grow, they may

83   escape gape limits of potential predators (Nilsson and Brönmark 2000; Feary et al. 2009),

84   develop increased escape abilities (Bishop and Brown 1992) or reduce the rate of

85   encounter with predators by shifting habitat use (Werner et al. 1983). Thus, as prey

86   grow, the value of learned information regarding predator identity should decrease.

87   Accordingly, we could predict that higher growth rates would lead to a more rapid

88   decline in the value of this information, thus faster growing prey should 'forget' sooner

89   than slower growing prey. As such, growth may be predicted to influence the retention

90   of overt behavioural responses to learned predator recognition. Alternatively, size may

91   play a similar role in the shaping of the response to learned predator cues.

92   In a series of laboratory trials, we tested the hypothesis that growth rate will shape

93   the 'memory window' of juvenile rainbow trout conditioned to recognize a novel

94   predator odour. We predicted that trout fed a high versus low food ration (hence high vs.

95   low growth rate) would exhibit similar responses when tested for learned recognition 24

96   hours post-conditioning due to the high degree of relevance of recently acquired

97   information. However, when tested 8 days post-conditioning, we predict that trout fed

98   the high food ration should show a reduced (or absent) response to the acquired cue when

99   compared to those fed the low food ration. In addition, we conducted companion

100   experiments to test if growth post-conditioning (Experiments 2 and 3) or absolute size

101   (Experiment 4) had an effect on the retention of learned predator recognition.

102

103   **General Methods**

104   *Test fish*

105   Juvenile rainbow trout were obtained from a commercial supplier (Pisciculture Arpents

106   des Vert, Ste Edwidge-de-Clifton, Quebec) and transported to the laboratory. Trout were

107   the offspring of brood stock reared in the hatchery for between two to four generations,

108   crossed with wild caught adults. Prior to testing, trout were housed in 390 L recirculating

109   holding tanks (~18° C, pH ~7.0) under a 14:10 L:D cycle and fed ad libitum daily with

110   commercial trout chow (Corey Mills). Adult pumpkinseed (*Lepomis cyanellus*), used as

111   predator odour donors, were collected from Canal Lachine, Montreal, Qc. Pumpkinseed

112   were held under in similar tanks and fed a diet of brine shrimp and commercial cichlid

113   pellets daily. Under laboratory conditions, juvenile rainbow trout typically retain the

114   learned response to a novel predator odour for up to three weeks (Brown and Smith 1998)

115   though the response begins to wane after approximately 10 to 14 days (Mirza and Chivers

116   2000). Thus, we chose to test either 24 hours or 8 days post-conditioning to ensure that

117   we would be able to detect a learned response if present.

118

119   *Stimulus production*

120   We generated trout alarm cues from 15 juvenile trout (mean ± SD fork length =

121   5.60 ± 0.41 cm). Donor trout were killed via cervical dislocation (in accordance with

122   Concordia University Animal Research Ethics Committee protocol #AREC-2008-

123   BROW) and skin fillets were removed from either side of the donors and immediately

124   placed into chilled distilled water. Skin fillets were then homogenized, filtered through

125   polyester filter floss, and diluted to the desired volume with the addition of distilled

126   water. We collected a total of 108 cm^2 of skin (in a final volume of 1080 ml of distilled

127   water). Chemical alarm cues were frozen in 20 ml aliquots at -20°C until needed. As a

128   control, we also froze 20 ml samples of distilled water.

129   In order to collect the odour of a novel predator, we placed four pumpkinseed

130   (11.20 – 14.5 cm S.L.) into unfiltered 37-L glass aquaria, filled with 15 L of

131   dechlorinated tap water for 3 days. Pumpkinseed were not fed during this period. For at

132   least one week prior to odour collection, we fed pumpkinseed only brine shrimp, to

133   ensure that the resulting predator odour did not contain any diet related cues that may

134   have been recognized by the test fish. After the 3-day period, the water from each donor

135   tank was pooled, filtered and frozen in 50 ml aliquots at -20°C until needed.

136

137   *Conditioning phase*

138   For each of the four experiments (see below), we placed groups of 10 juvenile

139   trout into each of four 'conditioning' tanks. Conditioning tanks consisted of a series of

140   60 l glass aquaria, each equipped with a power filter and a gravel substrate (~18° C, pH

141   ~7.0). Trout were placed in the tanks either 8 days (Experiments 1, 3, and 4) or 1 day

142   (Experiment 2) prior to conditioning. To condition trout to recognize a novel predator

143   odour, we introduced 20 ml of predator odour paired with 20 ml of trout alarm cue. As a

144   control, we 'pseudo-conditioned' equal numbers of trout by introducing 20 ml of distilled

145   water paired with 20 ml of predator odour. In all cases, we turned the power filter off

146   approximately 10 minutes prior to conditioning and left it off for 1 hour following

147   conditioning. Conditioning occurred between 10:00 and 11:00. The day of conditioning

148   (or pseudo-conditioning controls) is considered Day 1 of the experiment.

149

150   *Recognition phase*

151   Approximately 6 hours after conditioning, we transferred 4 or 5 individual trout

152   from each of the four conditioning tanks (i.e. each of the treatment combinations) to test

153   aquaria for recognition testing on Day 2. The remaining trout were fed on the appropriate

154   diet until Day 8 and then transferred to individual test tanks for recognition testing on

155 Day 9. Test tanks consisted of a series of 37 L glass aquaria, each equipped with a single

156 airstone and a gravel substrate. Test tanks were not filtered. In addition, we attached a 2

157 m length of plastic tubing to the back wall of the test tanks to allow for the introduction

158 experimental cues without disturbing test fish.

159   Recognition trials consisted of a 5 minute pre-stimulus and a 5 minute post-

160 stimulus observation period. Prior to the pre-stimulus observation, we withdrew and

161 discarded 60 ml of tank water through the plastic tubing. We then withdrew and retained

162 an additional 60 ml of water. Immediately following the pre-stimulus observation period,

163 we injected 10 ml of predator odour and slowly flushed it into the tank with the retained

164 water. During both the pre- and post-stimulus observation periods, we recorded the time

165 spent moving and the frequency of foraging attempts. We fed test fish approximately 30

166 minutes prior to testing, to ensure activity, with a standard amount of powdered trout

167 chow (~ 0.1 g of powdered chow). Sufficient food was presented such that there were

168 enough food particles remaining during the behavioural observations to quantify foraging

169 without the need for additional food (Vavrek et al. 2008). A feeding attempt was defined

170 as a pecking movement towards a food particle, either on the substrate or in the water

171 column. A reduction in movement and foraging rate are consistent with increased

172 predator avoidance behavior in juvenile salmonids (Martel and Dill 1993; Brown and

173 Smith 1997; Leduc et al. 2009). In all cases, the observers were blind to the treatment.

174

175 Experiment 1: different food rations **before and after** conditioning

176   In order to test the effects of growth on the strength of conditioning and

177 expression of learned recognition, we fed groups of trout either 1% or 5% of mean group

178 body mass day^{-1} for a period of 7 days prior to conditioning. For trout tested on Day 9,

179 we continued to feed the same relative diet, adjusted for the reduced number of fish

180 remaining in the conditioning tanks. We chose these diets because 1% represents a

181 minimum growth food ration and 5% represents an abundance of food, allowing for high

182 relative growth (Alsop and Wood 1997; Kamunde and Wood 2003; Brown et al. 2009a).

183 Following testing on Day 2 or Day 9, individuals were blotted on sterile paper and

184 measured (mass to the nearest 0.001 g and standard length to the nearest 0.5 mm). Each

185 conditioning block yielded four test fish per treatment combination. We tested four

186 complete blocks (n = 4 per conditioning stimulus, for a total of 16 trout per treatment

187 combination (N = 128)). Mean (± SD) fork length was 3.25 ± 0.31 cm (see Figure 1A for

188 mean mass).

189   We calculated the change in time spent moving and foraging attempts (post-

190 stimulus – pre-stimulus) and used these difference scores as dependent variables in all

191 analyses. We compared pre-stimulus time moving and foraging rates between the 1%

192 and 5% food ration treatments for trout tested on Day 2 (MANOVA: $F_{2, 61}$ = 0.78, P =

193 0.46) and Day 9 (MANOVA: $F_{2, 61}$ = 1.97, P = 0.15) and found no difference in baseline

194 activity, justifying the use of difference scores as dependent variables. We tested for the

195 overall effects of conditioning stimulus (alarm cue versus distilled water), diet (1%

196 versus 5%) and recognition test day (Day 2 versus Day 9) using univariate GLM

197 ANOVAs. To account for any effect of 'conditioning block', we included this as a

198 random variable. Given the significant overall interaction between the three independent

199 variables (see below), we tested for the effects of conditioning stimulus and diet for Day

200   2 and Day 9 separately. Data met the assumptions for parametric analysis (i.e. were

201   normally distributed and homoscedastic).

202

203   Experiment 2: different food rations **after** conditioning

204   In order to test for the possibility that the observed results of experiment 1 (see

205   below) were due to growth after conditioning, rather than differences in growth prior to

206   conditioning, we conducted this experiment. We placed groups of 10 trout into the

207   conditioning tanks 24 hours prior to conditioning, with no prior differences in food

208   ration. Following conditioning, trout were fed either 1% or 5% diets as in Experiment 1.

209   Sample sizes were the same as Experiment 1. Mean ($\pm$ SD) fork length at time of testing

210   was 3.42 ± 0.32 cm (Figure 1B for mean mass). Data met the assumptions of parametric

211   tests and were analyzed as above. As in Experiment 1, we found no difference in

212   baseline activity levels (MANOVA: $F_{2,61} = 0.94$, $P = 0.39$ and $F_{2, 61} = 0.26$, $P = 0.77$ Day

213   2 and 9 respectively).

214

215   Experiment 3: different food rations **prior** to conditioning

216   As a further control experiment, we fed groups of trout a diet of 1% or 5% mean

217   body mass for a period of 7 days prior to conditioning. Following conditioning, trout

218   were either tested on Day 2 or fed a common diet of 1% mean body mass day^{-1} for a

219   further 8 days. We tested a total of 4 blocks ($n = 5$ per block, $N = 160$). Mean ($\pm$ SD)

220   fork length at time of testing was 3.15 ± 0.28 cm (Figure 1C for mean mass). Data met

221   the assumptions of parametric tests and were analyzed as above. As in Experiment 1,

222   there was no difference in baseline activity (MANOVA: $F_{2,77} = 2.37$, $P = 0.10$ and $F_{2, 77} =$

223   1.52, $P = 0.23$, Day 2 and 9 respectively).

224

225   Experiment 4: different absolute size, same food rations.

226   In order to test the possibility that absolute size, rather than growth rate *per se*, is

227   driving our observed results, we tested juvenile rainbow trout of differing size under

228   similar conditions to experiment 1. We tested small (3.60 ± 0.34 cm) and large ($5.71 \pm$

229   0.22 cm) trout (Figure 1D for mean mass). We fed trout in the conditioning tanks a diet

230   of 1% mean body mass per day for a period of 7 days prior to conditioning. Trout were

231   conditioned as in Experiment 1 and those to be tested on Day 9, we continued the 1%

232   mean body mass day^{-1}. We tested a total of 4 blocks (n = 4 per block per treatment

233   combination, N = 128). Data met the assumptions of parametric tests and were analyzed

234   as above. Though there was a trend towards smaller trout being more active than larger

235   trout, baseline activity was not different for trout tested on Day 2 (MANOV: $F_{2,61} = 3.05$,

236    $P = 0.052$), nor Day 9 ($F_{2,61} = 2.97$, $P = 0.058$).

237

238   **Results**

239   Experiment 1:

240   Our initial analysis revealed no effect of the blocking variable for either the

241   change in time spent moving or foraging attempts ($F_{3, 117} = 1.51$, $P = 0.217$ and $F_{3, 117} =$

242   1.34, $P = 0.26$ respectively). We found significant three-way interactions between

243   conditioning stimulus, diet and recognition test day for both change in time moving ($F_{4,}$

244    $_{117} = 3.92$, $P = 0.005$) and foraging response variables ($F_{1, 117} = 2.51$, $P = 0.046$; Figure 2).

245   When testing for the effects of conditioning stimulus and diet for Day 2 recognition trials

246   alone, we found significant effects of conditioning stimulus for both change in time spent

247   moving ($F_{1, 57} = 36.42$, $P < 0.001$) and change in foraging attempts ($F_{1, 57} = 18.53$, $P <$

248   0.001), but no effect of diet for either behavioural measure (change in time moving: $F_{1, 57}$

249    $= 0.11$, $P = 0.74$; change in foraging attempts: $F_{1, 57} = 0.15$, $P = 0.70$). Moreover, there

250   was no significant two-way interaction for either change in time moving ($F_{1, 57} = 3.47$, P

251    $= 0.51$) or change in foraging attempts ($F_{1, 57} = 0.65$, $P = 0.42$; Figure 2). Trout fed on

252   both high and low food diets exhibited similar learned responses to the predator odour.

253   However, we found a considerably different response pattern when testing for

254   expression of learned predator odours on Day 9. We found significant conditioning

255   stimulus x diet interactions for change in time moving ($F_{1, 57} = 6.05$, $P = 0.017$) and

256   change in foraging attempts ($F_{1, 57} = 4.42$, $P = 0.04$), demonstrating that only the trout fed

257   the low food regime (1% mean body mass per day), and not the high food regime,

258   exhibited an overt antipredator response on day 9 (Figure 2).

259

260   Experiment 2:

261   When trout did not experience different feeding regimes prior to conditioning, we

262   found no evidence that diet post-conditioning (1% v. 5%) had an effect on the expression

263   of acquired predator recognition. Trout fed either the high versus low food diet following

264   conditioning exhibited similar responses to the learned predator odour on both Day 2 and

265   Day 9 of recognition testing. We found a significant effect of conditioning stimulus for

266   both change in time spent moving ($F_{1, 117} = 22.86$, $P < 0.001$) and change in foraging

267   attempts ($F_{1, 117} = 21.94$, $P < 0.001$; Figure 3), but no effect of diet (change in time

268   moving: $F_{1, 117} = 0.05$, $P = 0.82$; change in foraging attempts, $F_{1, 117} = 0.81$, $P = 0.37$) or

269   recognition test day (change in time moving: $F_{1, 117} = 0.004$, $P = 0.95$; change in foraging

270   attempts: $F_{1, 117} = 0.07$, $P = 0.78$). Moreover, there was no 3-way interaction (change in

271   time moving: $F_{4, 117} = 0.16$, $P = 0.96$; change in foraging attempts: $F_{4, 117} = 0.45$, $P =$

272   0.78). As with Experiment 1, there was no effect of the blocking variable for either

273   behavioral measure (change in time moving: $F_{3, 117} = 0.65$, $P = 0.56$; change in foraging

274   attempts: $F_{3, 117} = 1.79$, $P = 0.15$).

275

276   Experiment 3:

277   When trout were fed different feeding regimes for one week prior to conditioning

278   and then switched to the same proportional regime (1% mean body mass day^{-1}), the

279   results were strikingly similar to those of experiment 1. For the overall analyses, we

280   found significant interactions among conditioning stimulus, diet and recognition test day

281   for both change in time moving ($F_{4, 149} = 2.47$, $P = 0.047$) and the change in foraging

282   attempts ($F_{4, 149} = 2.52$, $P = 0.044$; Figure 4). When we separated the dataset based on

283   recognition test day (as in Experiment 1), we found no evidence of an interaction

284   between conditioning stimulus and diet on the recognition of predator cues on Day 2

285   (change in time moving: $F_{1, 73} = 0.43$, $P = 0.51$; change in foraging attempts: $F_{1, 73} =$

286   0.014, $P = 0.90$), but we did find a significant interaction for both change in time moving

287   ($F_{1, 73} = 4.03$, $P = 0.048$) and change in foraging attempts ($F_{1, 73} = 5.90$, $P = 0.018$; Figure

288   4) on Day 9. As in Experiment 1, there was no overall effect of the blocking variable for

289   either change in time moving ($F_{3, 149} = 0.64$, $P = 0.59$) or change in foraging attempts (F_3,

290    $_{149} = 0.41$, P = 0.74). This finding further supports the hypothesis that growth rate at the

291   time of conditioning influences expression of learned information.

292

293   Experiment 4:

294   When trout of different initial sizes were fed proportionally the same diet (1%

295   mean body mass day^{-1}), we found a significant effect of conditioning stimulus on the

296   learned recognition of the novel predator odour (change in time moving: $F_{1, 117} = 21.16$, P

297   < 0.001; change in foraging attempts: $F_{1, 117} = 38.16$, P < 0.001; Figure 5). There were no

298   significant effects of size class (change in time moving: $F_{1, 117} = 0.06$, P = 0.80; change in

299   foraging attempts: $F_{1, 117} = 0.08$, P = 0.77), day of recognition testing (change in time

300   moving: $F_{1, 117} = 0.25$, P = 0.62; change in foraging attempts: $F_{1, 117} = 0.08$, P = 0.77) nor

301   an interaction among the three (change in time moving: $F_{4, 117} = 0.14$, P = 0.97; change in

302   foraging attempts: $F_{4, 117} = 0.15$, P = 0.98; Figure 5). As above, there was no significant

303   effect of the blocking variable (change in time moving: $F_{3, 117} = 2.20$, P = 0.09; change in

304   foraging attempts: $F_{3, 117} = 2.15$, P = 0.10). These results suggest that absolute size, at

305   least within this size range, cannot explain the results of Experiment 1.

306

307   **Discussion**

308   Recent models concerning the retention of ecologically relevant learning predict

309   that the 'value' of information should decrease over time and that a suite of factors,

310   including growth rate, will influence this rate of information loss (McNamara and

311   Houston 1989; Mangel 1990; Hirvonen et al. 1999; Ferrari et al. 2010). Our current

312   study provides support for the prediction that an individual's rate of growth at the time it

313   acquires new information will influence its retention. The results of Experiment 1

314   suggest that growth rate at the time of conditioning influences the 'memory window' for

315   juvenile rainbow trout. Trout fed the higher food ration (hence higher growth rate) did

316   not differ from those fed the lower food ration when tested on day 2, demonstrating that

317   growth rate does not influence the strength of the learned response. However, when

318   tested on Day 9, only those fed the lower food ration exhibited expression of the response

319   to the predator odour; trout fed the high food ration did not differ from the pseudo-

320   conditioned controls. Alternatively, we might have predicted that energy costs associated

321   with retention may be a significant factor shaping the observed memory window (i.e.,

322   Fitting et al. 2008). If this were the case, however, we would expect trout on the lower

323   growth trajectory to show reduced retention. While it is tempting to state that our trout

324   fed the high food ration 'forgot' sooner than those fed the low food ration, we cannot

325   differentiate between the neurological loss of recognition (Speed 2000; Wixted 2004) and

326   the possibility that trout recognized the predator odour but fail to respond due to a

327   behavioural decision. Indeed, the absence of an overt response does not mean that the

328   memory of learned information is not present (Kraemer and Golding 1997; Skow and

329   Jakob 2006).   Regardless of the underlying mechanism, differences in the expression of

330   acquired predator recognition will likely have considerable functional consequences.

331   Ferrari et al. (unpublished ms) have found similar results with woodfrog tadpoles

332   (*Rana sylvatica*). Tadpoles with higher growth rates exhibited reduced retention

333   compared to tadpoles with lower growth rates. However, their study suggests that growth

334   rate at the time of conditioning and following conditioning both contribute to shape

335   retention. This differs from our current study since the results of Experiments 2 and 3

336   suggest that growth after the initial conditioning phase does not have any detectable

337   effect on the expression of an overt response to the predator cue. Alternatively, absolute

338   size and not growth rate *per se* may influence the expression of predator recognition. At

339   the time of conditioning (Experiment 1), trout on the high food ration were considerably

340   larger than those on the low food ration (0.85 ± 0.03 versus 0.66 ± 0.03 g). This,

341   however, is an unlikely explanation given the results of Experiment 4. When fed a

342   similar diet (% body mass day^{-1}), large and small trout exhibited similar retention

343   patterns. Moreover, it could be argued that low body condition is linked to reduced

344   retention. If this were the case, then we would have expected to see the trout on the

345   lower growth trajectory (presumably lower condition) to have lower retention. Taken

346   together, these results provide strong evidence that growth rate at the time of conditioning

347   fixes the memory window of learned predator recognition.

348   Regardless of the underlying mechanism, it is clear that factors that increase the

349   value of learned information should extend the 'memory window', while factors that

350   reduce its relative value should result in the loss of response. The model of a flexible

351   'memory window' predicts that learned or acquired information should be retained

352   (remain within the window) only as long as it is relevant (Cuthill et al. 1990; Kraemer

353   and Golding 1997). Moreover, factors that reinforce the relevance of information, either

354   positively or negatively, will influence how long they remain within the window (Kerr

355   and Feldman 2003; Dunlap et al. 2009). For example, retention of learned foraging

356   information such as food caches increases as the predictability of finding food decreases

357   (Cuthill et al. 1990; Prasvosudov and Clayton 2001).

358   Under variable habitat conditions, responding to learned information should be

359   considered adaptive, as it would increase flexibility in an individual's behavioural

360   repertoire (Kerr and Feldman 2003; Griffin 2004; Lima and Steury 2005). Recent models

361   (White 2001; Dunlap et al. 2009) suggest that as an individual's 'cost of living' varies,

362   the duration that learned information remains relevant (i.e., remains within the memory

363   window) should likewise vary. Factors such as variability in abundance or predictability

364   in finding suitable food sources and/or ambient predation pressure should be expected to

365   influence an individual's 'cost of living'. As the cost of living increases, the memory

366   window for acquired information should become longer as learned information remains

367   relevant (Kotler 1992; Kerr and Feldman 2003; Dunlap et al. 2009). Conversely, reduced

368   cost of living should result in extended memory windows (Dunlap et al 2009).

369   Presumably, factors such as reduced food availability or predictability and increased

370   predation pressure should result in increased pressure on individuals to balance threat-

371   sensitive trade-offs (Helfman 1989; Brown et al. 2006; 2009b; Roitberg et al. 2010). As

372   such, our observed differential response to learned predator recognition may result in

373   behavioural response patterns consistent with threat-sensitive trade-offs. When applied to

374   the question of retention of learned predator recognition, conditions leading to high

375   growth rates (high availability and/or predictability of food) likely translate to a relatively

376   low cost of living (Kerr and Feldman 2003; but see Biro and Stamps 2008; Adrianenssens

377   and Johnsson in press). At the same time, high growth should lead to a reduced

378   individual risk of predation due to greater ability to escape predator (Bishop and Brown

379   1992), reduced risk of gape limited predators (Nilsson and Brönmark 2000; Feary et al.

380   2009) and/or a higher energy reserve (Reinhardt and Healy 1999). As such, the retention

381   period should be expected to decrease (i.e., prey 'forget' sooner). Conversely, low

382   growth rates and the increased risk of predation associated with smaller body sizes and/or

383   reduced energy stores (Reinhardt and Healy 1999) may result in elevated costs of living

384   for prey individuals, leading to increased retention of acquired information (Dunlap et al.

385   1999). Overall, any devaluation of learned information may manifest as differing

386   behavioural strategies, with faster growing trout exhibiting a risk prone response pattern,

387   while slower growing trout showing a more risk aversive pattern.

388   Recent research shows that threat-sensitive trade-offs may shape the intensity of

389   learning in prey organisms (Ferrari et al. 2005; Ferrari and Chivers 2006; Zhao et al.

390   2006; Gonzalo et al. 2010). These studies demonstrate that the intensity of the learned

391   response to a novel predator cue is proportional to the strength of the initial conditioning.

392   Such a learning mechanism would allow prey to respond to acquired cues with a response

393   intensity proportional to the initial level of perceived risk. Our current study builds on

394   these results, suggesting that threat-sensitive trade-offs after the initial conditioning event

395   may also shape the response intensity. In each of the current studies, the initial strength

396   of the experimental conditioning phases (alarm cue + predator odour) was the same.

397   Thus, it is not surprising that we found no difference between the diet (size) treatments

398   when trout were tested on Day 2. Rather, the observed retention effects (Experiments 1

399   and 3) on Day 9 likely represent the product of threat-sensitive trade-offs at the time of

400   recognition testing and not due to differences in the initial conditioning events.

401   The applied goal of predator recognition training, at least for salmonids, has

402   always been to condition hatchery reared fish prior to stocking in hopes of increasing

403   post-stocking survival (Berejikian et al. 1999; Brown and Smith 1998; Brown and Laland

404   2001). There is some support that this methodology works to increase survival

405   (Berejikian et al. 1999; Mirza and Chivers 2000). However, recent studies by Wisenden

406   et al. (2004) and Hawkins et al. (2007) suggest that conditioned hatchery reared fish do

407   not exhibit any evidence of learned recognition of predator cues when tested under fully

408   natural conditions. Our current results suggest that one possible explanation for these

409   findings is that hatchery fish, which presumably would be fed on a high growth rate diet,

410   are simply not showing the expression of learned information due to diet-related threat-

411   sensitive trade-offs. An intriguing possibility would be to test the effects of dietary

412   restriction prior to conditioning and subsequent release. Future studies should address

413   this possibility.

414

415   **Acknowledgements**

423

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578

579   **Figure captions:**

580   Figure 1: Mean (± SE) mass of juvenile rainbow trout tested in each of the four

581   experiments. Experiment 1 (panel A): trout were fed either 1% or 5% mean body mass

582   per day for the duration of the study. Experiment 2 (panel B): trout were fed 1% or 5%

583   from the time of conditioning (Day 1) with no pre-conditioning feeding phase.

584   Experiment 3 (panel C): trout were fed 1% or 5% for one week prior to conditioning and

585   then fed only 1% until testing on Day 9. Experiment 4 (panel D): trout were fed similar

586   (1%) diets, but differed in initial mass.

587

588   Figure 2: Mean (± SE) change in time spent moving (panel A) and foraging attempts

589   (panel B) for trout tested in Experiment 1. Dark bars denote trout initially conditioned

590   with alarm cue + predator odour and open bars denote trout initially conditioned with

591   distilled water + predator odour. Trout fed on high food (5%) or low food (1%) diets

592   throughout the course of the experiment were tested for recognition of the predator odour

593   24 hours post-conditioning (Day 2) or eight days post-conditioning (Day 9). N = 16 for

594   each treatment combination.

595

596   Figure 3: Mean (± SE) change in time spent moving (panel A) and foraging attempts

597   (panel B) for trout tested in Experiment 2. Dark bars denote trout initially conditioned

598   with alarm cue + predator odour and open bars denote trout initially conditioned with

599   distilled water + predator odour. Trout were fed high food (5%) or low food (1%) diets

600   from Day 1 (conditioning) onwards and tested for recognition of the predator odour 24

601   hours post-conditioning (Day 2) or eight days post-conditioning (Day 9). N = 16 for each

602   treatment combination.

603

604   Figure 4: Mean (± SE) change in time spent moving (panel A) and foraging attempts

605   (panel B) for trout tested in Experiment 3. Dark bars denote trout initially conditioned

606   with alarm cue + predator odour and open bars denote trout initially conditioned with

607   distilled water + predator odour. Trout were fed high food (5%) or low food (1%) diets

608   for one week prior to conditioning; after which all trout to be tested on Day 9 were fed

609   the low food ration. Trout were tested for the recognition of the predator odour 24 hours

610   post-conditioning (Day 2) or eight days post-conditioning (Day 9). N = 20 for each

611   treatment combination.

612

613   Figure 5: Mean (± SE) change in time spent moving (Panel A) and foraging attempts

614   (panel B) for trout tested in Experiment 4. Dark bars denote trout initially conditioned

615   with alarm cue + predator odour and open bars denote trout initially conditioned with

616   distilled water + predator odour.   Trout differed in initial mass and were fed a similar

617   diet (1%) throughout the course of the experiment. Trout were tested for the recognition

618   of the predator odour 24 hours post-conditioning (Day 2) or eight days post-conditioning

619   (Day 9). N = 16 for each treatment combination.

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