

1 NOTICE: this is the author's version of a work that was accepted for
2 publication in *Animal Behaviour*. Changes resulting from the publishing
3 process, such as peer review, editing, corrections, structural formatting, and
4 other quality control mechanisms may not be reflected in this document.
5 Changes may have been made to this work since it was submitted for
6 publication. A definitive version was subsequently published in *Animal*
7 *Behaviour* 79: 1025-1030. DOI: 10.1016/j.anbehav.2010.01.017
8

9 **Colour change and assortment in the western rainbowfish**

10

11 Gwendolen M. Rodgers^{a,*}, Jennifer L. Kelley^{b,1} Lesley J. Morrell^a

12

13

14 ^a Institute of Integrative and Comparative Biology, University of Leeds

15 ^b School of Animal Biology, University of Western Australia

16 Received 7 October 2009

17 Initial acceptance 2 November 2009

18 Final acceptance 22 January 2010

19 MS. number: 09-00660

20

21

22 * Correspondence: G. M. Rodgers, Genetics, Ecology and Evolution Group,

23 Institute of Integrative and Comparative Biology, Miall Building, University of

24 Leeds, Leeds LS2 9JT, U.K.

25 E-mail address: bsgmr@leeds.ac.uk (G. M. Rodgers).

26

27 ¹ J. L. Kelley is at the Centre for Evolutionary Biology, Zoology Building,

28 School of Animal Biology (M092), The University of Western Australia,

29 Nedlands, WA 6009, Australia.

30

31 Grouping behaviour is widespread across the animal kingdom, and is known
32 to reduce an individual's risk of predation, for example through predator confusion.
33 Theory predicts that individuals that are different in appearance to the rest of the
34 group are at a greater risk of predation because they are more conspicuous to
35 predators (the 'oddity' effect). Thus, animals should choose group mates that are the
36 most similar in appearance to themselves. Another common antipredator tactic is
37 crypsis (camouflage). Fishes are capable of changing colour to match their visual
38 background, but few studies have examined how this might influence shoaling
39 decisions, particularly in the context of the oddity effect. We induced colour pattern
40 changes in a colourful species of freshwater fish, the western rainbowfish,
41 *Melanotaenia australis*, by maintaining fish in dark and pale aquaria for 2 weeks.
42 Analysis of the proportion of black body pigmentation confirmed that rainbowfish in
43 dark environments developed darker colour patterns than those held in pale
44 environments. We then conducted behavioural observations to determine whether
45 fish subsequently based their shoaling decisions on body coloration. We found that
46 rainbowfish preferred to shoal with similar individuals; fish that had been held in dark
47 aquaria preferred to shoal with other dark fish and fish from pale aquaria preferred
48 other pale fish. Our findings are consistent with the predictions of the oddity effect
49 and demonstrate how morphological colour pattern changes and behavioural
50 decisions interact to mediate antipredator tactics in fish.

51

52

53 Keywords

54 colour, colour change, *Melanotaenia australis*, oddity, shoaling, social behaviour,
55 rainbowfish

56

57 Animals that live in groups benefit through a reduction in predation risk, via a
58 variety of mechanisms (Krause & Ruxton 2002). Two of these, the 'odddity'
59 (Theodorakis 1989) and 'confusion' (Landeau & Terborgh 1986; Krakauer 1995)
60 effects rely at least partially on individuals within a group having similar phenotypic
61 characteristics. The oddity effect describes the preferential targeting of individuals
62 that are phenotypically or spatially distinct from others in the group (Ohguchi 1978),
63 while the confusion effect results from attempts by predators to track multiple moving
64 individuals within a group, and is reduced if some individuals differ from others
65 (Landeau & Terborgh 1986). Phenotypically distinct individuals, therefore, are at
66 enhanced risk of capture by predators, and predator preferences for odd phenotypes
67 should result in selection for behaviours that lead to assortment on the basis of
68 phenotypic characteristics. As a result, group-living animals assort into nonrandom
69 groups. This has been particularly well studied in shoaling fishes, where assortment
70 by species (Ward et al. 2002), body size (Theodorakis 1989), parasite load (Krause &
71 Godin 1996) and colour pattern is common.

72

73 Studies that have investigated the role of colour patterns in grouping
74 decisions in fishes have found preferences for shoalmates with similar coloration. For
75 example, black and white morphs of sailfin mollies, *Poecilia latipinna*, and black and
76 golden morphs of freshwater angelfish, *Pterophyllum scalare*, prefer to associate with
77 members of the same colour morph (McRobert & Bradner 1998; Gómez-Laplaza
78 2009). Similarly, shoal choice trials with different strains of zebrafish, *Danio rerio*,
79 have revealed preferences for shoalmates most similar to their own phenotype
80 (Engeszer et al. 2004; Rosenthal & Ryan 2005). However, these studies, and those
81 examining predation risk in relation to colour patterns, have used either dyed prey
82 (Landeau & Terborgh 1986), domestic morphs (McRobert & Bradner 1998; Gómez-
83 Laplaza 2009) or artificially selected laboratory strains (Rosenthal & Ryan 2005;

84 Engeszer et al. 2007), rather than drawing on natural variation in body coloration, on
85 which predators must base their choice of prey.

86

87 Some animals, in particular fishes, reptiles, amphibians and cephalopods, are
88 able to change their body colour by controlling the dispersion of colour pigments in
89 their skin (Parker 1948; Waring 1963; Bagnara & Hadley 1973). These colour
90 changes can be used for social signalling (Bradbury & Vehrencamp 1998) but are
91 more often associated with predator defence and, in particular, camouflage through
92 background matching, where the animal's body coloration matches the visual
93 background (Edmunds 1974; Endler 1978). For example, several species of octopus
94 demonstrate dynamic background matching when moving slowly over changing
95 substrates (Hanlon et al. 1999) and mimic various venomous animals when moving
96 more quickly or encountering specific predatory threats (Hanlon et al. 1999; Norman
97 et al. 2001). Specific predators have been seen to elicit different colour change
98 responses in other species too. For example in the neotropical treefrog,
99 *Dendropsophus ebraccatus*, colour pattern changes that enhance warning,
100 misleading or cryptic coloration are all possible (Touchon & Wartenkin 2008). In
101 juvenile pumpkinseed sunfish, *Lepomis gibbosus*, the opercular tab marking faded
102 when presented with the image of a predator (largemouth bass, *Micropterus*
103 *salmoides*; Rowland 1999).

104

105 Colour change according to background is particularly well known for fishes
106 and has been studied since the 1930s. These early experiments showed that
107 mosquitofish, *Gambusia patruelis* (now *G. affinis*) changed their body colour
108 (becoming darker or lighter) to match their background environment after being kept
109 in black or white tanks for 72 days (Sumner 1935a, b). Furthermore, Sumner (1935a,
110 b) demonstrated a survival benefit from this colour change as predators (green
111 sunfish, *Apomotis cyanellus*, now *Lepomis cyanellus*) were more likely to target light-

112 coloured mosquitofish than dark ones when trials were conducted in a black tank.
113 Sumner (1935b) noted that colour differences between the two treatment groups
114 were visibly reduced after several hours but remained discernable (to the human
115 eye) over several days when the fish were placed on a common background. These
116 morphological colour changes are under hormonal control (Sugimoto 2007) and last
117 a considerable period of time, during which the fish may have entered a different
118 habitat where its coloration no longer matches the visual background. However,
119 colour change is just one of a suite of antipredator tactics and fishes may also display
120 behavioural responses such as habitat avoidance, shoaling, hiding and predator
121 inspection (Godin 1997; Kelley 2008). An effective overall antipredator response
122 therefore involves the integration of morphological and behavioural defences, yet few
123 studies have taken this approach.

124

125 We investigated the relationship between colour pattern change and grouping
126 (shoaling) decisions in a colourful species of freshwater fish, the western rainbowfish,
127 *Melanotaenia australis*. First, we induced changes in fish colour patterns by exposing
128 individuals to differently coloured environments (dark or pale aquaria) for 2 weeks.
129 Second, we used image analysis to compare the photographs of individuals taken
130 before and after they were placed in the dark/pale treatment tanks to confirm that
131 colour pattern changes (i.e. background matching) have occurred. Third, we
132 performed shoal choice trials to determine whether shoaling decisions are based on
133 environment-induced colour changes and, in particular, whether individuals show a
134 preference for shoalmates with similar colour patterns (as predicted by the oddity
135 effect).

136

137

138 **<H1>METHODS**

139

140 <H2>Study System

141

142 The western rainbowfish is a small freshwater fish, widespread across
143 northwestern Australia, and is a particularly small member of one of the most
144 common genera in the country (*Melanotaenia* spp.). Rainbowfish live in small,
145 dynamic shoals, are amenable to handling by humans, and have received attention
146 as a suitable study system for behavioural experiments (e.g. Brown & Warburton
147 1997; Brown 2002). Rainbowfish are brightly coloured and their body coloration
148 varies within and between populations (Allen et al. 2002).

149

150 The rainbowfish used in this experiment were captured from Wittenoom
151 Gorge, a tributary of the Fortescue River, in the Pilbara region of northwestern
152 Australia in May 2006. This population is subjected to moderate predation risk from
153 fish predators such as spangled perch, *Leiopotherapon unicolor*, and flathead gobies,
154 *Glossogobius giurus* (M. Young, personal communication). Fish were captured as
155 juveniles or young adults with a seine net and transported to the University of
156 Western Australia (see Ethical note for more information). The population was
157 maintained in mixed-sex groups in stock tanks (85x45 cm, and filled to a water depth
158 of 30 cm) until taking part in the experiments. Stock aquaria were maintained at 26
159 ± 1 °C with a light cycle of 12:12 h; this is comparable to their conditions in the wild,
160 where at the time of capture, the water was 50 cm deep, and at 23 °C. In the wild,
161 rainfall and water temperature fluctuate seasonally, being moderate in May, highest
162 in January - February and lowest in August - September. Stock conditions, therefore,
163 approximated late spring or early autumn field conditions. We used male test fish in
164 all of our experiments because they are larger and more brightly coloured than
165 females and may therefore be at greater risk from visual predators (Brown 1999).
166 Although male-only shoals may not represent the situation in the wild (where female-
167 dominated shoals are more likely, Brown 2002), we wanted to avoid the potential

168 confounding effect of courtship in our trials. Furthermore, male rainbowfish have
169 previously been used in dichotomous choice trials and have shown shoaling
170 preferences for other males (Arnold 2000). After experiments all fish were returned to
171 mixed-sex stock tanks for breeding purposes.

172

173 <H2>Colour Change Experiment

174

175 Forty-eight male rainbowfish of between 42 and 55 mm (mean \pm SD= 49.50 \pm
176 2.68 mm) standard body length were anaesthetized using clove oil (dose of 80
177 mg/litre as for the product AQUI-S; see Young 2009) and photographed in standard
178 lighting conditions with a Kodak EasyShare V1003 digital camera, set to standard
179 settings, before being placed in an aerated recovery tank for a minimum of 20 min.
180 After recovery, these fish were allocated to either 'dark' or 'pale' experimental
181 treatments ($N=24$ fish per treatment). We set up six tanks (24.5 x 29 cm and 21 cm
182 high, filled to a depth of 17 cm) for each experimental treatment. Each tank contained
183 an airstone and was covered with a fine mesh to prevent the fish from jumping
184 between tanks. The dark and pale environments were created by adding coloured
185 back and side walls, coloured gravel and a coloured artificial plant made from wool to
186 each aquarium. 'Dark' treatment tanks had black side walls and gravel, and a dark
187 brown back wall and artificial plant. 'Pale' treatments had white gravel and walls, and
188 a cream-coloured artificial plant. One wall was not coloured to allow monitoring of the
189 condition of the fish. Natural rainbowfish habitats contain substrates (gravel,
190 boulders, silt, plant and algal material) of various colours and shades. These colour
191 treatments represent the extremes of natural colours of different microhabitats.

192

193 All aquaria were maintained at 26 ± 1 °C) under identical lighting conditions,
194 with a light cycle of 12:12 h light:dark. Fish were fed flake food daily. Four males
195 were placed into each experimental tank and we ensured that each fish could be

196 individually identified by size. All males in each experimental aquarium had
197 previously been housed in the same stock tank as each other and hence were
198 potentially familiar with one another. As we observed some male - male aggression
199 during the experiment, we also added four (nonfocal) females to each aquarium to
200 create more natural social conditions. These females played no further part in the
201 experiments. Some aggressive males were removed during the experiment (see
202 Ethical note), leaving 21 for the pale treatment and 17 for the dark treatment.
203 Experimental fish were photographed again (following the procedure above) after
204 they had spent 1 week in the pale or dark treatment tanks.

205

206 'Before' and 'after' colour treatment photographs were identified for the same
207 individuals based on size differences between the fish in each treatment tank.
208 Photographs were then analysed using two image analysis software programs:
209 ImageTool Uthscsa version 3.0 (<http://ddsdx.uthscsa.edu/dig/itdesc.html>) for
210 converting to greyscale and counting black and white pixels, and ImageJ 1.38x
211 (<http://rsbweb.nih.gov/ij/index.html>) for measuring sizes, standardizing white balance,
212 selecting relevant image sections and pixels darker than standard 'black'. Neither
213 program could perform all of these operations, so both were necessary. Images were
214 first standardized for white balance, and then converted to greyscale. The
215 percentage of body colour that was darker than a standard 'black' (a value of 70 on a
216 0 – 255 scale where 0 is 'true' black) was measured (hereafter referred to as 'black
217 coloration'). Digital image analysis has previously been used for analysing colour
218 patterns in animals (e.g. Touchon & Warkentin 2008) and this method provides a
219 simple way of summarizing changes in lightness without focusing on a particular
220 animal's visual system (Bennett et al. 1994; Stevens et al. 2007). Black was chosen
221 after initial observations (of fish assigned to the 'dark' treatment tanks) indicated that
222 body coloration became darker, and the extent of black pigmentation increased.
223 Indeed, changes in the dispersion of melanin (responsible for dark pigmentation) is a

224 typical response of fishes to changes in background coloration; on a light background
225 melanin is aggregated within the chromatophores, making the colour patterns appear
226 paler whereas on a dark background the melanin is dispersed, giving the fish darker
227 pigmentation (Sugimoto 2007).

228

229 <H2> Shoal Choice Experiment

230

231 Fish were returned to their experimental aquaria (in their previous colour
232 treatment tanks) where they remained for a further week. After these 2 weeks in the
233 dark and pale experimental tanks, the male fish were used in binary choice trials
234 (females played no further part in the experiment and were returned to stock
235 aquaria). We did not notice any further colour change as a result of the second week
236 spent in the dark/pale experimental treatment tanks. Individual fish from both colour
237 treatments (dark: $N=17$; pale: $N=16$) were presented with a choice between two
238 shoals of three fish: one shoal from the dark treatment and one shoal from the pale
239 treatment, in a standard binary choice arena (Brown 2002) adapted from Wright &
240 Krause (2006). Fish used in these experiments had a mean body depth (measured at
241 the deepest part of the body) \pm SD of 1.49 ± 0.129 cm. The choice tank measured
242 85×45 cm, and was filled with conditioned water to a depth of 10 cm above the gravel
243 substrate, allowing test fish adequate space to swim. Each tank contained brown
244 gravel identical to that in the stock tanks, to a depth of 5 cm, and two transparent
245 stimulus shoal cylinders (10 cm diameter). The stimulus shoal cylinders were
246 perforated to allow chemical cues from the stimulus shoals to pass into the rest of the
247 water, and positioned at opposite ends of the choice tank, so that their centres were
248 20 cm from the tank end. Each cylinder was surrounded by a 10 cm preference zone
249 (equivalent to two standard body lengths) which results in a conservative estimate of
250 shoaling tendency (Pitcher & Parrish 1993). The preference zones were marked with
251 depressions in the gravel.

252

253 In each trial, a dark stimulus shoal was placed into one of the cylinders and a
254 pale stimulus shoal in the other. Within a shoal, the three fish were taken from a
255 single experimental tank, and we ensured that the fish in the two stimulus shoals had
256 originally been sourced from the same stock tank. Thus, all fish within a stimulus
257 shoal would have potentially been familiar with one another, and may also have been
258 familiar with the opposite-coloured stimulus shoal. The test fish had not been housed
259 in the same experimental or source tank as any of the fish in stimulus shoals, and
260 was thus unfamiliar with both shoals. After the stimulus shoals had been added to the
261 cylinders, and given 5 min to acclimatize, the test fish was introduced in a net to the
262 centre of the choice tank.

263

264 Each trial began after the test fish had visited both stimulus shoals (swum
265 inside each preference zone) and returned to the neutral zone. Trials lasted 10 min.
266 Cumulative time in each preference zone was measured using stopwatches, and one
267 observer made all recordings. Half of the water in the binary choice tank was
268 changed after each trial, to reduce the build up of olfactory cues. After the trial, fish
269 were returned to their experimental tank. Fish that had previously been used as
270 stimulus fish were not later reused as test fish, but test fish could later be reused as
271 stimulus fish. Males appeared to retain their dark/pale colour patterns (according to
272 their experimental treatment) for the duration of the experiment.

273

274 <H2>Statistical Analysis

275

276 Data were analysed using the statistical analysis program R version 2.6.0 (R
277 Core Development Team, Vienna, Austria). Changes in fish colour patterns were
278 analysed with a general linear mixed-effects model (LME) with 'before' treatment
279 colour patterns, colour treatment (dark/pale) and standard body length as

280 explanatory variables. Fish identity (ID) nested in tank ID were random factors,
281 controlling for the use of multiple fish from the same tank. Two-tailed *t* tests were
282 used to test for differences in the colour patterns of fish both before and after their
283 allocation to the dark/pale treatment tanks. For the shoal choice trials, we calculated
284 the proportion of time spent with each colour shoal, which was then angular
285 transformed to meet the assumptions of normality. This was also analysed using a
286 general linear mixed-effects model with standard body length and change in body
287 colour as explanatory variables, and fish ID nested in tank ID as a random factor.

288

289 <H2>*Ethical note*

290

291 This work was approved by the University of Western Australia Animals
292 Ethics Committee. We ensured minimal stress to the fish during transport by using
293 fish transport bags (approximately 20 x 30 cm) that come lined with clove oil, which is
294 an effective sedative for these fish (Young 2009). Up to 12 fish were placed in each
295 bag which was filled with one-third water (containing a conditioning treatment,
296 Armour Coat) before being placed in an insulated polystyrene box, and transported to
297 the University by vehicle.

298

299 During the experiments, we observed no mortality or other adverse effects as
300 a result of the anaesthesia and photography procedures. During the colour change
301 experiment, tanks were monitored four times daily for signs of ill health or
302 aggression. Male - male aggression was observed in four of the 12 treatment tanks.
303 In these cases, any victims of the aggression (identified by damage to tail and fins;
304 torn fins as a result of 'nipping') were removed as soon as any damage was noted,
305 and housed singly in tanks with water containing Armour Coat, until recovered. Fins
306 damaged in this way grow back in a matter of weeks with no lasting damage to the
307 fish. Three fish were removed from the 'pale' treatment tanks and seven from the

308 'dark' treatment tanks. Three of these seven males were from a single 'dark'
309 treatment tank in which high levels of aggression were observed. In this case all four
310 male fish were removed and isolated to allow recovery of the victims. All victims of
311 aggression recovered after removal from the treatment tanks and no mortality
312 occurred.

313

314 <H1>RESULTS

315

316 <H2>Colour patterns

317

318 Before we placed test males in experimental colour tanks, fish that had been
319 allocated to the pale treatment did not differ in percentage black body colour from
320 those allocated to dark treatments ($t_{35.5} = -0.575$, $P=0.569$; Fig 1). Males showed
321 considerable variation in the percentage of black pigmentation on the body (range
322 1.83 - 50.06%; mean \pm SD = $16.46 \pm 13.58\%$, $N=48$). These data were positively
323 skewed, with the majority of males having <20% black pigmentation (median =
324 11.38%).

325 Following the week housed in experimental colour tanks, there were
326 significant effects on colour pattern change of colour treatment (LME: $F_{10}=192.777$,
327 $P<0.0001$), but not of standard body length (LME: $F_{24}=3.736$, $P=0.065$). Fish that had
328 been in dark treatment tanks had increased their black coloration ($t_{31.8} = -7.173$,
329 $P<0.0001$), and those in pale tanks had reduced it ($t_{30.0}=3.502$, $P=0.002$; Fig. 1).
330 Overall, fish that had been kept in dark treatments now had significantly more black
331 pigmentation than those kept in pale treatments ($t_{25.4}=12.1201$, $P<0.0001$). Examples
332 of this colour pattern change can be seen in Fig 2.

333

334 <H2> Shoal Choice

335

336 We found that fish from both the dark and pale treatments showed a
337 significant preference for shoalmates displaying similar colour patterns (i.e. levels of
338 black pigmentation). Fish from dark treatments preferred to shoal with others from
339 the dark treatment (LME: $F_{12}=10.729$, $P=0.007$), and those from the pale treatments
340 preferred to shoal with others from the pale treatment (LME: $F_{11}=8.304$, $P=0.015$; Fig
341 3). Change in body colour was not a significant predictor of social preference.

342

343 <H1>DISCUSSION

344

345 We have demonstrated that changes in the visual background influence the
346 colour patterns and shoaling behaviour of the western rainbowfish. Rainbowfish
347 exposed to dark aquaria displayed a higher proportion of black pigmentation in their
348 colour patterns and preferred to associate with other darkened fish. Likewise, fish
349 exposed to pale aquaria reduced the level of black pigmentation in their colour
350 patterns and showed a preference for shoalmates with lighter colour patterns. This is
351 consistent with classic work on colour pattern changes according to background (e.g.
352 Parker 1948) and the predictions of the oddity effect in showing that individuals prefer
353 shoalmates with colour patterns similar to their own (McRobert & Bradner 1998;
354 Engeszer et al. 2004; Rosenthal & Ryan 2005; Gómez-Laplaza 2009). Our
355 experiments combine two antipredator strategies and show that morphological colour
356 pattern changes can have an important bearing on subsequent behavioural
357 (shoaling) decisions.

358

359 Changing colour patterns is one way in which prey fishes can allow their
360 coloration to serve multiple functions, for example increasing colour pattern
361 conspicuousness to communicate with mates and competitors and enhancing crypsis
362 to avoid detection by predators. However, behaviour plays a critical role in
363 determining how colour patterns are perceived by both conspecifics and predators.

364 Morphological colour pattern changes, which occur over a relatively long period of
365 time (cf. physiological colour changes), may be disadvantageous if prey move
366 between different visual backgrounds faster than they can change colour.
367 Furthermore, even if the background environment is homogeneous, changes in the
368 light environment (e.g. diurnal fluctuations) will alter the visual properties of the prey's
369 colour pattern and its background, potentially reducing the level of background
370 matching (Ruxton et al. 2004). In these situations, prey may either need to adopt
371 other antipredator strategies to reduce their level of predation risk, or they may
372 achieve 'behavioural background matching' by choosing a substrate that is most
373 similar to their own coloration.

374

375 Endler (1978) noted that an animal's visual background can comprise not only
376 the habitat (substrate, open water, etc.) but also an individual's group mates when
377 animals form a dense group, such as a shoal of fish. In the current study, rainbowfish
378 may have achieved behavioural background matching by selecting a background of
379 shoalmates similar to their own coloration. This would effectively increase their level
380 of crypsis (when viewed against a background of shoalmates) and reduce their risk of
381 oddity (where odd prey animals within a group are more likely to be targeted by
382 predators, Theodorakis 1989). We acknowledge that this changeable aspect of body
383 coloration could also reveal an aspect of recent habitat to conspecifics. This may be
384 used as a cue to familiarity in a similar way to the olfactory diet and habitat cues seen
385 in three-spined sticklebacks, *Gasterosteus aculeatus* (Webster et al. 2007).

386

387 Changing colour to background match group mates and reduce the oddity
388 effect is an interesting idea which has received limited attention from researchers.
389 Cheney et al. (2008) showed that blue-striped fangblennies, *Plagiotremus*
390 *rhinorhynchos*, rapidly changed colour (within 30 min) to mimic juvenile cleaner fish,
391 *Labroides dimidiatus*, allowing them to attack reef fish (feeding off dermal tissue and

392 scales) that visit cleaner stations. However, the nonmimetic coloration of the
393 fangblennies resembled the colour patterns of other species, for example blue-green
394 chromis, *Chromis viridis*, which often occur in the same shoal. Crook (1999)
395 observed the shoaling decisions of juvenile bullethead parrotfish, *Chlorurus sordidus*,
396 which are able to change their colour patterns within seconds or minutes. Solitary fish
397 typically chose shoals containing other juvenile parrotfish with similar colour patterns
398 and only 4% of fish were observed to change colour after joining a shoal (Crook
399 1999). These studies suggest an interesting trade-off between behavioural and
400 physiological background matching.

401

402 The nature of this trade-off has been investigated in two closely related
403 species of salamander that differ in their ability to change colour (Garcia & Sih 2003).
404 The species showing the greater capacity for colour change (*Ambystoma barbouri*)
405 did not alter its behaviour according to its colour patterns but showed a preference
406 for dark substrates followed by a colour change (becoming darker). In contrast, *A.*
407 *texanum* (which has limited colour change ability) displayed behavioural background
408 matching and preferred substrates that were most similar to its own coloration.
409 Furthermore, use of a refuge under predation risk was dependent on body colour for
410 *A. texanum* (which spent less time in a refuge when its level of background matching
411 was high) but not for *A. barbouri* (Garcia & Sih 2003). This demonstrates how
412 antipredator behaviours in prey are influenced by both immediate levels of coloration
413 and the potential for colour pattern change.

414

415 The relationship between predation risk and group composition (i.e. the
416 proportion of odd prey) is also influenced by group size. Experiments with silvery
417 minnows, *Hybognathus nuchalis*, and largemouth bass predators showed that
418 solitary minnows were always captured by bass whereas attack success was
419 reduced to 50% if an odd individual was in a group of eight (Landeau & Terborgh

420 1986). However, shoals containing a few odd prey received more attacks than
421 homogeneous shoals, suggesting that the other group members incur a cost through
422 accepting odd shoalmates. In these experiments, the oddity effect did not persist in
423 larger groups, that is, odd prey in shoal sizes of 15 fish were not more vulnerable
424 than common prey types; however, work using computer simulations has found no
425 evidence that the oddity effect is confined to smaller groups (Ruxton et al. 2007). The
426 oddity effect operates in systems where predators display a preference for rare prey
427 (positive frequency-dependent selection). However, if predators are less likely to
428 detect and attack rare prey (for example because of lack of experience), rare prey
429 types have higher fitness (Ruxton et al. 2004). In this case, rare prey may reduce
430 their risk of predation by remaining solitary rather than joining a group comprising
431 common prey types. Grouping decisions are therefore contingent on the proportion of
432 odd prey in the population, which in the case of background matching may reflect the
433 heterogeneity of the local habitat.

434

435 Morphological colour pattern changes may be important in allowing
436 individuals to adapt to seasonal changes in their light environment. In rainbowfish
437 habitats, for example, changes in the light environment may be associated with
438 increased water turbidity during the cyclone season. The resulting colour pattern
439 changes could have implications for dispersal and mate choice, if for example
440 individuals become restricted to a particular habitat (in which they are background
441 matched) and become limited in their choice of mates. Although restrictions on
442 dispersal are an important mechanism of speciation (Wilson & Hessler 1987), a
443 recent study of coastrange sculpin, *Cottus aleuticus*, found that divergence in
444 background matching coloration was due to morphological plasticity rather than
445 genetic diversity (Whiteley et al. 2009).

446

447 Colour pattern changes also serve as important social and sexual signals in
448 many species of fish (Shibatta 2006; Korzan et al. 2008). For example, colour pattern
449 changes are used as a signal of subordination during aggressive interactions in
450 Atlantic salmon, *Salmo salar* (O'Connor et al. 1999). Assortment based on colour
451 patterns may therefore be complicated by the role that colour plays in other social
452 interactions, leading to interesting trade-offs between the antipredator benefits of
453 colour pattern matching, and the potential advantages associated with honest signals
454 of social status (Keys & Rothstein 1991). Observations made during the current study
455 suggest that black coloration may play a role in social dominance in western
456 rainbowfish, possibly signalling dominance status, which has an effect on group
457 organization and composition in many species. This would be consistent with findings
458 from other species where melanic forms are more aggressive (Price et al. 2008).

459

460 In summary, rainbowfish displayed morphological background matching after
461 being exposed to different light environments. Subsequent shoaling decisions were
462 based on these colour pattern changes with individuals showing a preference for
463 similarly coloured, background-matched shoalmates. These combined processes of
464 morphological and behavioural background matching amount to a sophisticated suite
465 of colour-mediated antipredator defences.

466

467

468 **ACKNOWLEDGMENTS**

469

470 We thank Cameron Duggin for practical advice and assistance with fish
471 husbandry, Mike Young for useful information on the study species, Stuart
472 Humphries for useful discussions and Jens Krause, Jolyon Faria, Steven Sait, Ben
473 Chapman and Christos Ioannou for comments on the manuscript. Comments from
474 two anonymous referees improved the manuscript tremendously. This work was

475 funded by the Royal Society, Company of Biologists, British Ecological Society,
476 NERC, BBSRC and the University of Western Australia.

477

478 References

479

480 **Allen, G., Midgely, S. & Allen, M.** 2002. *Guide to the Freshwater Fishes of*
481 *Australia*. Perth: Western Australian Museum.

482 Arnold, K.E. 2000. Kin recognition in rainbowfish (*Melanotaenia eachamensis*): sex,
483 sibs and shoaling. *Behavioral Ecology and Sociobiology*, **48**, 385-391.

484 Bagnara, J.T. & Hadley, M. E. 1973. *Chromatophores and Color Change*. Englewood
485 Cliffs, New Jersey: Prentice-Hall.

486 Bennett, A. T. D., Cuthill, I. C. & Norris, K. J.. 1994. Sexual selection and the
487 mismeasure of color. *American Naturalist* **144**:848-860.

488 Bradbury, J.W. & Vehrencamp, S.L.. 1998. *Principles of Animal Communication*.
489 Sunderland, Massachusetts: Sinauer Associates.

490 Brown, C. 1999. The behavioural ecology of predator avoidance in rainbowfish
491 (*Melanotaenia*). Ph.D. thesis, University of Queensland.

492 Brown, C. 2002. Do female rainbowfish (*Melanotaenia* spp.) prefer to shoal with
493 familiar individuals under predation pressure? *Journal of Ethology* **20**:89-94.

494 Brown, C. & Warburton, K.. 1997. Predator recognition and anti-predator responses
495 in the rainbowfish *Melanotaenia eachamensis*. *Behavioral Ecology and*
496 *Sociobiology* **41**:61-68.

497 Caro, T. 2005. The adaptive significance of coloration in mammals. *Bioscience*
498 **55**:125-136.

499 Caro, T. 2009. Contrasting coloration in terrestrial mammals. *Philosophical*
500 *Transactions of the Royal Society B* **364**:537-548.

501 Cheney, K.L. Grutter, A.S. & Marshall, N.J. 2008. Facultative mimicry: cues for colour
502 change and colour accuracy in a coral reef fish. *Proceedings of the Royal*
503 *Society B* **275**: 117-122.

504 Cott, H.B. 1940. *Adaptive Coloration in Animals*. London: Methuen.

505 Crook, A.C. 1999. Quantitative evidence for assortative schooling in a coral reef fish.
506 *Marine Ecology Progress Series* **176**: 17-23.

507 Edmunds, M. 1974. *Defence in Animals: A Survey of Antipredator Defences*. Harlow:
508 Longman.

509 Endler, J. A. 1978. A predator's view of animal color patterns. *Evolutionary Biology*
510 **11**:319-364.

511 Engeszer, R. E., Da Barbiano, L. A. Ryan, M. J. & Parichy, D. M. 2007. Timing and
512 plasticity of shoaling behaviour in the zebrafish, *Danio rerio*. *Animal*
513 *Behaviour* **74**:1269-1275.

514 Godin, J. –G.J. 1997. Evading predators. In: *Behavioural Ecology of Teleost Fishes*
515 (Ed. by J. –G.J. Godin), pp. 191-236. Oxford: Oxford University Press.

516 Gómez-Laplaza, L.M. 2009. Recent social environment affects colour-assortative
517 shoaling in juvenile angelfish (*Pterophyllum scalare*). *Behavioural Processes*
518 **82**: 39-44.

519 Hanlon, R. T., Forsythe, J. W. & Joneschild, D. E. 1999. Crypsis, conspicuousness,
520 mimicry and polyphenism as antipredator defences of foraging octopuses on
521 Indo-Pacific coral reefs, with a method of quantifying crypsis from video
522 tapes. *Biological Journal of the Linnean Society* **66**:1-22.

523 Kelley, J.L. 2008. Assessment of predation risk by prey fishes. In: *Fish Behaviour*
524 (Ed. by C. Magnhagen, V. A. Braithwaite, E. Forsgren & B. G. Kapoor), pp.
525 Enfield, New Hampshire: Science Publishers.

526 Keys, G. C. & Rothstein, S. I.. 1991. Benefits and costs of dominance and
527 subordination in white-crowned sparrows and the paradox of status signalling
528 *Animal Behaviour* **42**:899-912.

- 529 Korzan, W. J., Robison, R. R. B., Zhao, S. & Fernald, R. D. 2008. Color change as a
530 potential behavioral strategy. *Hormones and Behavior* **54**:463-470.
- 531 Krakauer, D. C. 1995. Groups confuse predators by exploiting perceptual
532 bottlenecks: a connectionist model of the confusion effect. *Behavioral Ecology*
533 *and Sociobiology* **36**:421-429.
- 534 Krause, J. & Godin, J.-G. J. 1996. Influence of parasitism on shoal choice in the
535 banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae). *Ethology*
536 **102**:40-49.
- 537 Krause, J. & Ruxton, G. D. 2002. *Living in Groups*. Oxford: Oxford University Press.
- 538 Landeau, L. & Terborgh, J. 1986. Oddity and the confusion effect in predation.
539 *Animal Behaviour* **34**:1372-1380.
- 540 McRobert, S. P. & Bradner, J. 1998. The influence of body coloration on shoaling
541 preferences in fish. *Animal Behaviour* **56**:611-615.
- 542 Marshall, N.J. 2000. Communication and camouflage with the same 'bright' colours in
543 reef fishes. *Proceedings of the Royal Society B* **355**: 1243-1248.
- 544 Mboko, S. K. & Kohda, M. 1995. Pale and dark dichromatism related to microhabitats
545 in a herbivorous Tanganyikan cichlid fish, *Telmatochromis temporalis*. *Journal*
546 *of Ethology* **13**:77-83.
- 547 Messmer, V., Jones, G. P., van Herwerden, L. & Munday, P. L. 2005. Genetic and
548 ecological characterisation of colour dimorphism in a coral reef fish.
549 *Environmental Biology of Fishes* **74**:175-183.
- 550 Norman, M. D., Finn, J. & Tregenza, T. 2001. Dynamic mimicry in an Indo-Malayan
551 octopus. *Proceedings of the Royal Society B* **268**:1755-1758.
- 552 O'Connor, K. I., Metcalfe, N. B. & Taylor, A. C. 1999. Does darkening signal
553 submission in territorial contests between juvenile Atlantic salmon, *Salmo*
554 *salar*? *Animal Behaviour* **58**:1269-1276.
- 555 Ohguchi, O. 1978. Experiments on selection against color oddity of water fleas by 3-
556 spined sticklebacks. *Zeitschrift für Tierpsychologie* **47**:254-267.

- 557 Parker, G.H. 1948. *Animal Colour Changes and their Neurohumours*. Cambridge:
558 Cambridge University Press.
- 559 Pitcher, T. J. & Parrish, J. K. 1993. Functions of behaviour in teleost fishes. In:
560 *Behaviour of Teleost Fishes* (Ed. by T. J. Pitcher), pp. 363-439. London:
561 Chapman & Hall.
- 562 Price, A. C., Weadick, C. J. Shim, J. & Rodd, F. H. 2008. Pigments, patterns, and fish
563 behavior. *Zebrafish* **5**:297-307.
- 564 Rosenthal, G. G. & Ryan, M. J. 2005. Assortative preferences for stripes in danios.
565 *Animal Behaviour* **70**:1063-1066.
- 566 Roulin, A. & Wink, M. 2004. Predator-prey polymorphism: relationships and the
567 evolution of colour a comparative analysis in diurnal raptors. *Biological*
568 *Journal of the Linnean Society* **81**:565-578.
- 569 Rowland, W. J. 1999. Studying visual cues in fish behavior: a review of ethological
570 techniques. *Environmental Biology of Fishes* **56**:285-305.
- 571 Ruxton, G. D., Sherratt, T. N. & Speed, M. P. 2004. *Avoiding Attack: the Evolutionary*
572 *Ecology of Crypsis, Warning Signals and Mimicry*. Oxford: Oxford University
573 Press.
- 574 Ruxton, G.D., Jackson, A.L. & Tosh, C.R. 2007. Confusion of predators does not rely
575 on specialist coordinated behaviour. *Behavioral Ecology* **18**: 590-596.
- 576 Shibatta, O. A. 2006. Social behavior of pira-brasilia, *Simpsonichthys boitonei*
577 Carvalho (Cyprinodontiformes, Rivulidae). *Revista Brasileira De Zoologia*
578 **23**:375-380.
- 579 Stevens, M., Parraga, C. A., Cuthill, I. C., Partridge, J. C. & Troscianko, T. S. 2007.
580 Using digital photography to study animal coloration. *Biological Journal of the*
581 *Linnean Society* **90**:211-237.
- 582 Sumner, F. B. 1935a. Evidence for the protective value of changeable coloration in
583 fishes. *American Naturalist* **69**:245-266.

584 Sumner, F. B. 1935b. Studies of protective color change III Experiments with fishes
585 both as predators and prey. *Proceedings of the National Academy of*
586 *Sciences, U.S.A.* **21**:345-353.

587 ter Pelkwijk, J. J. & N. Tinbergen. 1937. Eine Reizbiologische Analyse einiger
588 Verhaltenweisen von *Gasterosteus aculeatus* L. *Zeitschrift für*
589 *Tierpsychologie*, 193-200.

590 Theodorakis, C. W. 1989. Size segregation and the effects of oddity on predation risk
591 in minnow schools. *Animal Behaviour* **38**:496-502.

592 Touchon, J. C. & Warkentin, K. M. 2008. Fish and dragonfly nymph predators induce
593 opposite shifts in color and morphology of tadpoles. *Oikos* **117**:634-640.

594 Ward, A. J. W., Axford, S. & Krause, J. 2002. Mixed-species shoaling in fish: the
595 sensory mechanisms and costs of shoal choice. *Behavioral Ecology and*
596 *Sociobiology* **52**:182-187.

597 Waring, H. 1963. *Color Change Mechanisms of Cold-blooded Vertebrates*. New
598 York: Academic Press.

599 Webster, M. M., Goldsmith, J., Ward, A. J. W. & Hart, P. J. B. 2007. Habitat-specific
600 chemical cues influence association preferences and shoal cohesion in fish.
601 *Behavioral Ecology and Sociobiology* **62**:273-280.

602 Whiteley, A.R., Gende, S.M., Gharrett, A.J. & Tallmon, D.H. 2009. Background
603 matching and color-change in colonizing freshwater sculpin populations
604 following rapid deglaciation. *Evolution* **63**: 1519-1522.

605 Wilson, G. D. F. & Hessler, R. R. 1987. Speciation in the deep sea. *Annual Review of*
606 *Ecology and Systematics* **18**:185-207.

607 Wright, D. & J. Krause. 2006. Repeated measures of shoaling tendency in zebrafish
608 (*Danio rerio*) and other small teleost fishes. *Nature Protocols* **1**:1828-1831.

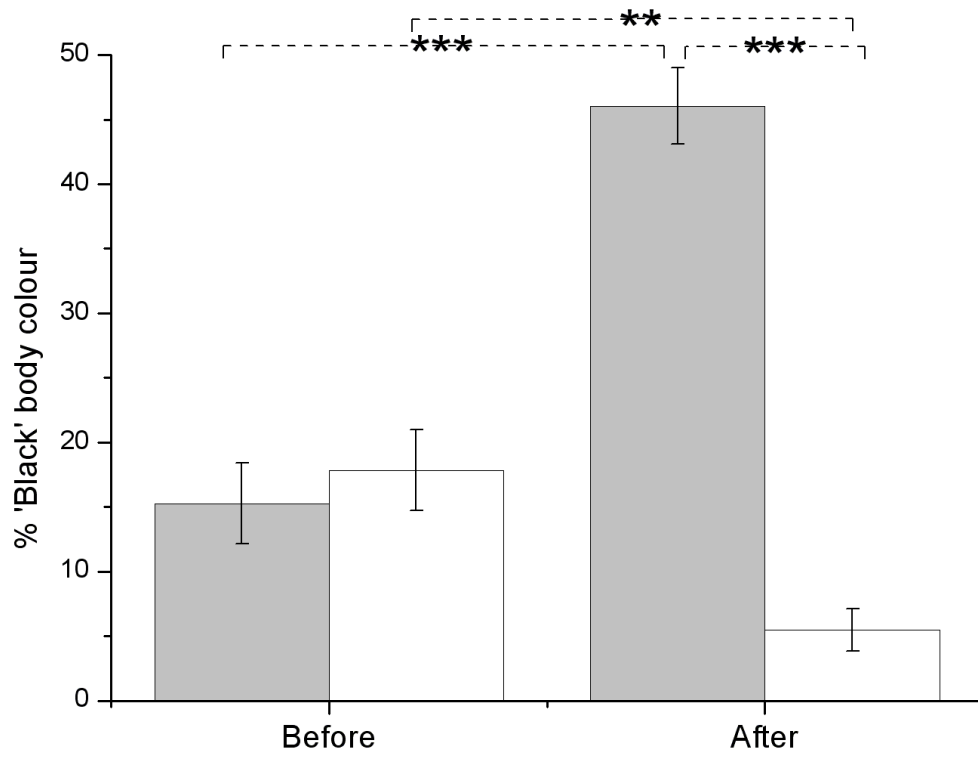
609 Young, M.J. 2009. The efficacy of the aquatic anaesthetic AQUI-S for anaesthesia of
610 a small freshwater fish, *Melanotaenia australis*. *Journal of Fish Biology* 75:
611 1888-1894.
612
613

614 Figure 1

615

616

617

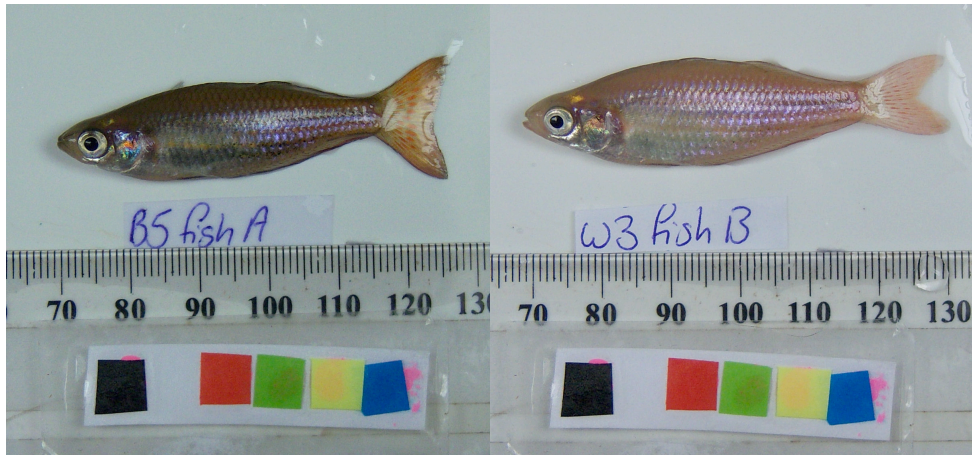


618

619

620

621 Figure 2



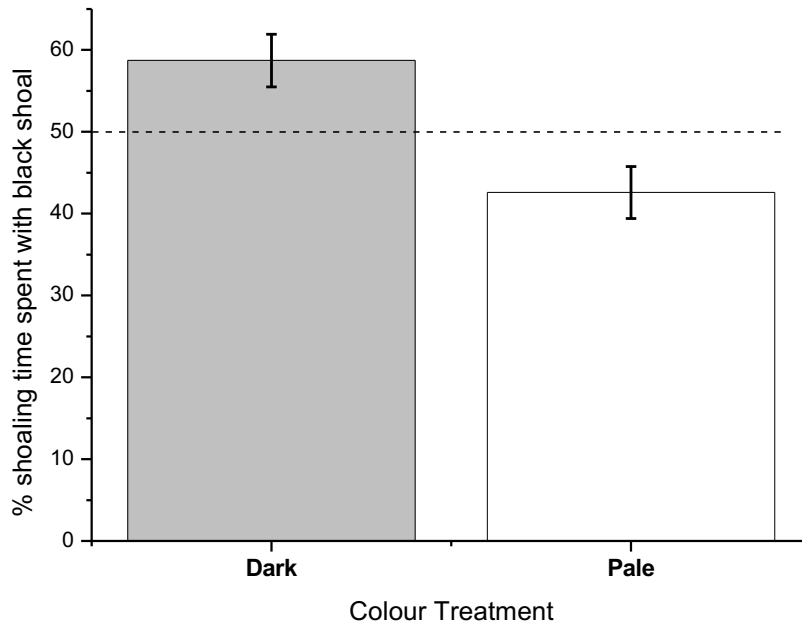
622

623

624 Figure 3

625

626



627

628

629 Figure legends

630

631 Figure 1. Percentage 'black' body coloration before and after being housed for 1
632 week in pale- (white bars) and dark-coloured habitats (grey bars). *** $P < 0.001$; ** P
633 < 0.01 . Error bars indicate ± 1 SE.

634

635

636

637 Figure 2. Examples of fish body colour after 1 week in (a) dark and (b) pale treatment
638 tanks. Images not standardized for white balance.

639

640

641 Figure 3. Percentage time spent shoaling with the dark stimulus shoal. The dashed
642 horizontal line represents no preference, above the line represents a preference for
643 the dark stimulus shoal and below the line represents a preference for the pale
644 stimulus shoal. Error bars represent ± 1 SE.