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4 **Turbidity weakens selection for assortment in body size in groups**

5 Helen S. Kimbell & Lesley J. Morrell

6 School of Biological, Biomedical and Environmental Sciences, University of Hull

7

8 **Address for correspondence:**

9 Helen S. Kimbell

10 School of Biological, Biomedical and Environmental Sciences

11 University of Hull

12 Kingston-upon-Hull

13 HU6 7RX

14

15 Email: h.kimbell@2011.hull.ac.uk

16 Tel: +44 (0)1482 465518

17

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24

25 **Turbidity weakens selection for assortment in body size in groups**

26

27 **Lay Summary**

28 Predators targeting groups often select “odd” individuals. This is mediated by body
29 size, with large individuals selected. However, this depends on a predator’s ability to
30 detect and target particular individuals. In turbid water, predators lose their
31 preference for large, odd individuals. This in turn alters shoaling decisions of prey, with
32 individuals no longer shoaling with size-matched groups. Turbidity alters predator-prey
33 interactions by altering levels of risk, and results in the formation of less uniform
34 groups.

35

36 **Abstract**

37 Prey animals commonly associate with similar-looking individuals to reduce predation
38 risk, via a reduction in predator targeting accuracy (the confusion effect) and
39 preferential targeting of distinct individuals (the oddity effect). These effects are
40 mediated by body size, as predators often preferentially select large bodied
41 individuals, which are therefore at an increased risk within a group. The selection
42 pressure to avoid oddity by associating with similar sized group-mates is stronger for
43 large individuals than small. This selection depends on the ability of both predators
44 and prey to accurately assess body size and respond accordingly. In aquatic systems,
45 turbidity degrades the visual environment and negatively impacts on the ability of

46 predators to detect (and consume) prey. We assessed the effect of algal turbidity on
47 predator-prey interactions in the context of the oddity effect from the perspective of
48 both predator and prey. From a predator's perspective, we find that 9-spined
49 sticklebacks preferentially target larger Daphnia in mixed swarms in clear water, but
50 not in turbid water, although the difference in attack rates is not statistically
51 significant. When making shoaling decisions, large sticklebacks preferentially associate
52 with size-matched individuals in clear water, but not turbid water, while small
53 individuals showed no social preference in either clear or turbid water. We suggest
54 that a reduced ability or motivation to discriminate between prey in turbid water
55 relaxes the predation pressure on larger prey individuals allowing greater flexibility in
56 shoaling decisions. Thus, turbidity may play a significant role in predator-prey
57 interactions, by altering predator prey interactions.

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60 **Key Words: group living, oddity, confusion effect, shoaling, visual environment**

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66 Introduction

67 Predator-prey interactions and anthropogenic change are two key factors influencing
68 community structure. Predation alters abundance, distribution and composition of
69 species in a community either directly through the consumption of prey (Holling 1959),
70 or indirectly by modifying prey behavior (Lima and Dill 1990). Interactions between
71 predators and prey are highly sensitive to disturbances in the environment. In aquatic
72 environments, increased turbidity, which may be naturally occurring or exacerbated by
73 anthropogenic activities, such as de-forestation, urbanisation and eutrophication,
74 degrades the visual environment, affecting how predators detect and target prey (De
75 Robertis et al. 2003) and how prey responds (Gregory 1993; Meager et al. 2006). This
76 can cause a shift in predator-prey interactions (Abrahams and Kattenfeld 1997),
77 ultimately changing community structure through altered levels of risk and survival.

78 In clear water aquatic predators often show active preferences for particular prey
79 types, such as size (Lehtiniemi et al. 2007; Rodgers et al. 2015), shape (Kislalioglu and
80 Gibson 1976) or colour (Ohguchi 1978); for example cichlids (*Aequidens pulcher*)
81 preferentially target brightly coloured male guppies (*Poecilia reticulata*) (Godin and
82 McDonough 2003). When there are no handling constraints, larger bodied prey are
83 generally more profitable (optimal foraging: MacArthur and Pianka 1966), and are
84 often over-represented in the diets of planktivorous fish (Li et al. 1985; Wetterer and
85 Bishop 1985). Thus, larger prey individuals are often at greater risk of predation
86 (Lehtiniemi et al. 2007; Manicom and Schwarzkopf 2011). In turbid water, however,
87 size selectivity is often impaired (Reid et al. 1999; Jonsson et al. 2013), as turbidity

88 directly affects a predator's ability to locate and target prey. Predator reaction
89 distances are shortened, which can lower capture success per unit of effort (Gregory
90 and Northcote 1993; Utne 1997), or the type of prey targeted may change, while
91 overall predation rates remain constant (Abrahams and Kattenfeld 1997; Shoup and
92 Wahl 2009). This alters the level of risk experienced by individuals; for prey animals
93 that aggregate, this may mean that while overall risk to the group remains constant,
94 relative risk to individuals within the group changes.

95 Group formation is a common and important response to the risk of predation. In
96 addition to reducing individual risk (the dilution effect: Foster and Treherne 1981),
97 groups of moving prey visually confuse predators, reducing targeting accuracy (the
98 confusion effect: Krakauer 1995, Tosh et al. 2009). This effect is enhanced in larger
99 groups and when prey individuals are morphologically or behaviorally similar to one
100 another (Landeau and Terborgh 1986). Predators are better able to overcome the
101 confusion effect if a distinct or 'odd' individual is present within the group (the oddity
102 effect: Theodorakis 1989). Predators preferentially target odd individuals as they are
103 easier to visually isolate, making them at increased risk within a group (Milinski 1977a;
104 Ohguchi 1978; Theodorakis 1989). Together, the confusion and oddity effects predict
105 that individuals should preferentially group with phenotypically similar individuals, a
106 phenomenon well-studied in shoaling fishes (Ranta et al. 1992; McRobert and Bradner
107 1998; Ward and Krause 2001; Rodgers et al. 2011), but observed in other taxa
108 including birds (Brightsmith and Villalobos 2011) and mammals (Meldrum and
109 Ruckstuhl 2009).

110 In the context of the confusion and oddity effects, predator selectivity for particular
111 prey phenotypes (e.g. large body size) means that the selection pressure to avoid
112 oddity should be stronger for preferred phenotypes than for less preferred ones
113 (Rodgers et al. 2015). In support of this, larger fish preferentially associate with large
114 conspecifics, while small individuals show no such preference (Svensson et al. 2000;
115 Rodgers et al. 2011), and larger fish are more risk averse than smaller ones when
116 foraging (Peuhkuri 1997; Peuhkuri 1998). Changes in prey selection by predators
117 associated with turbidity may thus alter the relative risk experienced by individuals
118 within groups, which may have significant consequences for group formation and
119 maintenance.

120 Here, we explore the effect of turbidity on predator-prey interactions in the context of
121 the oddity effect, from the perspective of both predators and prey. Firstly, we assess
122 predator (9-spine sticklebacks *Pungitius pungitius*) preferences for large prey *Daphnia*
123 *magna* individuals in mixed groups of small and large prey, predicting that preferences
124 for large individuals, particularly when they are odd (Rodgers et al. 2015), should be
125 reduced in turbid water due to the previously documented reduction in size selectivity
126 more generally. Secondly, we assess size-based association preferences of large and
127 small sticklebacks, predicting that because predator selectivity for large individuals is
128 reduced in turbid water, preferences for size-matched individuals in clear water should
129 again be weakened in turbid water.

130

131 **Methods**

132 Study species and husbandry

133 Approximately 250 9-spined sticklebacks were collected from Noddle Hill Nature
134 Reserve, Hull, (Grid Reference: 4111E, 5348N) in October 2013 and housed in groups
135 of 15-20 in 30 x 30 x 50cm aquaria (stock tanks) at the University Hull. All tanks were
136 connected on a closed re-circulating system, with external UV and bio-filters and a 20%
137 weekly water change. Tanks were kept at approximately 12°C on a 12:12 light:dark
138 cycle and fish were fed daily on defrosted frozen bloodworm (chironomid larvae) and
139 frozen *Daphnia* (purchased from Ings Lane Garden and Water Centre Ltd, Hull). Fish
140 used were not in reproductive condition, and therefore no effort was made to sex
141 individual fish. 30 fish were labelled as “stimulus fish” for the shoaling experiments
142 (see below) and not used as test fish in either experiment. All other fish were used in
143 both targeting and shoal choice experiments (see below), with at least a week
144 between experiments.

145 Turbid water was created using a unicellular, motile algae *Chlamydomonas* spp
146 (Phytotech lab, Kansas, USA), which has been previously used in studies disrupting
147 vision in fish (Jonsson et al. 2011b), and remained in suspension for the duration of the
148 trials. Algae was grown in a medium containing de-ionised water and Bold’s Basal
149 Medium Solution (Phytotech lab, Kansas, USA) at 20°C, in cylindrical culture vessels
150 (5cm in diameter, 50cm in length) with a constant light source and airflow. Cultures
151 were left to reach high concentrations (~200NTU) and then diluted with water from
152 the aquarium system for experiments to reach 12.5 NTU (± 2.5 NTU), which equated to

153 approximately 30cm visual depth (measured with a Secchi disc). Turbidity was
154 maintained in experiments using airstones.

155 Live *Daphnia magna*, an important food source for sticklebacks (Hynnes 1950;
156 Wootton 1976), were used as prey in targeting experiments (purchased from Ings Lane
157 Garden and Water Centre Ltd, Hull). Upon arrival to the lab *Daphnia* were placed in 20
158 x 10 x 15cm tanks containing a small quantity of algae (*Chlamydomonas sp.*, ~5NTU) for
159 a minimum of 5 days before experiments. This provided a food source for the *Daphnia*
160 (Ebert & Bethesda 2005) and ensured they were of a standardized colour for
161 experiments, which otherwise may affect detection by predators (Jonsson et al.
162 2011a). Before experiments two size classes of *Daphnia* (large: 2.5mm and small:
163 1.5mm) were separated from the main population into two size-matched pools (held
164 in tanks measuring 20 x 10 x 15cm).

165 Experiment 1: Targeting of individuals in groups

166 To investigate how groups of different compositions were targeted, we presented
167 sticklebacks (N = 216) with one of three different combinations of large and small live
168 *Daphnia*. Before the trials began, 12 sticklebacks from the same stock tank were
169 placed together in a test tank (30 x 30 x 50cm) containing either clear or turbid water
170 to a depth of 15cm and allowed to acclimatise for one hour. After acclimatisation, an
171 opaque barrier was carefully placed 30 cm from one end of the tank, dividing the tank
172 into a smaller holding area at the back and a larger experimental area, where the trials
173 took place (Ioannou and Krause 2008; Ioannou et al. 2009). All 12 fish were carefully
174 netted into the holding area and remained there for a further hour. During

175 experiments, fish were moved individually from the holding area into the experimental
176 area using a small hand net, and with minimal disturbance, and participated in the trial
177 one at a time.

178 At the end of the experimental area, a square array of *Daphnia*, consisting of 16 water-
179 filled 1cm³ transparent cubes arranged in a 4x4 grid, was positioned externally on the
180 end of the tank (Rodgers et al. 2013). This ensured visual, but not olfactory cues from
181 the prey were available to the fish, and that prey individuals remained separate and
182 could not physically interact during the experiment. Three treatments with different
183 ratios of *Daphnia* sizes were used; 1:15 large:small (large-bodied minority), 8:8
184 large:small (equal ratios), and 15:1 large:small (small-bodied minority). In the two
185 treatments with a single odd individual, the position of that individual in the grid was
186 rotated systematically between successful trials to control for any positional effects
187 (Krause 1994). In the equal ratios treatment, large and small individuals were placed in
188 the grid in an alternating pattern. Treatments were randomly assigned to individual
189 fish. A Microsoft LifeCam connected to a laptop was placed behind the array to record
190 the trials, and the test tank was screened by a curtain to minimise disturbance.

191 Before the trials commenced, the airstones were removed from the experimental area
192 of the tank, so disturbance from the air bubbles did not disturb targeting by the
193 stickleback. At the start of each trial, a single test fish was carefully netted over the
194 barrier from the holding area into the experimental area. This caused minimal
195 disturbance to the fish, with the majority (214/216) of fish resuming normal swimming
196 behavior less than 10 seconds after being transferred to the experimental area. Fish

197 that did not begin swimming within 2 minutes were excluded from the experiment
198 (N=2 fish). The fish was free to view the *Daphnia* array as soon as it was netted over
199 the barrier, and a further 10 minutes were allowed for the fish to attack an individual
200 within the array. From the videos, we recorded the size (large or small) of the first
201 *Daphnia* targeted, defined as the fish making a striking movement towards a particular
202 individual within the array and making contact with the glass of the tank. Once the first
203 attack had been made, the trial ended and the test fish was removed and returned to
204 the stock tanks. Each fish was only tested once and thus only experienced one of the
205 three combinations of *Daphnia* in one of the water conditions (clear or turbid). Fish
206 that had not made an attack within 10 minutes of being placed over the barrier were
207 excluded from the experiment (20/98 clear water trials, 50/118 turbid water trials).
208 During the turbid trials, turbidity was measured using a handheld Oakton Turbidity
209 Meter every other trial to ensure that it remained at 12.5 ± 2.5 NTU. The water in the
210 experimental tank was changed every 12 trials (i.e. when all the fish in the holding area
211 had been tested), and *Daphnia* were returned to their size matched pools. In clear
212 water we recorded N=25, N=22, N=24 successful attacks for 1:15 (large:small), 8:8 and
213 15:1 daphnia treatments respectively, and N=25, N=16 and N=24 successful attacks in
214 turbid water.

215 Experiment 2: Shoal choice

216 To investigate the effect of turbidity on social decisions, we carried out a series of
217 binary shoal choice tests (McRobert and Bradner 1998; Rodgers et al. 2011) in clear
218 and turbid water. Three days after being introduced and acclimatised to the aquarium,

219 15 fish measuring between 35-40mm (“large fish”) and 15 measuring between 25-
220 30mm (“small fish”) were placed in separate aquaria (on the circulating system) and
221 labelled “stimulus fish”. These fish were never used as test fish in either the targeting
222 experiment or shoal choice experiments. This setup controlled for familiarity (Griffiths
223 and Magurran 1999; Ward et al. 2005), as none of the test fish could become familiar
224 with the stimulus fish. These sizes were chosen as they were readily available in the
225 population, and because three-spine sticklebacks *Gasterosteus aculeatus*, can
226 distinguish between these size classes (Ward and Currie 2013).

227 The shoaling preference of each fish (N = 47) was assessed twice: once in clear water
228 and once in turbid water, such that half the fish were tested in clear water first, and
229 half in turbid water first. To allow us to identify individual fish between trials without
230 marking, test fish were moved in groups of 12 (6 large and 6 small) to 4 identical
231 holding tanks (40 x 20 x 20cm), each separated into 3 equal-sized compartments (each
232 13 x 20 x 20cm) 24 hours prior to experiments. Compartments were separated with
233 clear perforated barriers, which allowed visual and olfactory communication between
234 the test fish, to reduce possible stress caused by separation from conspecifics. Each
235 fish was placed individually in a holding tank compartment, with all compartments
236 within a holding tank containing fish of the same size (3 large or small fish per holding
237 tank). Fish were returned to their individual compartments for 24 hours between
238 experiments.

239 Shoal choice experiments were carried out in 60 x 20 x 30cm binary choice tanks. The
240 tank was split into 3 compartments by two solid glass barriers allowing the

241 transmission of visual but not olfactory cues, with one larger central compartment (30
242 x 20 x 20cm) set between two smaller compartments (15 x 20 x 20cm). The two
243 smaller compartments contained the stimulus shoals during the experiment. Two
244 10cm preference zones (approximately 3 body lengths; Pitcher and Parish 1993) were
245 drawn up beside each stimulus compartment. Test tanks were filled to a depth of
246 12cm (approximately 15L) using the turbid water (see above) or clear water taken from
247 the aquarium system and one air stone was placed in each compartment. Water was
248 changed between each set of experiments (12 test fish, 6 large and 6 small). As no
249 olfactory cues were exchanged between the stimulus shoals and test fish, it was not
250 necessary to change the water between each experiment to control for the build-up of
251 cues from the stimulus fish (which may relay information about size; Ward and Currie
252 2013). As the water for all experiments was taken from the aquarium system (with
253 concentrated algae added for the turbid water experiments), cues from sticklebacks of
254 all body sizes were present in the water.

255 One hour before experiments, test fish were transferred to individual 20 x 20 x 10cm
256 tanks containing either clear or turbid water to allow for acclimatisation to test
257 conditions. One stimulus shoal of 3 large fish and one stimulus shoal of 3 small fish,
258 selected haphazardly from the stimulus fish tanks, were placed in the two end
259 compartments of the binary choice tank and allowed to acclimatise for 15 minutes.
260 After this time the focal fish was placed in the centre compartment. Observations
261 began when the test fish resumed normal swimming behavior (between 30 - 120
262 seconds). One fish was excluded from the trial as it froze for 5 minutes. During a 20
263 minute observation period the time spent in each preference zone (defined as a fish

264 having more than 50% of its body within the preference zone) and the number of
265 times it moved between preference zones (a measure of activity; Fischer and
266 Frommen 2012, Rodgers et al. 2011) were recorded. The trial was observed from
267 behind a curtain using a Microsoft LifeCam attached to a laptop to minimise
268 disturbance. New stimulus shoals were taken from stimulus fish tanks after every third
269 experiment, and the side containing the shoal of large fish was systematically
270 alternated. To reduce the overall number of stimulus fish required, each individual was
271 used more than once over the course of experiments, but haphazard selection of
272 individuals from the stimulus stock tanks meant that it was unlikely the same
273 combination of fish was selected more than once. After each trial, test fish were placed
274 back into the holding tanks and fed defrosted frozen bloodworm. Stimulus fish were
275 fed on completion of the day's experiments.

276 Statistical Analysis

277 In the targeting experiment (experiment 1) we assessed whether sticklebacks targeted
278 particular body sizes more than would be expected by chance using exact binomial
279 tests. In each case, we compared the observed proportion of attacks on large *Daphnia*
280 to expected probabilities based on random targeting. Expected proportions for the
281 large minority, equal ratios and small minority treatments were 0.0625, 0.5 and 0.938
282 respectively. For example in a 1:15 ratio of large *Daphnia*: small *Daphnia*, the large
283 *Daphnia* would be targeted 6.25% of time ($1/16 \times 100$) if attack was random with
284 respect to body size. 95% confidence intervals for the proportion of attacks on large
285 *Daphnia* were calculated using the 'modified Wald' method recommended by Agresti

286 & Coull (1998). We then compared the proportion of large individuals targeted in each
287 of the treatments (large minority, equal ratios and small minority) between clear and
288 turbid water using proportion tests. The per capita risk to individuals was calculated as
289 the proportion of trials in which an individual was targeted, divided by the number of
290 size matched individuals present in the group (Rodgers et al 2014).

291 For the shoal choice experiments (experiment 2), we used a generalised linear mixed
292 effects model (GLMER) model with a binomial error distribution (as appropriate for
293 proportion data) to assess whether the proportion of time spent shoaling with size
294 matched individuals (shoal choice) was influenced by turbidity (clear or turbid water),
295 test fish body size and their interaction. Fish ID was included as a random factor to
296 account for the repeated measured design and an additional observation-level random
297 effect was used to account for overdispersion of the data (Harrison 2014). To assess
298 whether the shoaling preference exhibited by large and small fish in clear and turbid
299 water differed significantly from random expectation (50% of the time with each
300 shoal), one-sample tests were applied. Data was arcsin square root transformed to
301 meet the assumptions of normality where possible and a one-sample t-test was used;
302 otherwise we used a non-parametric Wilcoxon signed ranks test. The false discovery rate
303 (FDR) method was applied to correct for multiple testing (Benjamini and Hochberg
304 1995), and we present the adjusted p-values here. We used a linear mixed effects
305 (LME) model to assess the effect of turbidity, body size and their interaction on the
306 total time spent shoaling with both shoals. Fish ID was included as a random effect to
307 take into account the repeated measures design. Non significant interactions were
308 removed following Crawley (2007). Visual inspection of plots of residuals against fitted

309 values and quantile-quantile plots indicated that a normal error distribution was
310 appropriate here (Crawley 2007). Finally, to investigate if activity (the number of times
311 the fish switched between preference zones) was influenced by body size, turbidity
312 and their interaction we used a GLMER model with a Poisson error distribution (as
313 appropriate for count data) with fish ID included as a random factor. All analysis was
314 carried out in R 2.15.1 (R Development Core Team 2011).

315

316 **Results**

317 Experiment one: Targeting of individuals in groups

318 In clear water, large individuals were targeted significantly more than was expected by
319 chance in the equal ratios treatment (figure 1.1a: large *Daphnia* targeted in 20/22
320 trials, 91%, $P < 0.001$, with random expectation 0.5) and in the large minority
321 treatment (figure 1.1a: large individual targeted in 10/25 trials, 40%, $P < 0.001$, random
322 expectation 0.0625). In turbid water large individuals were no longer preferentially
323 targeted at either ratio, and were chosen with a rate consistent with chance (figure 1a:
324 equal ratios: large individual targeted 12/16 trials, $P = 0.08$, with a random expectation
325 of 0.5, large minority: 4/25, $P = 0.076$, random expectation 0.0625). There was no
326 significant difference in attack rate at either ratio between clear and turbid water
327 (equal ratios: $X^2 = 0.77$, $df = 1$, $p = 0.38$, large minority, $X^2 = 2.48$, $df = 1$, $p = 0.12$)
328 When large individuals made up the majority of a group (15:1) large *Daphnia* were

329 attacked at a rate consistent with chance in both clear and turbid water (figure 1a:
330 100% of trials. 25/25 in clear and 21/21 in turbid water).

331 Per capita risk for large individuals is greatest when they form the minority in the
332 group, and decreases as the number of large individuals increases, and in turbid water
333 (figure 1.1b). In small individuals, per capita risk increases as their number within the
334 group increases. Small individuals are slightly more at risk in turbid water (figure 1.1c)
335 although they are still at lower risk overall compared to large individuals.

336

337 Experiment two: Shoal choice

338 We found a significant interaction between water treatment and body size on the
339 proportion of time individuals spent associating with size matched shoals (GLMER: $Z =$
340 2.22 , $P = 0.027$, figure 2a, table 1a). Large individuals preferred to associate with size
341 matched shoals in clear water ($t = 3.99$, adjusted $P = 0.0024$), but not in turbid water (t
342 $= 0.56$, adjusted $P = 0.36$). Small individuals showed no active preference for either
343 sized shoal in clear ($V = 94$, adjusted $P = 0.64$) or turbid water ($V = 122$, adjusted $P =$
344 0.64 , figure 2b). Both large and small test fish spent significantly more time shoaling
345 overall (total time spent shoaling) in turbid water compared to clear (LME: $F_{1,44} = 14.52$,
346 $P < 0.001$, figure 2b, table 1b), but there was no effect of body size and no interaction.
347 Finally, we found a significant interaction between water treatment and fish size on
348 activity levels (GLMER: $z=3.07$, $p = 0.002$, figure 2c, table 1c). Large test fish had a
349 higher level of activity in clear water compared to small fish, but both large and small
350 fish reduced their activity to similar levels in turbid water. Examining the data more

351 closely, we found that fish in turbid water were more likely to remain in one
352 preference zone for the duration of the trial than fish in clear water (5/46, 11% clear
353 water trials, 15/46, 33% turbid water trials).

354

355 **Discussion**

356 Our results suggest that turbidity may weaken predator preferences for targeting odd,
357 large-bodied individuals, and relaxes the pressure on large bodied prey to associate
358 with similarly-sized groupmates. We suggest there is weakened selection pressure for
359 behavioral assortment in prey, driven by confusion and oddity effects, in turbid water.
360 Reflecting previous work (Rodgers et al. 2015; Gibson 1980; Li et al. 1985; Wetterer
361 and Bishop 1985), we found strong predator selectivity for large prey in clear water,
362 particularly when they were in equal ratios with small-bodied prey or were in the
363 minority (odd) within the group. In turbid water, this preference was absent and
364 targeting of large individuals occurred at a rate consistent with chance, although we
365 did not detect a significant reduction in preference between turbidity treatments. A
366 similar absence of preference in turbid water has also found in more ecological studies
367 of dietary preference (Vinyard and Yuan 1996; Rowe et al. 2003). We suggest that
368 turbidity may relax predation pressure on large individuals within groups, reducing the
369 benefits of assorting by size, and resulting in a loss of the preference for associating
370 with size-matched conspecifics seen in clear water when turbidity increases, as
371 observed in experiment 2.

372 Large prey may be less at risk in turbid water due to visual constraints: in low-visibility
373 conditions, predator-prey interactions occur at closer distances (Miner and Stein 1996;
374 Meager et al. 2006), meaning predators may have reduced choice or reduced time for
375 selection of prey from a group. Algae absorb photosynthetically active wavelengths
376 and scatter light (Kirk 2011), reducing the contrast between objects and their
377 background (Utne-Palm 2002), which negatively affects long-distance detection
378 substantially more than short distance detection (De Robertis et al. 2003). For large
379 individuals, therefore, detection distances are reduced to a greater extent than for
380 small individuals, which may reduce size selectivity by altering encounter rates (Utne-
381 Palm 2002; Jonsson et al. 2013). Turbidity may impact on predator confusion: if
382 detection distances are reduced, prey swarms may appear less dense or numerically
383 smaller, and predators therefore less susceptible to confusion effects (which are
384 enhanced in larger and denser groups; Milinski 1977b; Ioannou et al. 2009). The
385 importance of oddity for successful predation would therefore also be reduced, and
386 preferences for odd individuals in groups weakened. Here, we focused on visual cues
387 alone, but predators that use alternative cues to hunt are also susceptible to confusion
388 effects (Jeschke and Tollrian 2007). Tactile predators, for example, may suffer from
389 confusion effects as they lack the high spatial resolution thought necessary to single
390 out and target individuals from within a group (Jeschke and Tollrian 2007). If exposure
391 to a degraded visual environment means predators switch to alternative cues more
392 susceptible to confusion effects, how predators respond to groups of prey may be
393 changed in other ways. Further work teasing apart how different cues influence group
394 detection and targeting for predators using different sensory modalities is needed.

395

396 If predators are less selective in turbid water, then prey could be expected to respond
397 appropriately to the altered risk environment. Our results suggest that shoaling fish
398 adjust their shoal choices in response to their immediate environment, with large fish
399 losing their preference for size-matched shoals under turbid conditions. For large
400 individuals, at higher risk of predation, association with size-matched individuals
401 reduces risk by reducing oddity and enhancing confusion effects (Theodorakis 1989;
402 Ranta et al. 1992; Rodgers et al. 2011), while for small individuals, risk is lowered
403 through association with larger prey (Rodgers et al. 2015). If large-bodied prey is at
404 lower risk in turbid water, and the anti-predator benefits of size-matching are reduced,
405 we would expect to see a reduction in the strength of association preferences.
406 Association with large-bodied individuals carries a number of costs – particularly
407 increased competition for food (Metcalf and Thomson 1995; Hoare 2000), which can
408 be avoided under potential relaxed selection pressures for assortment in turbid water,
409 although large fish did not make a complete switch to take advantage of this by
410 shoaling with small individuals, suggesting other factors also influence assortment
411 decisions (Rodgers et al. 2013).

412 Turbid water is often associated with a reduced perception of overall risk in fish
413 (Gregory 1993; Engström-Öst and Mattila 2008), resulting in reduced anti-predator
414 behavior, including weakened escape responses (Gregory 1993; Meager et al. 2006),
415 reduced use of shelter (Abrahams and Kattenfeld 1997) and decreased shoal cohesion
416 (Kimbell & Morrell 2015) even if actual risk remains unchanged (Reid et al. 1999;

417 Shoup and Wahl 2009). A reduction in shoaling preferences could be attributed to this
418 effect: sticklebacks perceive that overall, rather than individual, risk is reduced and
419 adjust their social behavior accordingly. However, our finding that fish both increased
420 the total time they spent in association with other shoals (figure 4.2b) and reduced
421 their activity levels (figure 4.2c) suggests an enhanced, rather than reduced,
422 perception of overall risk levels in turbid water for 9 spined sticklebacks. In birds, the
423 degradation or obstruction of visual cues can mean predators are more difficult to
424 detect (Whittingham et al. 2004), and individuals increase vigilance and decrease
425 foraging (Metcalfe 1984; Whittingham et al. 2004; Devereux et al. 2008), consistent
426 with an enhanced perception of risk. Reduced activity levels are thought to reduce
427 encounter rates with predators and have previously been observed in shoaling fish
428 (Fischer and Frommen 2012). By remaining with a shoal, rather than moving between
429 shoals, individuals reduce their exposure to predators under situations where they are
430 at increased risk through isolation (Landeau and Terborgh 1986).

431 As predators, fish are affected by visual constraints in turbid water, and thus the same
432 constraint might be expected for fish as prey. Our test fish may have been unable to
433 detect both shoals simultaneously, although the turbidity levels in our experiment
434 (12NTU, equivalent to a secchi depth of 30cm) were chosen so that fish should be able
435 to view both shoals simultaneously from any location within the test tank. It remains
436 possible that distinguishing the body sizes of conspecifics is more difficult in turbid
437 water, particularly from a distance. As a result, individuals may be unable to assess
438 whether the shoal they were not associating with at any given time was in fact a better
439 'match' for them, phenotypically, and shoals are therefore formed via chance

440 encounters rather than active choice. Further work is needed to tease apart the
441 precise mechanisms underlying the changes in shoal preference we observed.

442 Overall, we have shown that weakened size selectivity by predators and reduced shoal
443 preference by prey are both consequences of increasing turbidity in aquatic
444 environments. Together, these changes both reduce the selection pressure for prey to
445 associate with phenotypically matched individuals, and weaken those association
446 preferences. The confusion and oddity effects are thought to be strong drivers in the
447 evolution of behaviors leading to the formation of phenotypically associated groups,
448 but under turbid water we anticipate a reduction in phenotypic assortment in groups,
449 leading to more diverse, less assorted groups. As assortativeness is associated with
450 behaviors other than predator avoidance, such as enhanced foraging efficiency
451 (Lindstrom and Ranta 1993; Ranta et al. 1994) and synchronisation of activity (Conradt
452 and Roper 2000), a reduction in the pressure for assortment may increase the costs
453 associated with other activities for animals that live in groups.

454

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620

621 Table 1: Results of the analyses of the effects of turbidity treatment (clear or turbid),
622 test fish body size (large or small) and their interaction on a) the proportion of time
623 spent associating with the size matched shoal (GLMER with binomial errors), b) the
624 total time spent shoaling (LME) and c) the number of times the test fish moved
625 between the preference zones associated with the two shoals (activity levels; GLMER
626 with poisson errors). Significant p-values are highlighted in bold text.

Fixed effects	Test statistic	DF	P
a) Proportion of time spent with size matched shoal			
Turbidity	Z = -3.17		0.002
Size	Z = -1.73		0.085
Turbidity * Size	Z = 2.21		0.027

b) Total shoaling time

Turbidity	F = 14.52	1,45	<0.001
Size	F = 0.32	1,44	0.57
Turbidity*Size	F = -3.52	1,44	0.067

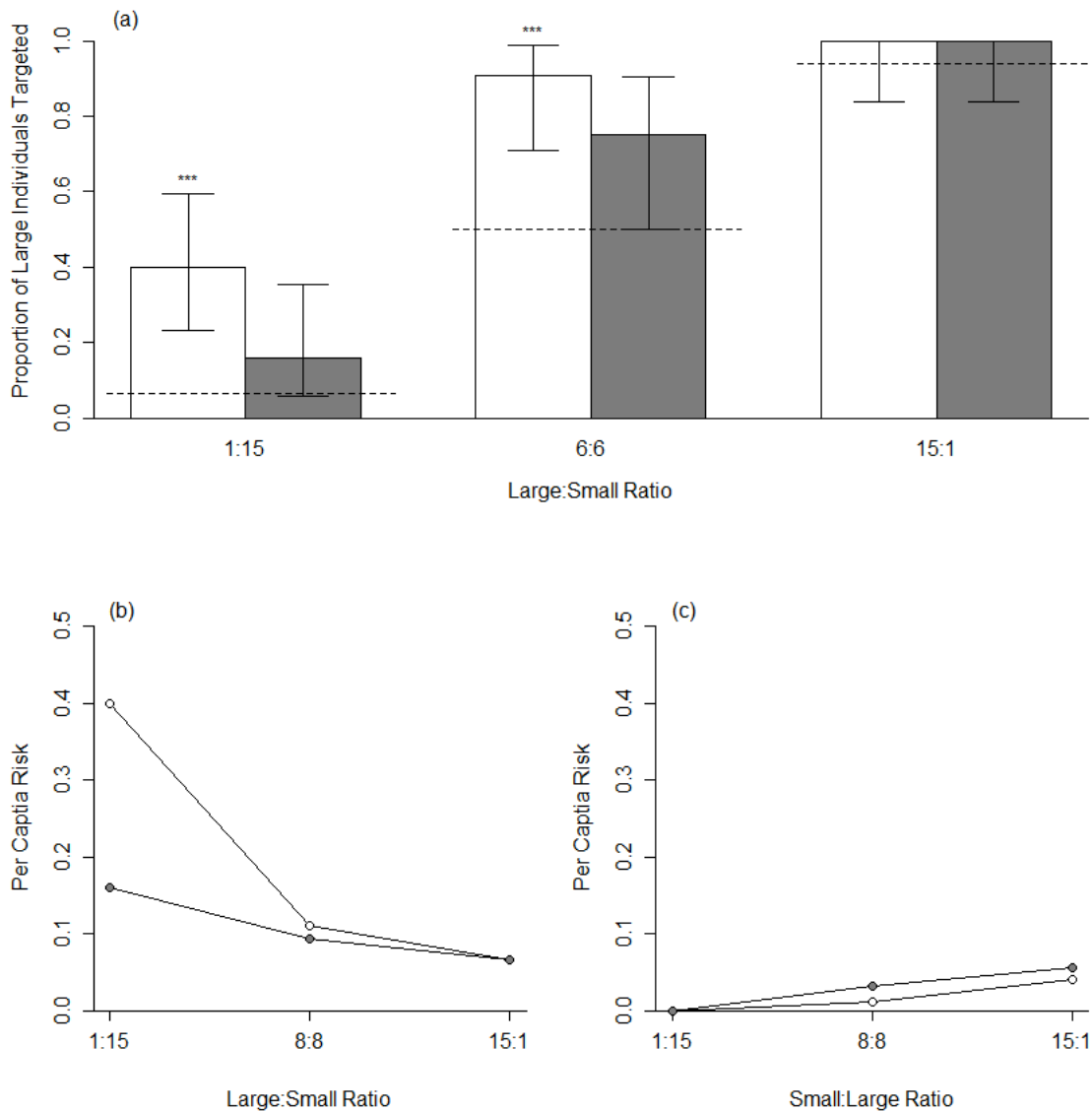
c) Activity (number of preference zone switches)

Turbidity	Z = -10.05		<0.001
Size	Z = -2.79		0.005
Turbidity * Size	Z = 3.07		0.002

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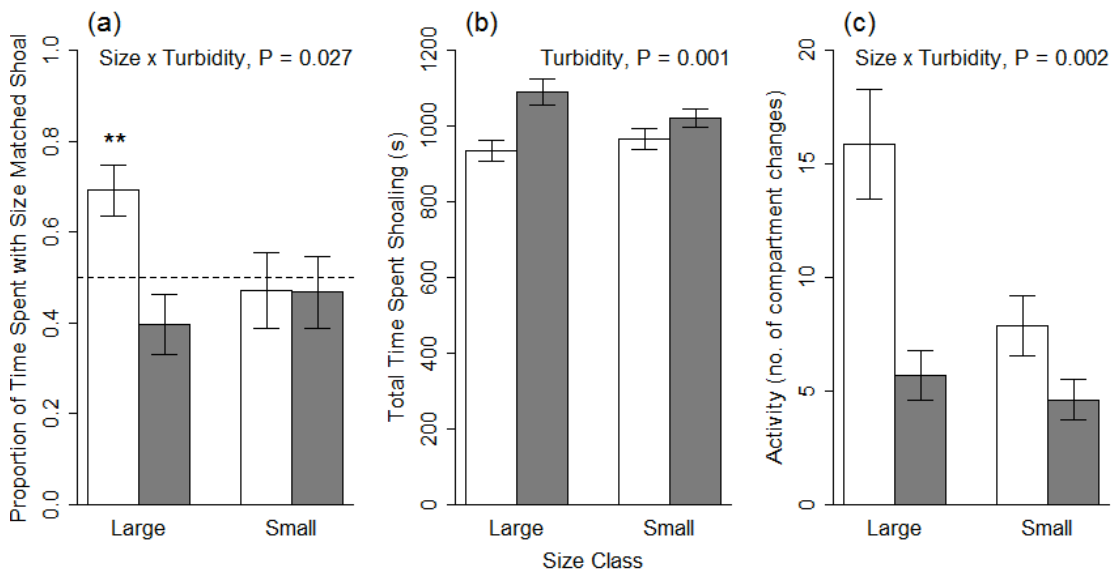


630

631 Figure 1: **(a)** Proportion of attacks on large individuals when large *Daphnia* were the
 632 minority (1:15), equal ratios (8:8) and majority (15:1) within the group (\pm 95% C.I.) in
 633 clear (light bars) and turbid (dark bars) water. Dashed lines represent the proportion
 634 expected if prey selection were random according to each prey group composition.
 635 Asterisks indicate significant differences from random expectation ($P < 0.001$). **(b)** The
 636 per capita risk for large *Daphnia* for each of the treatments (large minority, equal

637 rations and large majority). **(c)** The per capita risk for small *Daphnia* for each of the
 638 treatments (small minority, equal rations and small majority). Open circles represent
 639 clear treatments and dark circles represent turbid treatments. Lines connecting points
 640 are for ease of visualisation.

641



642

643 Figure 2. **(a)** Proportion of time spent shoaling with a size matched shoal for both large
 644 and small fish in clear (light bars) and turbid (dark bars) water (\pm S.E.). Dashed line
 645 represents the proportion expected if fish chose shoals by chance (0.5). **(b)** The total
 646 time spent shoaling by large and small fish in clear and turbid water (\pm S.E.). **(c)** Activity
 647 (number of compartment changes) by large and small fish in clear and turbid water
 648 (\pm S.E.)

649