Turbidity weakens selection for assortment in body size in groups

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Lay Summary

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

Abstract

Prey animals commonly associate with similar-looking individuals to reduce predation risk, via a reduction in predator targeting accuracy (the confusion effect) and preferential targeting of distinct individuals (the oddity effect). These effects are mediated by body size, as predators often preferentially select large bodied individuals, which are therefore at an increased risk within a group. The selection pressure to avoid oddity by associating with similar sized group-mates is stronger for large individuals than small. This selection depends on the ability of both predators and prey to accurately assess body size and respond accordingly. In aquatic systems, turbidity degrades the visual environment and negatively impacts on the ability of
predators to detect (and consume) prey. We assessed the effect of algal turbidity on predator-prey interactions in the context of the oddity effect from the perspective of both predator and prey. From a predator’s perspective, we find that 9-spined sticklebacks preferentially target larger Daphnia in mixed swarms in clear water, but not in turbid water, although the difference in attack rates is not statistically significant. When making shoaling decisions, large sticklebacks preferentially associate with size-matched individuals in clear water, but not turbid water, while small individuals showed no social preference in either clear or turbid water. We suggest that a reduced ability or motivation to discriminate between prey in turbid water relaxes the predation pressure on larger prey individuals allowing greater flexibility in shoaling decisions. Thus, turbidity may play a significant role in predator-prey interactions, by altering predator prey interactions.

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

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

In clear water aquatic predators often show active preferences for particular prey types, such as size (Lehtiniemi et al. 2007; Rodgers et al. 2015), shape (Kislalioglu and Gibson 1976) or colour (Ohguchi 1978); for example cichlids (Aequidens pulcher) preferentially target brightly coloured male guppies (Poecilia reticulata) (Godin and McDonough 2003). When there are no handling constraints, larger bodied prey are generally more profitable (optimal foraging: MacArther and Pianka 1966), and are often over-represented in the diets of planktivorous fish (Li et al. 1985; Wetterer and Bishop 1985). Thus, larger prey individuals are often at greater risk of predation (Lehtiniemi et al. 2007; Manicom and Schwarzkopf 2011). In turbid water, however, size selectivity is often impaired (Reid et al. 1999; Jonsson et al. 2013), as turbidity
directly affects a predator’s ability to locate and target prey. Predator reaction
distances are shortened, which can lower capture success per unit of effort (Gregory
and Northcote 1993; Utne 1997), or the type of prey targeted may change, while
overall predation rates remain constant (Abrahams and Kattenfeld 1997; Shoup and
Wahl 2009). This alters the level of risk experienced by individuals; for prey animals
that aggregate, this may mean that while overall risk to the group remains constant,
relative risk to individuals within the group changes.

Group formation is a common and important response to the risk of predation. In
addition to reducing individual risk (the dilution effect: Foster and Treherne 1981),
groups of moving prey visually confuse predators, reducing targeting accuracy (the
confusion effect: Krakauer 1995, Tosh et al. 2009). This effect is enhanced in larger
groups and when prey individuals are morphologically or behaviorally similar to one
another (Landeau and Terborgh 1986). Predators are better able to overcome the
confusion effect if a distinct or ‘odd’ individual is present within the group (the oddity
effect: Theodorakis 1989). Predators preferentially target odd individuals as they are
easier to visually isolate, making them at increased risk within a group (Milinski 1977a;
Ohguchi 1978; Theodorakis 1989). Together, the confusion and oddity effects predict
that individuals should preferentially group with phenotypically similar individuals, a
phenomenon well-studied in shoaling fishes (Ranta et al. 1992; McRobert and Bradner
1998; Ward and Krause 2001; Rodgers et al. 2011), but observed in other taxa
including birds (Brightsmith and Villalobos 2011) and mammals (Meldrum and
Ruckstuhl 2009).
In the context of the confusion and oddity effects, predator selectivity for particular prey phenotypes (e.g. large body size) means that the selection pressure to avoid oddity should be stronger for preferred phenotypes than for less preferred ones (Rodgers et al. 2015). In support of this, larger fish preferentially associate with large conspecifics, while small individuals show no such preference (Svensson et al. 2000; Rodgers et al. 2011), and larger fish are more risk averse than smaller ones when foraging (Peuhkuri 1997; Peuhkuri 1998). Changes in prey selection by predators associated with turbidity may thus alter the relative risk experienced by individuals within groups, which may have significant consequences for group formation and maintenance.

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

**Methods**
Study species and husbandry

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

Turbid water was created using a unicellular, motile algae *Chlamydomonas spp* (Phytotech lab, Kansas, USA), which has been previously used in studies disrupting vision in fish (Jonsson et al. 2011b), and remained in suspension or the duration of the trials. Algae was grown in a medium containing de-ionised water and Bold’s Basal Medium Solution (Phytotech lab, Kansas, USA) at 20°C, in cylindrical culture vessels (5cm in diameter, 50cm in length) with a constant light source and airflow. Cultures were left to reach high concentrations (~200NTU) and then diluted with water from the aquarium system for experiments to reach 12.5 NTU (±2.5NTU), which equated to
approximately 30cm visual depth (measured with a Secchi disc). Turbidity was maintained in experiments using airstones.

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

**Experiment 1: Targeting of individuals in groups**

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

At the end of the experimental area, a square array of *Daphnia*, consisting of 16 water-filled 1cm$^3$ transparent cubes arranged in a 4x4 grid, was positioned externally on the end of the tank (Rodgers et al. 2013). This ensured visual, but not olfactory cues from the prey were available to the fish, and that prey individuals remained separate and could not physically interact during the experiment. Three treatments with different ratios of *Daphnia* sizes were used; 1:15 large:small (large-bodied minority), 8:8 large:small (equal ratios), and 15:1 large:small (small-bodied minority). In the two treatments with a single odd individual, the position of that individual in the grid was rotated systematically between successful trials to control for any positional effects (Krause 1994). In the equal ratios treatment, large and small individuals were placed in the grid in an alternating pattern. Treatments were randomly assigned to individual fish. A Microsoft LifeCam connected to a laptop was placed behind the array to record the trials, and the test tank was screened by a curtain to minimise disturbance.

Before the trials commenced, the airstones were removed from the experimental area of the tank, so disturbance from the air bubbles did not disturb targeting by the stickleback. At the start of each trial, a single test fish was carefully netted over the barrier from the holding area into the experimental area. This caused minimal disturbance to the fish, with the majority (214/216) of fish resuming normal swimming behavior less than 10 seconds after being transferred to the experimental area. Fish
that did not begin swimming within 2 minutes were excluded from the experiment (N=2 fish). The fish was free to view the Daphnia array as soon as it was netted over the barrier, and a further 10 minutes were allowed for the fish to attack an individual within the array. From the videos, we recorded the size (large or small) of the first Daphnia targeted, defined as the fish making a striking movement towards a particular individual within the array and making contact with the glass of the tank. Once the first attack had been made, the trial ended and the test fish was removed and returned to the stock tanks. Each fish was only tested once and thus only experienced one of the three combinations of Daphnia in one of the water conditions (clear or turbid). Fish that had not made an attack within 10 minutes of being placed over the barrier were excluded from the experiment (20/98 clear water trials, 50/118 turbid water trials).

During the turbid trials, turbidity was measured using a handheld Oakton Turbidity Meter every other trial to ensure that it remained at 12.5±2.5NTU. The water in the experimental tank was changed every 12 trials (i.e. when all the fish in the holding area had been tested), and Daphnia were returned to their size matched pools. In clear water we recorded N=25, N=22, N=24 successful attacks for 1:15 (large:small), 8:8 and 15:1 daphnia treatments respectively, and N=25, N=16 and N=24 successful attacks in turbid water.

Experiment 2: Shoal choice

To investigate the effect of turbidity on social decisions, we carried out a series of binary shoal choice tests (McRobert and Bradner 1998; Rodgers et al. 2011) in clear and turbid water. Three days after being introduced and acclimatised to the aquarium,
15 fish measuring between 35-40mm ("large fish") and 15 measuring between 25-30mm ("small fish") were placed in separate aquaria (on the circulating system) and labelled “stimulus fish”. These fish were never used as test fish in either the targeting experiment or shoal choice experiments. This setup controlled for familiarity (Griffiths and Magurran 1999; Ward et al. 2005), as none of the test fish could become familiar with the stimulus fish. These sizes were chosen as they were readily available in the population, and because three-spine sticklebacks Gasterosteus aculeatus, can distinguish between these size classes (Ward and Currie 2013).

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

Shoal choice experiments were carried out in 60 x 20 x 30cm binary choice tanks. The tank was split into 3 compartments by two solid glass barriers allowing the
transmission of visual but not olfactory cues, with one larger central compartment (30 x 20 x 20cm) set between two smaller compartments (15 x 20 x 20cm). The two smaller compartments contained the stimulus shoals during the experiment. Two 10cm preference zones (approximately 3 body lengths; Pitcher and Parish 1993) were drawn up beside each stimulus compartment. Test tanks were filled to a depth of 12cm (approximately 15L) using the turbid water (see above) or clear water taken from the aquarium system and one air stone was placed in each compartment. Water was changed between each set of experiments (12 test fish, 6 large and 6 small). As no olfactory cues were exchanged between the stimulus shoals and test fish, it was not necessary to change the water between each experiment to control for the build-up of cues from the stimulus fish (which may relay information about size; Ward and Currie 2013). As the water for all experiments was taken from the aquarium system (with concentrated algae added for the turbid water experiments), cues from sticklebacks of all body sizes were present in the water.

One hour before experiments, test fish were transferred to individual 20 x 20 x 10cm tanks containing either clear or turbid water to allow for acclimatisation to test conditions. One stimulus shoal of 3 large fish and one stimulus shoal of 3 small fish, selected haphazardly from the stimulus fish tanks, were placed in the two end compartments of the binary choice tank and allowed to acclimatise for 15 minutes. After this time the focal fish was placed in the centre compartment. Observations began when the test fish resumed normal swimming behavior (between 30 - 120 seconds). One fish was excluded from the trial as it froze for 5 minutes. During a 20 minute observation period the time spent in each preference zone (defined as a fish...
having more than 50% of its body within the preference zone) and the number of
times it moved between preference zones (a measure of activity; Fischer and
Frommen 2012, Rodgers et al. 2011) were recorded. The trial was observed from
behind a curtain using a Microsoft LifeCam attached to a laptop to minimise
disturbance. New stimulus shoals were taken from stimulus fish tanks after every third
experiment, and the side containing the shoal of large fish was systematically
alternated. To reduce the overall number of stimulus fish required, each individual was
used more than once over the course of experiments, but haphazard selection of
individuals from the stimulus stock tanks meant that it was unlikely the same
combination of fish was selected more than once. After each trial, test fish were placed
back into the holding tanks and fed defrosted frozen bloodworm. Stimulus fish were
fed on completion of the day’s experiments.

Statistical Analysis

In the targeting experiment (experiment 1) we assessed whether sticklebacks targeted
particular body sizes more than would be expected by chance using exact binomial
tests. In each case, we compared the observed proportion of attacks on large Daphnia
to expected probabilities based on random targeting. Expected proportions for the
large minority, equal ratios and small minority treatments were 0.0625, 0.5 and 0.938
respectively. For example in a 1:15 ratio of large Daphnia: small Daphnia, the large
Daphnia would be targeted 6.25% of time (1/16 x 100) if attack was random with
respect to body size. 95% confidence intervals for the proportion of attacks on large
Daphnia were calculated using the ‘modified Wald’ method recommended by Agresti
& Coull (1998). We then compared the proportion of large individuals targeted in each of the treatments (large minority, equal ratios and small minority) between clear and turbid water using proportion tests. The per capita risk to individuals was calculated as the proportion of trials in which an individual was targeted, divided by the number of size matched individuals present in the group (Rodgers et al 2014).

For the shoal choice experiments (experiment 2), we used a generalised linear mixed effects model (GLMER) model with a binomial error distribution (as appropriate for proportion data) to assess whether the proportion of time spent shoaling with size matched individuals (shoal choice) was influenced by turbidity (clear or turbid water), test fish body size and their interaction. Fish ID was included as a random factor to account for the repeated measured design and an additional observation-level random effect was used to account for overdispersion of the data (Harrison 2014). To assess whether the shoaling preference exhibited by large and small fish in clear and turbid water differed significantly from random expectation (50% of the time with each shoal), one-sample tests were applied. Data was arcsin square root transformed to meet the assumptions of normality where possible and a one-sample t-test was used; otherwise we used a non-parametric Wilcox signed ranks test. The false discovery rate (FDR) method was applied to correct for multiple testing (Benjamini and Hochberg 1995), and we present the adjusted p-values here. We used a linear mixed effects (LME) model to assess the effect of turbidity, body size and their interaction on the total time spent shoaling with both shoals. Fish ID was included as a random effect to take into account the repeated measures design. Non significant interactions were removed following Crawley (2007). Visual inspection of plots of residuals against fitted
values and quantile-quantile plots indicated that a normal error distribution was appropriate here (Crawley 2007). Finally, to investigate if activity (the number of times the fish switched between preference zones) was influenced by body size, turbidity and their interaction we used a GLMER model with a Poisson error distribution (as appropriate for count data) with fish ID included as a random factor. All analysis was carried out in R 2.15.1 (R Development Core Team 2011).

Results

Experiment one: Targeting of individuals in groups

In clear water, large individuals were targeted significantly more than was expected by chance in the equal ratios treatment (figure 1.1a: large Daphnia targeted in 20/22 trials, 91%, $P < 0.001$, with random expectation 0.5) and in the large minority treatment (figure 1.1a: large individual targeted in 10/25 trials, 40%, $P < 0.001$, random expectation 0.0625). In turbid water large individuals were no longer preferentially targeted at either ratio, and were chosen with a rate consistent with chance (figure 1a: equal ratios: large individual targeted 12/16 trials, $P = 0.08$, with a random expectation of 0.5, large minority: 4/25, $P = 0.076$, random expectation 0.0625). There was no significant difference in attack rate at either ratio between clear and turbid water (equal ratios: $X^2 = 0.77$, df = 1, $p = 0.38$, large minority, $X^2 = 2.48$, df = 1, $p = 0.12$) When large individuals made up the majority of a group (15:1) large Daphnia were
attacked at a rate consistent with chance in both clear and turbid water (figure 1a: 100% of trials. 25/25 in clear and 21/21 in turbid water).

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

Experiment two: Shoal choice

We found a significant interaction between water treatment and body size on the proportion of time individuals spent associating with size matched shoals (GLMER: Z = 2.22, P = 0.027, figure 2a, table 1a). Large individuals preferred to associate with size matched shoals in clear water (t = 3.99, adjusted P = 0.0024), but not in turbid water (t = 0.56, adjusted P = 0.36). Small individuals showed no active preference for either sized shoal in clear (V = 94, adjusted P = 0.64) or turbid water (V = 122, adjusted P = 0.64, figure 2b). Both large and small test fish spent significantly more time shoaling overall (total time spent shoaling) in turbid water compared to clear (LME: F_{1,44} = 14.52, P < 0.001, figure 2b, table 1b), but there was no effect of body size and no interaction.

Finally, we found a significant interaction between water treatment and fish size on activity levels (GLMER: z=3.07, p = 0.002, figure 2c, table 1c). Large test fish had a higher level of activity in clear water compared to small fish, but both large and small fish reduced their activity to similar levels in turbid water. Examining the data more
closely, we found that fish in turbid water were more likely to remain in one preference zone for the duration of the trial than fish in clear water (5/46, 11% clear water trials, 15/46, 33% turbid water trials).

**Discussion**

Our results suggest that turbidity may weaken predator preferences for targeting odd, large-bodied individuals, and relaxes the pressure on large bodied prey to associate with similarly-sized groupmates. We suggest there is weakened selection pressure for behavioral assortment in prey, driven by confusion and oddity effects, in turbid water. Reflecting previous work (Rodgers et al. 2015; Gibson 1980; Li et al. 1985; Wetterer and Bishop 1985), we found strong predator selectivity for large prey in clear water, particularly when they were in equal ratios with small-bodied prey or were in the minority (odd) within the group. In turbid water, this preference was absent and targeting of large individuals occurred at a rate consistent with chance, although we did not detect a significant reduction in preference between turbidity treatments. A similar absence of preference in turbid water has also found in more ecological studies of dietary preference (Vinyard and Yuan 1996; Rowe et al. 2003). We suggest that turbidity may relax predation pressure on large individuals within groups, reducing the benefits of assorting by size, and resulting in a loss of the preference for associating with size-matched conspecifics seen in clear water when turbidity increases, as observed in experiment 2.
Large prey may be less at risk in turbid water due to visual constraints: in low-visibility conditions, predator-prey interactions occur at closer distances (Miner and Stein 1996; Meager et al. 2006), meaning predators may have reduced choice or reduced time for selection of prey from a group. Algae absorb photosynthetically active wavelengths and scatter light (Kirk 2011), reducing the contrast between objects and their background (Utne-Palm 2002), which negatively affects long-distance detection substantially more than short distance detection (De Robertis et al. 2003). For large individuals, therefore, detection distances are reduced to a greater extent than for small individuals, which may reduce size selectivity by altering encounter rates (Utne-Palm 2002; Jonsson et al. 2013). Turbidity may impact on predator confusion: if detection distances are reduced, prey swarms may appear less dense or numerically smaller, and predators therefore less susceptible to confusion effects (which are enhanced in larger and denser groups; Milinksi 1977b; Ioannou et al. 2009). The importance of oddity for successful predation would therefore also be reduced, and preferences for odd individuals in groups weakened. Here, we focused on visual cues alone, but predators that use alternative cues to hunt are also susceptible to confusion effects (Jeschke and Tollrian 2007). Tactile predators, for example, may suffer from confusion effects as they lack the high spatial resolution thought necessary to single out and target individuals from within a group (Jeschke and Tollrian 2007). If exposure to a degraded visual environment means predators switch to alternative cues more susceptible to confusion effects, how predators respond to groups of prey may be changed in other ways. Further work teasing apart how different cues influence group detection and targeting for predators using different sensory modalities is needed.
If predators are less selective in turbid water, then prey could be expected to respond appropriately to the altered risk environment. Our results suggest that shoaling fish adjust their shoal choices in response to their immediate environment, with large fish losing their preference for size-matched shoals under turbid conditions. For large individuals, at higher risk of predation, association with size-matched individuals reduces risk by reducing oddity and enhancing confusion effects (Theodorakis 1989; Ranta et al. 1992; Rodgers et al. 2011), while for small individuals, risk is lowered through association with larger prey (Rodgers et al. 2015). If large-bodied prey is at lower risk in turbid water, and the anti-predator benefits of size-matching are reduced, we would expect to see a reduction in the strength of association preferences. Association with large-bodied individuals carries a number of costs – particularly increased competition for food (Metcalfe and Thomson 1995; Hoare 2000), which can be avoided under potential relaxed selection pressures for assortment in turbid water, although large fish did not make a complete switch to take advantage of this by shoaling with small individuals, suggesting other factors also influence assortment decisions (Rodgers et al. 2013).

Turbid water is often associated with a reduced perception of overall risk in fish (Gregory 1993; Engström-Öst and Mattila 2008), resulting in reduced anti-predator behavior, including weakened escape responses (Gregory 1993; Meager et al. 2006), reduced use of shelter (Abrahams and Kattenfeld 1997) and decreased shoal cohesion (Kimbell & Morrell 2015) even if actual risk remains unchanged (Reid et al. 1999;
Shoup and Wahl 2009). A reduction in shoaling preferences could be attributed to this effect: sticklebacks perceive that overall, rather than individual, risk is reduced and adjust their social behavior accordingly. However, our finding that fish both increased the total time they spent in association with other shoals (figure 4.2b) and reduced their activity levels (figure 4.2c) suggests an enhanced, rather than reduced, perception of overall risk levels in turbid water for 9 spined sticklebacks. In birds, the degradation or obstruction of visual cues can mean predators are more difficult to detect (Whittingham et al. 2004), and individuals increase vigilance and decrease foraging (Metcalfe 1984; Whittingham et al. 2004; Devereux et al. 2008), consistent with an enhanced perception of risk. Reduced activity levels are thought to reduce encounter rates with predators and have previously been observed in shoaling fish (Fischer and Frommen 2012). By remaining with a shoal, rather than moving between shoals, individuals reduce their exposure to predators under situations where they are at increased risk through isolation (Landeau and Terborgh 1986).

As predators, fish are affected by visual constraints in turbid water, and thus the same constraint might be expected for fish as prey. Our test fish may have been unable to detect both shoals simultaneously, although the turbidity levels in our experiment (12NTU, equivalent to a secchi depth of 30cm) were chosen so that fish should be able to view both shoals simultaneously from any location within the test tank. It remains possible that distinguishing the body sizes of conspecifics is more difficult in turbid water, particularly from a distance. As a result, individuals may be unable to assess whether the shoal they were not associating with at any given time was in fact a better ‘match’ for them, phenotypically, and shoals are therefore formed via chance
encounters rather than active choice. Further work is needed to tease apart the precise mechanisms underlying the changes in shoal preference we observed.

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

References


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

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**b) Total shoaling time**
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c) Activity (number of preference zone switches)

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<td>Size</td>
<td>-2.79</td>
<td>0.005</td>
</tr>
<tr>
<td>Turbidity * Size</td>
<td>3.07</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Figure 1: (a) Proportion of attacks on large individuals when large *Daphnia* were the minority (1:15), equal ratios (8:8) and majority (15:1) within the group (± 95% C.I.) in clear (light bars) and turbid (dark bars) water. Dashed lines represent the proportion expected if prey selection were random according to each prey group composition. Asterisks indicate significant differences from random expectation (*P* < 0.001). (b) The per capita risk for large *Daphnia* for each of the treatments (large minority, equal
rations and large majority). (c) The per capita risk for small *Daphnia* for each of the treatments (small minority, equal rations and small majority). Open circles represent clear treatments and dark circles represent turbid treatments. Lines connecting points are for ease of visualisation.

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