Turbidity influences individual and group level responses to predation in guppies (*Poecilia reticulata*)

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Abstract

Increasing turbidity (either sedimentary or organic) from anthropogenic sources has significant negative impacts on aquatic fauna, both directly and indirectly by disrupting behaviour. In particular, anti-predator responses of individuals are reduced, which has been attributed to a reduced perception of risk. Here, we explore the effect of turbidity on shoaling behaviour, which is known to carry important anti-predator benefits, predicting that fish in turbid water should show reduced shoal cohesion (increased inter-individual distances) and reduced responses to a simulated predatory threat. We explore both the individual and shoal level responses to a predation threat at 4 different levels of turbidity. At the shoal level, we find that shoals are less cohesive in more turbid water, but that there is no effect of turbidity on shoal-level response to the predation threat. At an individual level, guppies in turbid water were more likely to freeze (rather than dart then freeze), and those that darted moved more slowly and over a shorter distance than those in clear water. Fish in turbid water also took longer to recover from a predation threat than fish in clear water. We suggest that because fish in turbid water behaved in a manner more similar to that expected from lone fish than to those in a shoal, the loss of visual contact between individuals in turbid water explains the change in behaviour, rather than a reduced perception of individual risk as is widely supposed. We suggest that turbidity could lead to a reduced collective response to predators and a loss of the protective benefits of shoaling.

<u>Keywords:</u>

Collective behaviour, *Poecilia reticulata*, predation, turbidity, vision

Introduction

Intensified agricultural practices, urbanisation and deforestation are increasing levels of turbidity from suspended sediment and algal overgrowth in fresh water environments (Smith et al., 2006). This can have multiple negative implications for aquatic communities and is thought to be a significant contributor to declines in aquatic fauna worldwide (reviewed in Richter et al. 1997; Henley et al. 2000). At high levels of turbidity, particles can directly affect growth and survival: sediment particles can damage gills leading to infection (Sutherland & Meyer, 2007) and large algal blooms can deoxygenate water (Bruton, 1985). Turbidity at lower levels acts indirectly by altering the behaviour of aquatic organisms due to the degradation of the visual environment. Turbidity can disrupt communication signals (Van Der Sluijs et al., 2011), impair mate choice (Engstrom-Ost & Candolin, 2007; Sundin et al., 2010) and reduce the ability to detect food resources (Aksnes & Giske, 1993; Utne-Palm, 2002).

Crucially, turbidity can mean that individuals can no longer accurately detect predation threats (Swanbrow Becker et al., 2012), leading to changes in anti-predator behaviour and survival. In highly turbid environments, individual Atlantic cod (*Gadus morhua*) display poorly timed, weakened anti predatory responses (Meager et al., 2006). Other species increase behaviours often viewed as more risky (Van De Meutter et al., 2005) and decrease use of shelter in the presence of a predator (Abrahams & Kattenfeld, 1997; Lehtiniemi et al., 2005). This may indicate a reduced perception of risk for some species, suggesting that turbidity acts as a shelter for prey, affording them protection from predators (Engstrom-Ost et al., 2006; Engström-Öst & Mattila, 2008; Gregory & Northcote, 1993; Snickars et al., 2004). In support of this theory, some visually-orientated predators can suffer from reduced capture success in turbid water (Ljunggren & Sandstrom, 2007; Utne, 1997), however many do not (Abrahams & Kattenfeld, 1997; Gregory & Levings, 1998; Jonsson et al., 2013; Reid et al., 1999). Primarily, turbidity acts to shorten the distance at which predators and prey detect each other so although prey detection by predators is impaired, those prey that are detected have less time to respond. This can make escape manoeuvres less effective (Gregory, 1993; Meager et al., 2006), although shorter distances are required to move prey out of sight of predators in turbid conditions (De Robertis et al., 2003).

While changes in behaviour on an individual level have been well documented, less is known about responses to turbidity in the context of shoaling, a common and important anti-predatory tactic among many fish species (Krause & Ruxton, 2002; Magurran, 1990). Groups of animals detect potential threats more quickly (Godin et al., 1988; Magurran et al., 1985), perform coordinated evasive manoeuvres (Magurran & Pitcher, 1987), dilute individual risk of predation (Godin, 1986; Treherne & Foster, 1981) and visually confuse predators, resulting in reduced targeting success (Ioannou et al., 2009; Krakauer, 1995; Landeau & Terborgh, 1986). Groups become larger, tighter and more polarised when at risk from predation (Caraco et al., 1980; Magurran & Pitcher, 1987; Seghers, 1974; Watt et al., 1997), enhancing these benefits. Thus, a reduced perception of risk in turbid water (Engstrom-Ost et al., 2006; Engström-Öst & Mattila, 2008; Gregory & Northcote, 1993; Snickars et al., 2004) could lead to reduced shoal cohesion. However, most fish rely strongly on vision for shoaling (Partridge & Pitcher, 1980) meaning reduced visual distances could also disrupt this important anti-predator tactic: at low light intensities fish shoals tend to break apart (Ryer & Olla 1998, Einfolt et al 2012, Paciorek and McRobert 2013, Miyazaki et al 2000). Increasing turbidity is therefore expected to lead to reduced levels of shoal cohesion through either reduced perception of risk or reduced visual distances. Empirical data suggests that while moderate levels of turbidity may enhance shoaling in some species with well-developed eyes (Ohata et al., 2013), in highly turbid water, fish lose their preference for shoals composed of more individuals (Fischer & Frommen, 2012) and form looser aggregations (Ohata et al., 2013).

During a predation attempt, fish in shoals perform a fast burst of motion to accelerate themselves away from the threat, leading to the flash expansion of the group (although some species remain highly cohesive during this response; Radakov, 1973). Individuals then regroup to form more cohesive shoals (Ryer and Olla, 1998). Increased cohesion reduces risk through increased predator confusion (Ioannou et al., 2009; Krakauer, 1995; Milinski, 1977) and selfish herd effects, where individuals seek cover behind other shoal members (Hamilton, 1971). If turbidity disrupts shoal cohesion (Fischer & Frommen, 2012; Ohata et al., 2013) and reduces the perception of risk (Engström-Öst & Mattila, 2008; Gregory, 1993), responses to a predation event may be negatively impacted, increasing predation risk. Here, we explore how shoaling patterns of guppies (*Poecilia reticulata*) are influenced by increasing turbidity and in particular, how turbidity affects both individual and shoal level responses to the visual detection of a simulated aerial predation threat. Anti-predatory behaviour is well studied in this species: guppies form loose, uncoordinated shoals (as opposed to tightly polarised schools) of 2 – 47 individuals (Croft et al., 2003), and respond to predators by using escape responses or freezing, and by increasing shoal cohesion (Fischer et al., in press; Magurran, 2005). Guppies have excellent vision (Endler, 1991), responding strongly to visual predator cues (Kelley & Magurran, 2003), making them an ideal species for this study. Our aim is to assess whether increased turbidity is likely to have negative impacts on grouping as an anti-predator response, by changing the way fish within shoals respond to a threat when they can no longer easily detect one another.

Methods

Study species and housing

All fish used in this experiment were descendants of wild-caught guppies from Trinidad. Fish were maintained in aquaria (20 x 40 x 40 cm) on a recirculating system at the University of Hull at approximately 26^{o}C (±1^{o}C) on a 12:12hr light:dark cycle and fed daily on ZM small granular feed (0.5-0.8mm ZM Systems, Hampshire, UK). Shoals consisting of 4 guppies were created by taking female fish of similar size (all fish in a shoal measured within 0.5cm of each other) from stock tanks and moving them to separate holding tanks 20cm x 20cm x 20cm (26 shoals in total). Mean body size of individuals within shoals ranged from 1.4cm to 2.5cm. Only females were used as they form the core of guppy shoals (Croft et al., 2004) and to reduce the confounding effect of sexual behaviour on association patterns. Shoals were left in these tanks for 14 days before experiments began to allow fish to become familiar with one another (Griffiths & Magurran, 1999), as familiarity can enhance anti-predator responses (Chivers et al., 1995).

Experimental design

Each shoal was exposed to 4 turbidity treatments (0, 50, 100 or 200 ± 10NTU) in a randomised order, with one week between exposures to allow for recovery. Thirty min before each trial, shoals of fish were moved to separate cylindrical holding tanks (diameter 10cm, depth 33cm) to allow the fish to acclimitise to the turbidity level. Turbidity levels were chosen as turbidity is known to reach 200NTU during rainy seasons in Trinidad (Luyten

& Liley 1990), making the levels ecologically relevant. Turbidity was created using a bentonite clay-water solution and measured using an Oakton T100 portable turbidity meter. A concentrated bentonite clay solution was created using 100g of clay suspended in 4 litres of purified water. This was filtered through fine mesh to remove larger, heavier particles that sank faster when placed in suspension, to allow for the maintenance of turbidity levels over the course of the experiment. The resulting filtrate (>1000NTU) was further diluted with water taken from the aquarium system to obtain the desired turbidity level. Turbidity was maintained in the acclimatisation tanks by pumping air into the bottom of the cylindrical tank, which re-suspended any particles that fell to the bottom.

After the acclimatisation period shoals were transferred to a white circular tank with grey sides (diameter 40cm, depth 15cm, filled to a depth of 2cm with water of the required turbidity). A monofilament fishing line ran above the centre of the tank at a 30^{o} angle, the end of which was attached to the back of the tank 10cm above the waterline. From this a model bird predator could be dropped such that it passed over the centre and came to rest against back of the tank. This approach elicits a rapid escape response in fish (Chapman et al., 2010a, Seghers, 1974), leads them to initiate aggregation (Krause & Tegeder, 1994) and increase shoaling tendency (Krause et al., 1998). Thus, fish respond to the approaching aerial stimulus as if it were a predation threat, without the need for a predation event to occur. Guppies are predated on by a number of bird species in their natural habitat (Magurran, 2005) and preliminary trials in clear water indicated it was effective in eliciting a behavioural response in our study fish, and thus would be appropriate for investigating the effect of turbidity on responses to a visually-detected predator. It is possible fish may respond to a mechanical stimulus caused by the model coming to rest at the back of the tank rather than the visual stimulus from flying overhead, but the majority of individuals responded when the model bird was overhead (0NTU =79%, 50NTU = 76%, 100NTU = 73%, 200NTU = 77%).

The water in the tank was kept at a depth of 2cm to allow for observation of the fish in highly turbid water and to minimise vertical movement (increasing accuracy in measuring inter-individual distances). Guppies are found in very shallow pools and streams in their natural environment, making the depth used ecologically relevant across at least some of their habitat (Luyten & Liley, 1985). The shallow water also ensured that guppies were not impeded significantly in the detection of the stimulus, and that any differences in behavioural response at different turbidity levels were not due to differences in the detection of the threat. Turbidity was maintained in the test tank by gently circulating water using an external filter. A Microsoft webcam suspended 60cm above the tank was used to record trials at 15 frames/s, and the tank was illuminated from above to ensure that a shadow passed over the tank when the predator was released.

A model bird predator was used to elicit a startle response in the guppy shoals. The model predator was an oval piece of black card 10cm long and 4cm at its widest point attached to a small circular ring at its centre through which the monofilament line was threaded. At the start of the trial, the predator was positioned at the highest point of the wire, out of sight of the tank. Fish were acclimatised in the test tank for 5 min (all fish had begun swimming normally by this point) and then their shoaling behaviour was recorded for 10 min. To initiate a startle response after the 10 min of shoaling, the model predator was released. Video recording continued through the simulated predation event and for 2 min afterwards, when trials were terminated. Water in the test tank was changed between each trial to remove any olfactory cues.

Shoal cohesion was defined as the mean inter-individual distance between individuals within a group (Miller & Gerlai, 2007). As guppies form lose shoals, rather than schools, we did not measure alignment or activity synchrony, which may also component of overall cohesion. To measure shoal cohesion, videos files were converted into an image stack using VirtualDub (http://www.virtualdub.org) at 1 frame every 10 s (analysis at different frame intervals confirmed this gave an accurate representation of shoaling behaviour), which were then manually analysed in ImageJ (http://imagej.nih.gov/ij/). A scale bar drawn on the base of the tank and the tank diameter allowed accurate setting of scale for each video (pixels/cm). The XY coordinate of every fish (taken from the nose of each individual) was recorded every 10 s for 12 min (10 min before the simulated predation event, and 2 min after) and the average pairwise distance between the 4 fish calculated.

At an individual level, we observed two responses to the predator: fish would either freeze immediately ('freeze'), or dart away from their position, using a fast burst of motion, and then freeze ('dart then freeze'), and we recorded the number of each type of response performed by each individuals. All individuals showed one of these two responses: no individuals darted without freezing, and no individuals remained swimming normally or showed another response. For fish that darted and then froze, we recorded their response in detail, by tracking individual movement of each of the 4 fish in the shoal for 15 consecutive frames (1 second) immediately after the predator had passed over the tank, using the plugin MtrackJ (http://www.imagescience.org/meijering/software/mtrackj/) for ImageJ. The latency to respond to the attack (defined as the number of frames taken till the fish responded by either darting or freezing), the distance moved (cm), the maximum speed reached (cm/s) and the time taken to regain normal swimming (when the fish had moved more than approximately one body length from the frozen position) were recorded for each fish in each shoal.

Individual fish were not identifiable between turbidity treatments, although shoal membership remained constant throughout the experiment. 7 fish died during the experiments and these shoals were excluded from further treatments. 2 videos were excluded as the fish were disturbed before the startle response.

Statistical analysis

Shoal cohesion was measured as the mean of the inter-individual distances between each pair of fish within the shoal at each time point for 10 min before the predator release and 2 min after. The effect of the appearance of a predator on shoal cohesion was assessed using a linear mixed effects model (LME) with time (before and after) and turbidity set as the main effects and shoal identity as a random effect to account for repeated measures and non-independence of individuals within a shoal. No interaction between time and turbidity treatment was observed, so this was removed to give the minimum adequate model (MAM; Crawley, 2007). Model assumptions were checked by visual inspection of plots of residuals and were found to conform to the assumptions of normality. In order to test for differences between each of the turbidity treatments, the model was repeated using each turbidity level as the main intercept (re-levelled data).

To assess the effect of turbidity on the proportion of individuals freezing we used a generalised linear mixed effects model (GLMER) with a binomial error distribution. Turbidity was set as the main effect and shoal identity as a random factor. A GLMER with a poisson error distribution was used to analyse the latency to respond, with turbidity set as the main effect and shoal identity as a random factor. An additional observation-level random effect was used to account for overdispersion of the data (Harrison, 2014). For fish that responded with the darting response, individual startle responses (distance moved, maximum speed) were analysed using LME models with turbidity included as a main effect and shoal as a random effect to account for both repeated measures and non-independence of individuals within a shoal. Fish that froze were excluded from this analysis as they did not move as part of their response. In order to assess whether there was a difference between each pairwise of the turbidity treatments, each model was repeated using each turbidity level as the main intercept. All analysis was carried out in R 2.15.1 (R Development Core Team 2011)

Ethical note

This project was approved by the ethical review committees of the School of Biological Biomedical and Environmental Sciences and the Faculty of Science and Engineering at the University of Hull (reference numbers U021 and U023).

Results

Shoal Cohesion

Shoals were observed to expand and contract (decrease and increase cohesion) throughout the experiment, resulting in a large variability in shoal cohesion over time. Despite this variation, there was a significant effect of turbidity (LME: $F_{3,142} = 4.98$, $P = 0.0026$), but no effect of time (before or after predator simulation) on shoal cohesion (LME: $F_{1,142} = 0.15$, $P =$

0.7) and no interaction. The expansion of the shoals during the predator exposure was well within the normal shoaling range, with post-predator exposure shoals showing similar patterns to the pre-exposure distances.

Over the course of the experiment (before and after the aerial predation attempt), fish were found to shoal more cohesively in the lowest two levels of turbidity (0 and 50NTU) compared to the highest two level of turbidity (100 and 200NTU) (Figure 1: 0NTU vs 100NTU: t = 2.7, P = 0.0077; 0NTU vs 200NTU: t = 2.79, P = 0.006; 50NTU vs 100NTU: t = 2.67, P = 0.0084; 50NTU vs 200NTU, t = 2.77, P = 0.0064). No significant differences in shoal cohesion were observed between 0NTU and 50NTU (t = 0.09, P = 0.93) or 100NTU and 200NTU (t = 0.048, P = 0.96)

Individual responses to simulated predation threat

There was a significant effect of turbidity on the proportion of fish freezing (rather than darting then freezing) immediately after the predator attack (figure 2a). With a higher proportion of fish found to freeze in highly turbid water compared to clear water (0NTU vs 200NTU; z = 3.05, P = 0.0023) and the 50NTU treatment (50NTU vs 200NTU, z = 2.43, P = 0.015; table 1).

We found no effect of turbidity treatment on the latency to respond to the predation threat (LME: $F_{3,233}$ = 1.28, P = 0.28, table 1), however, the strength of response differed depending on turbidity. Fish reached a higher maximum speed within the first second of movement when in clear water compared to turbid (LME: $F_{3,233}$ = 2.95, P = 0.034, figure 2b). Fish in clear water (0NTU) moved more quickly during the escape manoeuvre than fish in 50NTU (t

= -2.60, *P* = 0.01) and 200NTU (*t* = -2.75, *P* = 0.0064), but not 100NTU (*t* = -1.85, *P* = 0.065). No differences were observed between the 3 turbid treatments (table 1).

The total distance moved in the first second also differed between the turbidity treatments (LME: $F_{3,\ 233}$ = 4.98, *P* = 0.0023, figure 2c). Significant differences were found between clear water and highly turbid water (0NTU vs 200NTU, *t*= -3.74, *P* <0.001, table 1d) and the lowest level of turbidity (0NTU vs 50NTU, *t* = -2.08, *P* = 0.038), with fish in the clear treatment swimming increased distances in response to the threat. A difference was also observed between the two highest turbidity treatments; 200NTU and 100NTU (*t*= -2.57, *P* = 0.010), with fish moving further in 100NTU compared to 200NTU (table 1).

Individuals took less time to recover from the simulated predation in clear water compared to all turbid treatments (Figure 2d. LME: $F_{3,327}$ = 5.01, *P* = 0.002). Fish recovered (began swimming normally) significantly faster in clear water compared to 200NTU (*t* = 3.85, *P* < 0.001), 100NTU (*t* = 2.06, *P* = 0.046) and 50NTU (*t* = 2.39, *P* = 0.017). No differences were observed between any of the turbid treatments (table 1).

Discussion

Increasing levels of turbidity influenced the behaviour of guppies at both the individual and group level. Shoals were less cohesive in highly turbid water, but we found no effect of turbidity on the shoal level response to a simulated aerial predation threat. At an individual level turbidity had a strong influence on anti-predator behavioural responses. In more turbid water, individuals were more likely to freeze (rather than dart then freeze), and those that did show darting behaviour had a slower escape speed and moved a shorter distance than those in clear water, even though there was no difference in the time to initially respond to the predator. Fish in turbid water also took longer to recover from the predation threat than fish in clear water. Our finding that turbidity reduced shoal cohesion and caused individual fish to display weaker darting responses to a predator could either be explained by either a reduced perception of risk in turbid water (Gregory, 1993; Miner & Stein, 1996) or by constraints caused by the poor visual environment (Abrahams & Kattenfeld, 1997). The finding that fish in turbid water had increased recovery times, however, contradicts the reduced perception of risk theory.

Weakened anti-predator responses in turbid water displayed by fish (Abrahams & Kattenfeld, 1997; Engström-Öst & Mattila, 2008; Gregory, 1993; Meager et al., 2006; Snickars et al., 2004) have been attributed to a reduced perception of risk. This may be due to turbidity reducing the probability of encountering a predator (Gregory & Levings, 1998) indicating that individuals are safer in turbid water, or reducing the ability of individuals to detect a predator (Meager et al., 2006) meaning the level of actual risk may remain unchanged. Studies reporting the true level of risk in turbid water show mixed results; in some cases (particularly for small juvenile fish) turbidity appears to act as a refuge, protecting prey from predators (Engström-Öst & Mattila, 2008; Snickars et al., 2004), with some individuals actively seeking out turbid water (Gregory & Levings, 1998). Although perception of risk may be reduced, actual risk may not: capture rates often remain the same in clear and turbid water (Jonsson et al., 2013; Reid et al., 1999) as predators compensate by using alternative cues to locate prey (Johannesen et al., 2012) or increase searching activity (Meager & Batty, 2007), and as a result of inappropriate or less effective anti-predatory behaviours displayed by prey.

Our finding that fish in turbid water took longer to recover from a threat contradicts the predictions of the reduced perception of risk theory, which would predict a reduced recovery time in turbid water (Gregory, 1993). Instead, the reduced visual distances in turbid water may cause fish to act as individuals rather than members of a shoal, since they are no longer able to easily detect and respond to their group-mates using vision. Fish in shoals have been found to recover more quickly than individual fish (Magurran & Pitcher, 1987), supporting the suggestion that longer recovery periods in turbid environments in our experiment could indicate that the guppies' anti-predator responses are more comparable to those of lone fish. The idea that altered behaviour is due to physical constraints imposed by turbidity is further supported by our finding that, in higher turbidity, a greater proportion of individuals froze rather than darting: freezing and hiding behaviours are more often associated with lone individuals than individuals in larger groups (Magurran & Pitcher, 1987; Rangeley & Kramer, 1998), although Fischer et al (in press) found lone fish were more likely to dart in response to a simulated predation threat compared to fish in a shoal. In a shoal, the darting response may add to predator confusion (Ioannou et al., 2012), reducing predator attack success and decreasing risk to prey. For lone fish, however, freezing or hiding may help an individual reduce risk by avoiding detection (Magurran & Pitcher, 1987). In high turbidity, the combination of reduced visual range and more dispersed shoals suggests fish are no longer able to easily detect conspecifics causing them to switch anti-predatory tactics to those more commonly associated with isolated individuals.

In contrast to previous work, we did not see an increase in shoal cohesion after the simulated attack in any of the groups (selfish herd effect: Hamilton, 1971, Krause et al., 1998). This may have been because guppies tended to move to the edges of the tanks (pers.

obs.), which could potentially be used or perceived as a possible refuge location by the fish, as the grey colour of the sides of the tank made individuals more cryptic against the background (Rodgers et al., 2013). In a more open environment, we may have seen different effects. However, the shallow streams that guppies inhabit in Trinidad mean that use of refuge areas is likely to be a component of their anti-predator response. Guppies may also have received directional cues from the over-head predator stimulus and moved away from a possible attack location (Viscido et al., 2001), rather than towards their shoal-mates. An individual at risk from predation would need to balance the relative costs and benefits of protection in a group (e.g. through dilution, confusion and selfish herd effects; Krause & Ruxton 2002) against the benefits of seeking refuge as an individual. The low number of guppies in the shoal (n=4) may have also influenced behaviour within the group, although small shoals are commonly found in guppies (Croft et al., 2003). To our knowledge the interaction between group size and turbidity on anti-predatory behaviours has not been investigated, and represents an interesting avenue for future study.

In turbid water, the benefits of shoaling may be reduced as the distance at which predators can detect prey is shortened (Utne-Palm, 2002), reducing the benefits of coordinated evasion and resulting in weakened shoaling preferences (Fischer & Frommen, 2012) and the reduced shoal cohesion observed here and in previous studies (Ohata et al., 2013; Ryer & Olla, 1998). Poor visual environments may cause predators to rely more strongly on olfactory cues (Chapman et al., 2010), meaning that the anti-predator benefits of grouping as a means of avoiding detection (the encounter-dilution effect; Wrona & Dixon, 1991) are reduced (Kunin, 1999; Treisman, 1975; Whitton et al., 2012; Wilson & Weissburg, 2012). The reduction in shoal cohesion in turbid water may thus be an adaptive response to changed predation tactics, although some evidence suggests that aggregation could beneficial in avoiding detection even in turbid water (Johannesen et al., 2014).

Our study focuses on anti-predatory responses mediated by the visual detection of an over-head threat. In turbid water, however, fish may rely on alternative senses such as olfaction and the lateral line to detect and respond to both conspecifics and predators (Brown & Godin, 1999; Stewart et al., 2013, Partridge & Pitcher, 1980). The cues used and the responses shown will also depend on the type of predator encountered, as aquatic predators will produce different cues and may be detected at different times by members of a shoal. Spiny damselfish (*Acanthochromis polyacanthus*), for example, respond more strongly to the olfactory cues of an aquatic predator in turbid water compared to clear (Leahy et al., 2011). Interestingly, the guppies in our study were able to maintain similar shoal cohesiveness and anti-predatory responses to that of clear water until relatively high levels of turbidity, suggesting individuals can use alternative cues to compensate for the poor visual environment. Olfactory cues may be important in maintaining cohesive shoals; disruption to olfactory cues through pollution interferes shoaling behaviour (Ward, 2008) and alters individual responses to predators (Dixon et al., 2010), and therefore may affect group level responses to predators. The lateral line may also compensate for the lack of vision, by providing cues to the speed and direction of other shoal members (Partridge & Pitcher, 1980), although to our knowledge this remains untested in guppies, and warrants further investigation.

Our study suggests that the reduced visual distances in turbid water constrains individual responses to an aerial predation threat and may result in a reduced collective response to predators and a loss of protection gained by shoaling. This may have implications for individual survival during a predation event and for other behaviours linked to the benefits of grouping, such as anti-predator vigilance (‘many eyes effect’), enhanced foraging success, transfer of information and energy conservation (reviewed in Krause & Ruxton, 2002). How predators respond to the combination of changed prey behaviour and changed environmental conditions represents an interesting avenue for future study.

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Table 1. Individual level responses of guppies to a simulated aerial predation threat at the 4 levels of increasing turbidity (0, 50, 100, 200NTU)

Turbidity (NTU)		**DF**	***z*- or *t*-value**	***P*-value**
Proportion of individuals freezing				
0	50	3	0.86	0.38
0	100	3	1.86	0.063
0	200	3	3.03	**0.002**
50	100	3	-1.10	0.27
50	200	3	-2.42	**0.015**
100	200	3	-1.17	0.24
Latency to respond (frames)				
0	50	3	-0.63	0.49
0	100	3	-0.99	0.73
0	200	3	-0.96	0.15
50	100	3	1.59	0.28
50	200	3	-0.59	0.47
100	200	3	-1.88	0.078
Maximum speed (cm/s)				
0	50	3, 233	-2.60	**0.01**
0	100	3, 233	-1.85	0.065
0	200	3, 233	-2.75	**0.006**
50	100	3, 233	0.61	0.54
50	200	3, 233	-1.45	0.15
100	200	3, 233	-0.83	0.41
Total distance travelled (cm)				
0	50	3, 233	-2.43	**0.015**
0	100	3, 233	-1.03	0.30
0	200	3, 233	-3.77	**<0.001**
50	100	3, 233	1.034	0.21
50	200	3, 233	-0.25	0.80
100	200	3, 233	-2.61	**0.01**
Recovery time (s)				
0	50	3, 327	2.23	**0.027**
0	100	3, 327	1.96	**0.05**
0	200	3, 327	3.64	**<0.001**
50	100	3, 327	-0.23	0.88
50	200	3, 327	1.46	0.14
100	200	3, 327	1.55	0.12

Pairwise comparisons from the GLMER (proportion of individuals freezing and latency to respond) and LME (maximum speed, total distance travelled and recovery time) models for the individual level responses of guppies to a simulated aerial predation threat at the 4 levels of increasing turbidity. Bold *P*-values represent significant results.

Figures

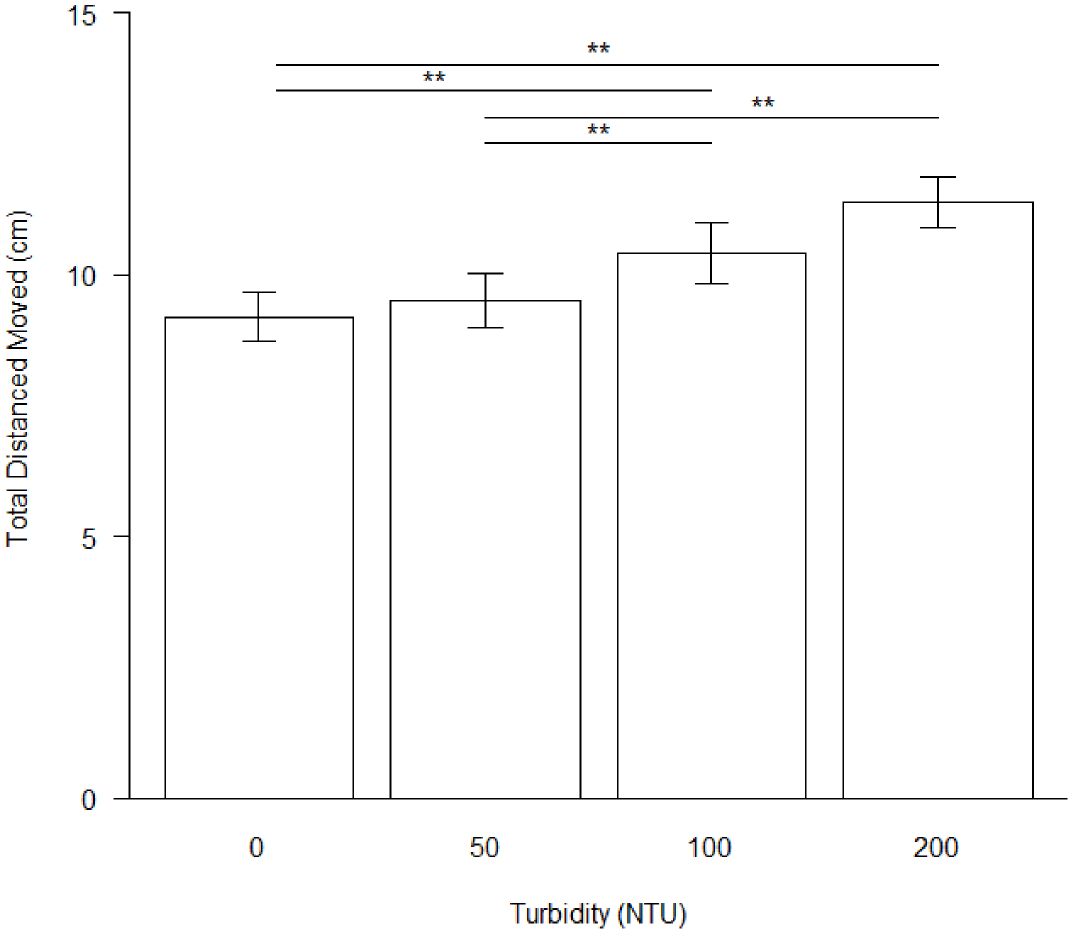

Figure 1: Mean shoal cohesion (cm) of fish within a shoal, measured every 10 s for 12 min in increasing levels of turbidity (±S.E.). Asterisks indicate $p < 0.01$

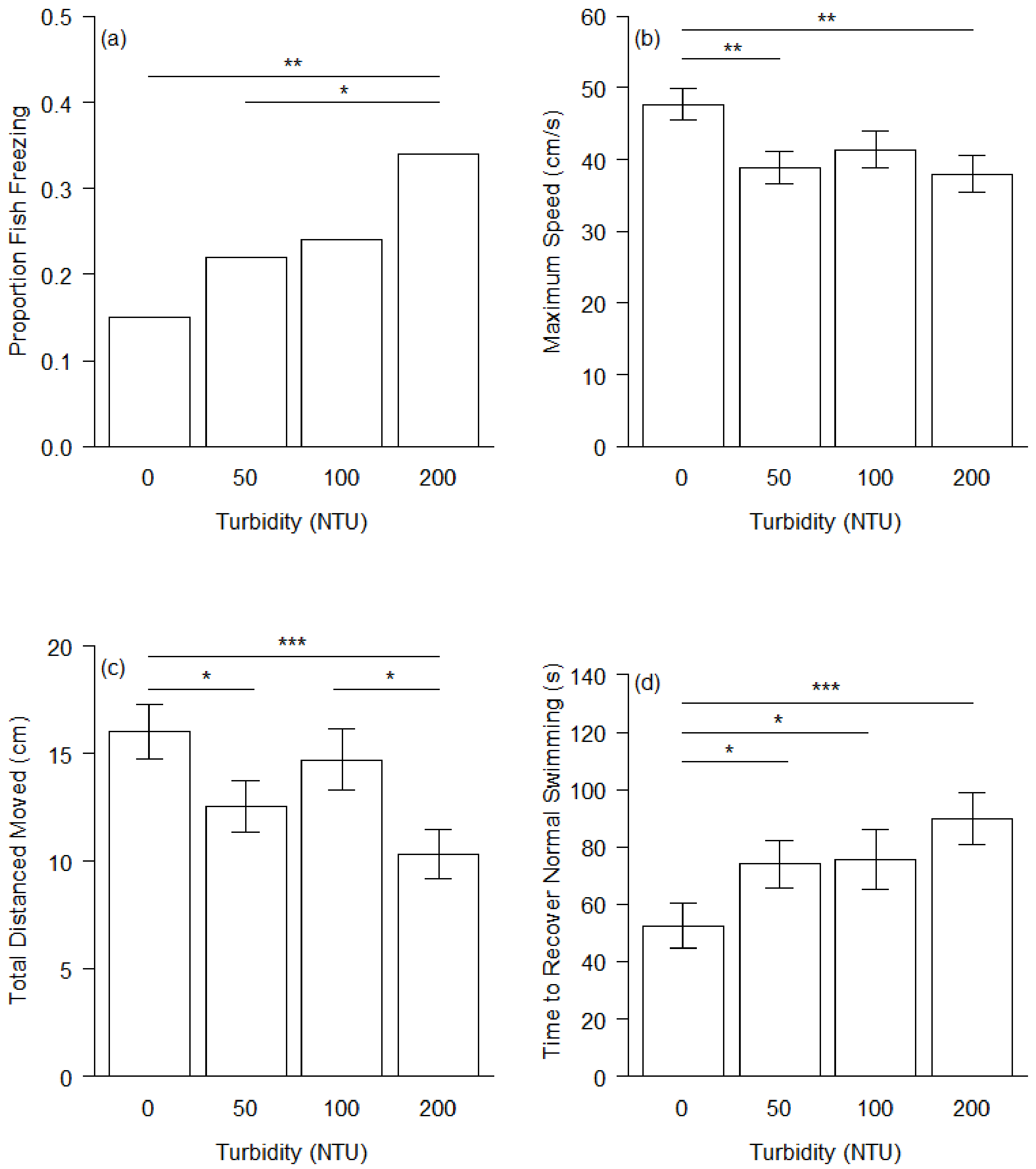

Figure 2: Individual level responses. a) Proportion of fish freezing in response to a simulated predator attack. b) Maximum speed (cm/s) reached in the first second of movement, c) Total distance moved (cm) within the first second of response and d) Time taken to recover normal swimming (s) after the predator simulation (±S.E.). Asterisks indicate significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.