Olfactory cue use by three-spined sticklebacks foraging in turbid water: prey detection or prey location?

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Foraging, when senses are limited to olfaction, is composed of two distinct stages; the detection of prey and the location of prey. While specialist olfactory foragers are able to locate prey using olfactory cues alone, this may not be the case for foragers who rely primarily on vision. Visual predators in aquatic systems may be faced with poor visual conditions such as natural or human-induced turbidity. The ability of visual predators to compensate for poor visual conditions by using other senses is not well understood although it is widely accepted that primarily visual fish, such as three spined sticklebacks (Gasterosteus aculeatus) can detect and use olfactory cues for a range of purposes. We investigated the ability of sticklebacks to a) detect the presence of prey and b) to precisely locate prey, using olfaction, in clear and turbid (two levels) water. When provided with only a visual cue, or only an olfactory cue, sticklebacks showed a similar ability to detect prey, but a combination of those cues improved their performance. In open-arena foraging trials, a dispersed olfactory cue added to the water (masking cues from the prey) improved foraging success, contrary to our expectations, while activity levels and swimming speed did not change as a result of olfactory cue availability. We suggest that olfaction functions to allow visual predators to detect rather than locate prey, and that olfactory cues also have an appetitive effect, enhancing motivation to forage.

Keywords: olfaction, predator-prey interactions, turbidity, Gasterosteus aculeatus, vision
Predators use a range of senses to find prey including vision, olfaction and the detection of electric fields (Goerlitz et al. 2008; Nakata 2010; Patullo & Macmillan 2010; Gracheva et al. 2010). For predators using visual cues to forage, detecting and locating a prey item occur simultaneously. For predators using olfactory cues, however, the detection of a cue may convey very little information about the location of a prey item (Conover 2007). In such systems, finding a prey item (or mate, or other resource) using olfaction can be considered as two discrete steps: detection, where an individual is alerted to the presence of food in the vicinity; and location, where detected item is found. The step from detection to location when using olfaction may depend on factors such as wind or flow speed and turbulence, the strength of the cue, and the sensitivity of chemoreception by the individual (Conover 2007; Carthey et al. 2011). For example in mice (Mus domesticus), cue patchiness is an important factor determining foraging success (Carthey et al. 2011) and plume tracking insects need both an olfactory cue and wind direction in order to successfully navigate to the source of the cue (Cardé & Willis 2008).

In aquatic systems, many fish predators rely primarily on vision, yet visual cues can be highly limited, as water is often turbid or too deep to allow light to penetrate (Davies-Colley & Smith 2001; Utne-Palm 2002). Fish also use olfaction in a range of behaviours, including mate choice (cichlids: Plenderleith et al. 2005, sticklebacks: Rafferty & Boughman 2006; Heuschele et al. 2009), as a social cue (sticklebacks: Ward et al. 2004; Ward et al. 2005, perch: Behrmann-Godel et al. 2005) to detect predators (rainbow trout: Brown et al., 2011, minnows: Ferrari, Lysak, & Chivers, 2010) and to detect prey (cod: Løkkeborg 1998). Thus, changes to the visual (e.g. through turbidity; Quesenberry, Allen, & Cech, 2007; Utne, 1997) or olfactory (e.g. through altered pH; Heuschele & Candolin, 2007;
Moore, 1994) environment can negatively impact on the ability of fish to detect
and locate prey items.

Turbid conditions can be caused by natural events, such as algal blooms
due to seasonal shifts in temperature and light availability; and from
anthropogenic activities such as excess fertiliser from agriculture reaching
waterways, or erosion caused by deforestation or construction (Richter et al.
1997; Henley et al. 2000; Donohue & Molinos 2009). Highly turbid water is known
to be detrimental to a visual forager: in high-production lakes lowered encounter
rates between predators and prey lead to fewer large fish predators in
comparison to low-production lakes (Turesson & Brönmark 2007). Across a
range of fish species, reaction distance to prey decreases with increasing
turbidity (Utne 1997; Sweka & Hartman 2003; Pekcan-Hekim & Lappalainen
2006; Quesenberry et al. 2007) and increased turbidity decreases foraging
success (Gregory & Northcote 1993; Sweka & Hartman 2003; Granqvist & Mattila
2004).

However, in some cases, high turbidity has little impact on foraging
success (Miner & Stein 1993; Grecay & Targett 1996; Granqvist & Mattila 2004;
Quesenberry et al. 2007). This may be related to the size of the predator and its
prey (Utne-Palm 2002): A small predator feeding on plankton will often find itself
close to prey, so reaction distances can be short without negatively affecting the
 predator. In contrast, larger predators that eat sparser prey are more likely to be
negatively affected by turbidity (Turesson & Brönmark 2007). While some
predators are not adversely affected by turbidity because of their size and prey
density, others may be able to compensate for the loss of available visual cues
with changes in behaviour (Andersen et al. 2008) or through developmental
plasticity, making use of other senses such as olfaction (Chapman et al. 2010).
Here, we investigated whether three-spined sticklebacks (*Gasterosteus aculeatus*) can use olfaction to compensate for a reduction in the availability of visual foraging information due to increased turbidity. The three-spined stickleback is a visual predator occupying a wide range of habitats including very turbid water (Wootton 1976; Utne-Palm 2002; Engström-Öst & Candolin 2006; Webster et al. 2007b). Sticklebacks are known to use olfaction across a range of behaviours: they compensate for poor visual conditions by using olfactory cues in mate choice, allowing them to accurately assess male quality (Reusch et al. 2001), and base shoaling preferences on habitat-derived olfactory cues (Ward et al. 2004; Ward et al. 2005). Webster et al. (2007a) demonstrated that sticklebacks performed more poorly in a foraging task when olfactory cues from prey were concealed by an excess of prey cue added to the water, indicating a key role for olfaction in foraging in this species. Thus, as primarily visual foragers, but with a well-documented sense of smell, sticklebacks are an ideal model system in which to test the hypothesis that olfaction allows individuals to compensate for the reduced availability of visual cues in turbid water.

Here, we used two complementary approaches to investigate the use of visual and olfactory cues in stickleback foraging, in the context of both prey detection and prey location. In the first ‘prey detection’ experiment we tested the hypotheses that a) sticklebacks can use olfaction to detect prey; b) reliance on olfactory cues to detect prey increases with increasing turbidity. In the second, ‘foraging success’ experiment, we tested the hypotheses that a) increasing turbidity reduces the ability of fish to locate prey items and b) this effect is increased when olfactory prey cues are masked by the addition of excess prey cue to the water (thus providing no information about the location of prey items). Together, these experiments allowed us to test the general hypothesis that
sticklebacks compensate for poor visual conditions by using olfactory cues to detect and locate dispersed prey.
METHODS

Study Species and Housing

250 three-spined sticklebacks (*Gasterosteus aculeatus*) 45-55 mm long were caught using small (single or two person) seine nets from water bodies near Saltfleet, Lincolnshire, UK (53° 25' 59.55"N, 0° 10' 49.41"E). Fish were placed in commercial fish transportation bags at maximum density of 5 fish per litre. Each bag was filled with 25% water from the source water body, and 75% air (total bag volume of 20 litres), and bags were packed into plastic boxes. Fish were returned by car to the laboratory in Leeds, and no fish died during transportation. At our facilities, the fish were kept in groups of between 50 and 150 fish in fresh water holding tanks (60 x 90 x 45 cm) on a 10:14 hour light/dark cycle at a temperature of 16 ± 1 °C and pH was 6.5 – 7.0. To control for any potential confounding effect of social background, fish from each holding tank were evenly distributed between treatments. The holding tanks were enriched with gravel substrate and artificial plants. They were fed defrosted frozen bloodworm (chironomid larvae) once daily. The fish were maintained in the laboratory for 18 months after which they were released again where caught in agreement with the Home Office and DEFRA. The prey species used in our experiments were live bloodworm (*Chironomidae spp*) sourced from a local pet shop (prey detection) and frozen bloodworm sourced from a commercial fish food provider (foraging success).

Experiment 1: Prey detection

To investigate whether sticklebacks could use olfactory cues to detect prey, we used a binary choice design (similar to that of Chapman et al, 2010). Fish were presented with two containers, one containing prey and one without prey. We used 3 cue-availability treatments (olfactory, visual and combined...
(cues), each repeated in three turbidity environments (clear, medium and high; see below for details) with 25 trials in each group (a total of 225 trials). Some trials (N = 47) were excluded due to the fish not entering a selection zone (see below), giving a total sample size of 178. A web-cam positioned above the arena and connected to a laptop next to the experimental arena was used to monitor the fish during acclimatisation and record the trials.

The choice arena (54 x 34 cm, filled to a depth of 5 cm; figure 1a) contained two prey containers, positioned at opposite ends, 10 cm from the tank wall, and an opaque shelter positioned in the tank centre. Around each prey container we marked a 5 cm wide 'selection zone'. Each prey container was constructed from a 100 ml plastic beaker divided vertically into two equal sections, one transparent and one opaque (see figure 1a for positioning of the containers). Live bloodworm prey placed into the transparent section provided visual cues to the predator (in the visual only and combined cue treatments), while prey placed in the opaque section (in the olfactory only treatment) did not. Live prey were used as movement is an important visual cue (Utne-Palm 2002).

For treatments where an olfactory cue was available (the olfactory and combined cue treatments), the containers were perforated with 1 mm holes spread at 1 cm intervals across the entire surface of the container. For the visual only treatment, the container remained unperforated. In each trial, one container held prey while the other did not. The side containing the prey was randomised between trials to control for any potential side bias.

To facilitate the transmission of olfactory cues from the container in to the surrounding water (for the olfactory only and combined treatments), an additional olfactory cue was dripped via airline tubing into the container containing prey at a rate of 1 drop per 10 seconds amounting to approximately 5 ml of drip per trial.
dripping into approximately 9 litres of water in the arena. A control drip of water was added to the container without prey. We performed a series of pilot trials using water dyed with food colouring to visualise patterns of cue dispersal, prior to the start of experimental trials. These pilot trials indicated that over the course of 30 minutes, the cue would disperse to create a cylindrical odour plume approximately 2 cm wide around the container with a sharp concentration gradient. These pilots indicated no visually detectable current caused by the olfactory cue drip. To control for the presence of the tubing, it was left in place for the visual only treatments, but no cue was added.

The olfactory cue was generated from the water in which the live bloodworm were stored. The bloodworm were supplied in small plastic bags containing approximately 150 ml of water, and we housed the bloodworm in this water in the laboratory for up to 2 days after purchase (bloodworm survived for no more than 3 days in the laboratory). Thus, the water used for the olfactory cue used was generated by housing bloodworm in water for 3-5 days. In order to achieve the required volume of olfactory cue, the water used to house the bloodworm was diluted immediately before use one part water, one part bloodworm housing water. As the cue water had a slight pink tinge, a small amount of red food colouring was added to the control water. Pilot trials indicated that there was no effect of the food colouring on fish response to the water.

In addition to randomly assigning the side containing the food cue, we also carried out cue treatments in a random order. Trials were recorded on video and analysed blind to cue treatment and the side containing the cue. A separate spreadsheet held information on cue treatment and on which container held prey items for each trial. Although much was done to ensure randomisation, all clear water trials were carried out before the turbid trials. The initial experiment in clear
water was designed to test whether sticklebacks could detect the olfactory cue in
our experimental set up. This pilot indicated that detection of the prey when
olfactory cues were available was similar to detection when both cues were
available (ANOVA: $F_{1,59} = 1.45, P = 0.24$), and so these results were incorporated
into the full experiment. Within the clear water trials, cue treatment was
randomised and videos analysed blind, as for the main experiment.

Turbidity was created by dissolving industrial clay (Commercial Clay Ltd)
in conditioned water (Abrahams & Kattenfeld 1997; Ferrari et al. 2010). High
turbidity ($488.69 \pm 5.46$ NTU) was created from 1g of clay per litre of water and
medium turbidity using 0.5g/L ($296.51 \pm 4.77$ NTU). Turbidity dropped to $437.05 \pm$
7.96 NTU and $250.63 \pm 5.10$ NTU respectively over a period of 15 minutes (5
minutes acclimatisation plus 10 minutes trial time). Turbidity differed significantly
between high turbidity and medium turbidity treatments (ANOVA: $F_{1,112} = 682.9$, $P$
< 0.001). The clay did not alter the pH of the water used in our trials. Clear water
treatments contained no clay (~0.1 NTU). The fish showed no symptoms of ill
health during or following experiments. It is likely that the turbidity levels chosen
for these experiments were higher than is usually seen in the wild, but as the
trials ran in small volumes of water, high turbidity was necessary to prevent the
fish from seeing prey at short distances. At the turbidities we used, the secchi
disk distance (indicative of the distance the fish would be able to see through the
water) was approximately 3 cm for high turbidity and 10 cm for medium turbidity.

Fish were starved for 24 hours prior to trials in order to standardise
motivation to feed. Individual fish were placed in the shelter and left for 5 minutes
to acclimatise, in order to minimise decrease in turbidity and in line with other
studies (Engström-Öst & Candolin 2006; Webster et al. 2007a; Quesenberry et
al. 2007). After the acclimatisation period, the video recording was started and
the fish was released into the arena by raising the shelter above water level using a remote pulley system. Each trial lasted 10 minutes, after which the fish was caught and measured, and the trial number assigned to the video. The arena was emptied of water and refilled for each subsequent trial to remove olfactory cues from previous trials. Total time spent in each selection zone was recorded from the video.

Analysis

Statistical analysis was carried out in R version 2.13.0 (R Development Core Team 2011) using a generalised linear model (glm) with quasibinomial errors to analyse the proportion of time spent in the selection zone with the container holding food as opposed to the empty container selection zone. The model was run with interactions first and when an interaction was found between turbidity and treatment, post hoc glms in each turbidity level were run with a Bonferroni correction for multiple tests in order to test for main effects of cue availability.

Experiment 2: Foraging success

As detecting prey in a binary choice test does not necessarily equate to the ability to locate prey, we carried out a second experiment, in which predators located and consumed prey in an open arena, again under 3 differing turbidity levels (as above).

Foraging success trials were carried out in a 100x100 cm arena with a water depth of 5 cm (figure 1b). A 10x10 cm floating polystyrene shelter was positioned in the centre of the arena, held in place by lengths of white sewing
thread attached to the centre of two opposite sides of the arena. 8 bloodworms were placed at evenly spaced predetermined spots (25 cm from the arena wall and 25 cm from the nearest neighbouring worm) surrounding the shelter. Defrosted frozen bloodworms were used as prey to prevent excessive movement away from these locations during the trial. A high definition webcam (Logitech Webcam Pro 9000), suspended above the arena was used to remotely monitor and record trials.

We used two cue availability treatments: “visual and olfactory” and “visual only”. The visual and olfactory treatment allowed the stickleback to use both senses (although visual cue availability was dependent on turbidity levels – we make no assumptions about the effect of clay on the olfactory cue available). In the visual only treatment, we prevented the use of olfactory cues to locate prey by adding additional olfactory cue to the water used to fill the arena, ensuring that the cue was well-mixed with the water before the arena was filled. The added olfactory cue was created following the methodology in Webster et al (2007a) from the filtered extract of macerated frozen bloodworm (1g of bloodworm per 20 L of water final concentration). The added olfactory cue was intended to override any olfactory cue emanating from the prey items, thus preventing the fish from using this cue to locate the prey. Fish were fed then starved for 24 hours preceding trials to standardise motivation to feed. Each cue availability treatment was carried out in three different turbidity treatments, as above. On each day of experimentation, we carried out 2-3 trials at each turbidity level. Within a day, turbidity levels were grouped (for logistical reasons), but between days, the order in which different turbidity levels were trialled was randomised.

Individual fish were released under the shelter, where they would hide. Any fish that did not hide under the shelter or did not emerge from the shelter
within 15 minutes were removed and excluded from the experiment ($N = 92$ fish).

The 15 minute emergence limit was imposed in order to avoid overlap in turbidity treatments due to settling of clay over time. We recorded the time taken for the fish to emerge, defined as the time at which the full extent of its body was free of the shelter. Mean time until emergence from the shelter did not differ significantly between cue or turbidity treatment groups (cox proportional hazards survival model, likelihood ratio test $\chi^2 = 3.38$, $P = 0.34$). Turbidity was measured (for the majority of trials) before the fish was released and after the trial was complete.

Turbidity decreased over time from $646.38 \pm 12.74$ (mean $\pm$ SE) NTU to $460 \pm 20.69$ (high turbidity, $N = 18$ & 26 respectively), and from $391.15 \pm 9.35$ NTU, to $286.83 \pm 9.1$ NTU (medium turbidity, $N = 29$ in both cases) over a maximum of 35 minutes (maximum time permitted in the shelter plus maximum foraging time).

Thus, despite decreases in turbidity over time, turbidity in the medium and high turbidity treatments differed significantly ($F_{1,53} = 63.06$, $P < 0.001$). Once the fish had emerged, we started video recording and the fish was allowed to forage until all worms were eaten or for 20 minutes, at which point the trial was terminated.

Fish were measured to the nearest mm (total body length) at the end of each trial.

Data were manually extracted from videos using Etholog (2.2.5) and Windows Media Player. The time spent engaged in each of the 4 behaviours outlined in table 1 was recorded. In addition, we recorded the time taken to emerge from the shelter (see above) and the time of consumption of each individual worm.

**Analysis**

All analysis was carried out in R (R Development Core Team 2011). Cox Proportional Hazards Survival Models (Therneau & Lumley 2011) and Mixed
Effects Cox Models (Therneau 2011) were used to analyse our three response variables: the total time until emergence from shelter, the total time until first worm was eaten and the total time until each worm was eaten, as a function of turbidity and cue availability treatment.

In a subsequent analysis, we focused only on the time when the fish was actively swimming in the arena, excluding time when the fish was hiding, inactive or swimming around the edges of the arena. This measure best represents active search for food, as all other behaviours were counterproductive to locating the bloodworm. Swimming time analyses were also carried out using Mixed Effects Cox Models, but using swimming time instead of total time until consumption of each worm. Both time until consumption of first and all worms were analysed.

A Mixed Effects Generalised Linear Model using the R package lme4 (Bates et al. 2011) with binomial errors was used to test for difference in number of worms eaten. Size of fish as a random factor (to account for the fact that smaller fish might eat fewer worms) and an observation level random variable was included to account for over dispersion (Bates et al. 2011). No interaction between cue and environment was found, so this was removed and the minimum adequate model (MAM) is presented.

Each behaviour recorded represented a proportion of the total time budget recorded, so the measurements were not independent, with the increase of time spent on one behaviour necessarily causing the decrease in one or more of the others. As this type of data may cause spurious correlations, it is best treated like a composition (Aitchison 1982). Therefore, the compositions package in R (Boogaart et al. 2011a) was used to transform the data (using the isometric log ration transform in the package) into a composition suitable for linear analysis.
(Boogaart & Tolosana-Delgado 2006; Boogaart 2008; Boogaart et al. 2011b), and using a MANOVA to test for differences in time budgets. Individual behaviours were analysed using generalised linear models with quasibinomial errors. Swimming speeds were analysed using a linear model with two factors (turbidity and cue availability) after log transforming the data in order to meet the assumptions of a linear model.
RESULTS

Experiment 1: Prey detection

There was a significant interaction between turbidity and cue (ANOVA: $F_{4,169} = 2.455, P = 0.048$). High turbidity affected time spent with the food container when a visual cue only was available (figure 2). Single factor analysis on treatments at separate turbidity levels, using a Bonferroni correction for multiple tests, revealed that fish in the visual only and olfactory only treatments spent significantly less time with the correct cup compared to when both cues were available, when turbidity levels were high (Binomial GLM: Olfactory only: $t_{60} = -2.467, P = 0.017$; Visual only: $t_{60} = -4.233, P < 0.001$; figure 2). There was no significant difference between treatments in clear water and medium turbidity (ANOVA: $F_{2,59} = 1.45, P = 0.24$ and $F_{2,52} = 2.22, P = 0.12$ respectively).

Experiment 2: Foraging success

More worms were eaten in treatments with an added olfactory cue compared to treatments without an added olfactory cue (Mixed effects GLM, $z = 1.976, N = 90, P = 0.048$) and fewer worms were eaten at high turbidity ($z = -4.053, N = 90, P < 0.001$) but not medium turbidity ($z = -0.898, N = 90, P = 0.369$) compared to clear water. There was no significant interaction between cue treatment and turbidity level on the number of worms eaten. Comparison of the $z$ values indicates a greater effect of turbidity than presence/absence of olfactory cue.

There was no significant difference in the total time until the first worm was eaten between clear water and high turbidity ($z = -0.658, N = 90, P = 0.51$).
or between added cue and no added cue ($z = 1.165, N = 90, P = 0.24$), in a cox proportional hazards model (fig. 3a). There was, however, a significant difference between clear and medium turbidity, with medium turbidity leading to a decrease in the time taken until the capture of the first worm ($z = 2.95, N = 90, P = 0.003$).

When looking at swimming time only to the first worm being eaten (fig 3b), high turbidity leads to a significant increase in the time taken until the first worm is eaten, compared to clear water ($z = -3.219, N = 90, P = 0.001$). The other treatment combinations do not differ significantly from clear water with no added cue (medium turbidity: $z = 1.369, N = 90, P = 0.17$ and added cue: $z = -0.109, N = 90, P = 0.91$).

Worms survived longer (total time) in medium and highly turbid water than in clear water and with an added olfactory cue they were eaten sooner than with no added cue (mixed effects cox model, cue: $z = 2.86, N = 90, P = 0.004$, turbidity: medium: $z = -2.24, N = 90, P = 0.025$, high: $z = -7.36, N = 90, P < 0.0001$; figure 3c), but there was no interaction between turbidity and cue availability. Repeating this analysis using active swimming time only revealed a significant interaction between turbidity and cue availability on the survival of worms ($z = 3.27, N = 90, P = 0.001$, figure 3d). The interaction effect suggests that at high turbidity, the addition of the olfactory cue increases the 'hazard' (the risk to the worm of being eaten). Post hoc tests (with Bonferroni correction for multiple tests) revealed that added cue significantly shortened the lives of worms in clear water ($z = 2.66, N = 30, P = 0.008$) but no effect was found at medium turbidity ($z = -0.4, N = 30, P = 0.69$). Both with and without added cue, increasing turbidity increased the time until worms were eaten (no added cue: medium: $z = -5.68, N = 45, P < 0.001$, high: $z = -12.98, N = 45, P < 0.001$; added cue: medium: $z = -5.80, N = 45, P < 0.001$, high: $z = -9.73, N = 45, P < 0.001$).
We found no significant interaction effect between olfactory cue and turbidity level on time budgets (MANOVA following transformation using compositions $F_{6,166} = 1.34, P = 0.242$). There was a highly significant main effect of turbidity ($F_{6,170} = 4.84, P < 0.001$) but no effect of olfactory cue treatment ($F_{3,84} = 1.49, P = 0.224$) on behaviour. The above analysis looks at the effect on activity budget as a whole, and when looking at individual behaviours, fish spent a significantly larger proportion of time actively swimming in medium and high turbidity than in clear water (Quasibinomial GLM, $t_{89} = 3.45, P < 0.001$ and $t_{89} = 3.80, P < 0.001$ respectively; figure 4a). In time spent hiding, there was no significant interaction between added olfactory cue and turbidity ($F_{2,84} = 2.09, P = 0.13$). After removing the interaction term, the fish spent significantly less time in hiding in both medium and high turbidity than they did in clear water ($t_{86} = -5.28, P < 0.001$ and $t_{86} = -5.17, P < 0.001$, figure 4b).

Swimming speeds did not differ between added cue and no added cue ($F_{1,59} = 0.832, P = 0.365$), but speeds were higher at medium and high turbidity than in clear water (medium: $t_{58} = 2.908, P = 0.005$, high: $t_{58} = 2.990, P = 0.004$).
DISCUSSION

Our results suggest that olfaction plays an important role in foraging, particularly in turbid waters. At high turbidity, prey detection was enhanced by the presence of both visual and olfactory cues compared to one cue type alone. Surprisingly, we found that when foraging in highly turbid waters, the addition of a masking olfactory cue did not hamper the location of prey items, instead increasing predation risk on the bloodworm. Based on the results of a previous study (Webster et al. 2007a) we predicted that flooding the arena with olfactory cues from bloodworm would conceal the location of the prey to a predator using olfaction to find them. Instead, foraging success was increased with the addition of this olfactory cue in our study. We suggest that the added olfactory cue may have had an appetitive effect on the fish, stimulating them to actively search for or consume prey. We found no difference, however, in the time spent actively foraging, or swimming speed between the two olfactory cue treatments.

In line with previous studies, overall foraging success was decreased in highly turbid waters (Pekcan-Hekim & Lappalainen 2006; Nurminen et al. 2010b; Nurminen et al. 2010a). However, our prey detection results in particular suggest that the detrimental effect of turbidity may be mediated by the use of olfactory cues from prey for foraging sticklebacks. Previous work has found that when juvenile guppies (Poecilia reticulata) are reared in an environment where visual cues are limited, individuals increase reliance on olfactory cues in foraging, to the extent that their overall foraging success is not negatively impacted by reduced visual information (Chapman et al. 2010). Such an increased reliance on olfaction provides a way for individuals to compensate for potentially detrimental effects of environmental change on foraging success and survival, although the mechanisms underlying this are not yet known (Chapman et al. 2010).
Sticklebacks are often found naturally in highly turbid water, so early experience of this environment may allow for enhanced use of olfactory cues.

While our study finds a negative effect of high turbidity on the ability of sticklebacks to detect and locate prey, other studies have found that a moderate level of turbidity can have a positive effect on foraging success (Gregory & Northcote 1993) as well as reaction distance (Utne 1997; Utne-Palm 1999). While an increase in reaction distance can be explained by how prey will sometimes stand out more against a turbid background than clear water (Utne-Palm 2002), this cannot explain why juvenile chinook salmon (*Oncorhynchus tshawytscha*) have higher foraging success when foraging for benthic or surface prey. These prey do not have a turbid water background, so would not be more easily detected for this reason. Turbidity causes a decreased anti-predator response in fathead minnows (*Pimephales promelas*) and chinook salmon (Gregory 1993; Abrahams & Kattenfeld 1997) and it may well be that improved foraging at moderate turbidity is at least partly due to change in foraging behaviour caused by a decreased perception of risk.

The contrast between our results and those of Webster et al (2007a) is interesting, and may be explained by the configuration of the prey in the different experiments. In Webster et al’s (2007a) experiment, prey items (sections of bloodworm) were partially concealed within a darker coloured substrate, while the prey in our experiment were in high contrast to the flat white background of the test arena. High turbidity reduces the long-range availability of visual cues (Berg & Northcote 1985; Mazur & Beauchamp 2003; Quesenberry et al. 2007), but once close to the prey, the short-range availability of cues will be affected by small-scale habitat structure: worms concealed within the substrate are less likely to be located than those clearly visible, when using visual cues alone. We
suggest that for our fish, the appetitive effect of the added olfactory cue, combined with the availability of short-range visual cues, allowed for increased consumption of prey.

It is possible that the clay used to create turbidity in our experiments may have affected the availability of olfactory cues, which we did not control for in our trials. However, if the clay had a strong negative effect on the availability or perception of olfactory cues, we would expect to see a decrease in the availability/use of olfactory cues in the medium and high turbidity treatments, and a reduced effect of the added olfactory cue in the foraging experiment in turbid water treatments, and this is not reflected in our results. Therefore, while the clay used in our trials may have had some effect on olfactory cue, the primary effect seems to be in limiting visual cues.

Olfaction is known to play a key role in a number of other behaviours in sticklebacks specifically, and in other fish species. Olfactory cues are an important component of social decision-making (Ward et al. 2004; Ward et al. 2005) and mate choice (Rafferty & Boughman 2006; Heuschele & Candolin 2007; Heuschele et al. 2009). In sticklebacks, increased algal turbidity leads to an increased reliance on olfactory cues in mate choice in comparison to clear water, where visual cues are of primary importance, with knock-on implications for mate selection and the direction of sexual selection (Heuschele et al. 2009). Roach (Rutilus rutilus), when exposed to olfactory predator cues from either pike (Esox lucius) or perch (Perca fluviatilis), are able to successfully identify the predator species and take suitable species dependent evasive action (Martin et al. 2010). Together with previous studies, our results suggest that sticklebacks are able to flexibly rely on olfactory cues, although this may not always compensate for the reduction in visual cue availability caused by turbidity.
Our results suggest that in sticklebacks, olfactory cues are used primarily for prey detection, with vision used for final prey location. Where there is no water movement, pervasive olfactory cues alert the fish to the presence of prey in the immediate environment. Highly localised cues may be of less use, as they remain undetected until the predator is very close to the cue source, where vision may successfully be used to locate prey. Where wind or water flow disperses cues, olfactory predators may use anemo- or rheotaxis (upstream movement) in addition to chemotaxis to locate prey (Zimmer-Faust et al. 1995), utilising information provided by moving air or water to follow an odour plume to its source, but this information may be disrupted by turbulence (Weissburg et al. 2002). How and whether primarily visual foragers like sticklebacks utilise flow to track odour plumes is unknown (however, see Cripps et al. 2011 and Løkkeborg 1998).
510 References


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Tables

Table 1. Behaviours recorded in the foraging trials.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swimming</td>
<td>Moving around in the arena including saltatory and steady movement, but not along the edges of the arena.</td>
</tr>
<tr>
<td>Hiding</td>
<td>The fish is under the shelter and invisible to the observer</td>
</tr>
<tr>
<td>Edge</td>
<td>Continuous swimming along the edge of the arena</td>
</tr>
<tr>
<td>Inactive</td>
<td>Time spent immobile for at least 5 seconds in one bout</td>
</tr>
</tbody>
</table>
Figure legends

Fig. 1 Illustration (not to scale) of the (a) Binary choice arena. (A) indicates the cue containers, one half opaque (shaded) and one half transparent (unshaded). The containers were perforated for the olfactory and combined treatment, but intact for the visual treatment. (B) indicates the selection zones of 5cm and (C) indicates the containers holding the cue drip positioned at the side of the arena with tubing (dashed lines). (D) indicates the opaque cylinder for acclimatisation. (b) Foraging arena. (E) is floating shelter at centre of arena held in place with sewing thread (dashed lines). (X) mark the predetermined spots where prey were placed prior to trials. The distance between each prey and to either shelter or arena edge, was approximately 25 cm.

Fig. 2 Mean proportion of time spent with the food container with error bars of two standard errors. Grey is olfactory cue only, white is combined cues and black is visual cue only. Significant effects are marked with an asterisk.

Fig. 3 Survival curves for total time to first worm (A), swimming time to first worm (B), total time for all worms (C) and swimming time to all worms (D). Lines are: solid line = high turbidity, added olfactory cue; long dashes = high turbidity, no added cue; dotted line = clear water, added olfactory cue, short dashes = clear water, no added cue; dash-dot = clear water, added olfactory cue; dash-double dot = clear water, no added cue).

Fig. 4 The proportion of time spent swimming (A) and hiding (B) in the six treatment groups. Black bars are no added cue, and white bars are added olfactory cue. The error bars are two standard errors. Level of turbidity had an effect on behaviours whereas olfactory cue did not.