NOTICE: this is the author's version of a work that was accepted for publication in *Animal Behaviour*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Animal Behaviour* 84: 151-158. DOI: 10.1016/j.anbehav.2012.04.024

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

Asa Johannesen*[a], Alison Dunn[a], Lesley Morrell[b]

[a]University of Leeds, UK

[b]University of Hull, UK

Correspondence

Miss Asa Johannesen

Address: Irene Manton 8.17, Faculty of Biological Sciences, University of Leeds, LS2 9JT

Telephone: +44(0) 7708340710

Email: bsaj@leeds.ac.uk

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).

79

80 Here, we investigated whether three-spined sticklebacks (*Gasterosteus*
81 *aculeatus*) can use olfaction to compensate for a reduction in the availability of
82 visual foraging information due to increased turbidity. The three-spined
83 stickleback is a visual predator occupying a wide range of habitats including very
84 turbid water (Wootton 1976; Utne-Palm 2002; Engström-Öst & Candolin 2006;
85 Webster et al. 2007b). Sticklebacks are known to use olfaction across a range of
86 behaviours: they compensate for poor visual conditions by using olfactory cues in
87 mate choice, allowing them to accurately assess male quality (Reusch et al.
88 2001), and base shoaling preferences on habitat-derived olfactory cues (Ward et
89 al. 2004; Ward et al. 2005). Webster et al. (2007a) demonstrated that
90 sticklebacks performed more poorly in a foraging task when olfactory cues from
91 prey were concealed by an excess of prey cue added to the water, indicating a
92 key role for olfaction in foraging in this species. Thus, as primarily visual foragers,
93 but with a well-documented sense of smell, sticklebacks are an ideal model
94 system in which to test the hypothesis that olfaction allows individuals to
95 compensate for the reduced availability of visual cues in turbid water.
96

97 Here, we used two complementary approaches to investigate the use of
98 visual and olfactory cues in stickleback foraging, in the context of both prey
99 detection and prey location. In the first 'prey detection' experiment we tested the
100 hypotheses that a) sticklebacks can use olfaction to detect prey; b) reliance on
101 olfactory cues to detect prey increases with increasing turbidity. In the second,
102 'foraging success' experiment, we tested the hypotheses that a) increasing
103 turbidity reduces the ability of fish to locate prey items and b) this effect is
104 increased when olfactory prey cues are masked by the addition of excess prey
105 cue to the water (thus providing no information about the location of prey items).
106 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

193 water was designed to test whether sticklebacks could detect the olfactory cue in
194 our experimental set up. This pilot indicated that detection of the prey when
195 olfactory cues were available was similar to detection when both cues were
196 available (ANOVA: $F_{1,59} = 1.45$, $P = 0.24$), and so these results were incorporated
197 into the full experiment. Within the clear water trials, cue treatment was
198 randomised and videos analysed blind, as for the main experiment.
199
200 Turbidity was created by dissolving industrial clay (Commercial Clay Ltd)
201 in conditioned water (Abrahams & Kattenfeld 1997; Ferrari et al. 2010). High
202 turbidity (488.69 ± 5.46 NTU) was created from 1g of clay per litre of water and
203 medium turbidity using 0.5g/L (296.51 ± 4.77 NTU). Turbidity dropped to 437.05 ±
204 7.96 NTU and 250.63 ± 5.10 NTU respectively over a period of 15 minutes (5
205 minutes acclimatisation plus 10 minutes trial time). Turbidity differed significantly
206 between high turbidity and medium turbidity treatments (ANOVA: $F_{1,112} = 682.9$, P
207 < 0.001). The clay did not alter the pH of the water used in our trials. Clear water
208 treatments contained no clay (~0.1 NTU). The fish showed no symptoms of ill
209 health during or following experiments. It is likely that the turbidity levels chosen
210 for these experiments were higher than is usually seen in the wild, but as the
211 trials ran in small volumes of water, high turbidity was necessary to prevent the
212 fish from seeing prey at short distances. At the turbidities we used, the secchi
213 disk distance (indicative of the distance the fish would be able to see through the
214 water) was approximately 3 cm for high turbidity and 10 cm for medium turbidity.
215
216 Fish were starved for 24 hours prior to trials in order to standardise
217 motivation to feed. Individual fish were placed in the shelter and left for 5 minutes
218 to acclimatise, in order to minimise decrease in turbidity and in line with other
219 studies (Engström-Öst & Candolin 2006; Webster et al. 2007a; Quesenberry et
220 al. 2007). After the acclimatisation period, the video recording was started and

221 the fish was released into the arena by raising the shelter above water level using
222 a remote pulley system. Each trial lasted 10 minutes, after which the fish was
223 caught and measured, and the trial number assigned to the video. The arena was
224 emptied of water and refilled for each subsequent trial to remove olfactory cues
225 from previous trials. Total time spent in each selection zone was recorded from
226 the video.
227
228 *Analysis*
229 Statistical analysis was carried out in R version 2.13.0 (R Developement
230 Core Team 2011) using a generalised linear model (glm) with quasibinomial
231 errors to analyse the proportion of time spent in the selection zone with the
232 container holding food as opposed to the empty container selection zone. The
233 model was run with interactions first and when an interaction was found between
234 turbidity and treatment, post hoc glms in each turbidity level were run with a
235 Bonferroni correction for multiple tests in order to test for main effects of cue
236 availability.
237
238 *Experiment 2: Foraging success*
239
240 As detecting prey in a binary choice test does not necessarily equate to
241 the ability to locate prey, we carried out a second experiment, in which predators
242 located and consumed prey in an open arena, again under 3 differing turbidity
243 levels (as above).
244
245 Foraging success trials were carried out in a 100x100 cm arena with a
246 water depth of 5 cm (figure 1b). A 10x10 cm floating polystyrene shelter was
247 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$_3$ = 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 ± 12.74 (mean ± SE) NTU to 460 ± 20.69 (high turbidity, N = 18 & 26 respectively), and from 391.15 ± 9.35 NTU, to 286.83 ± 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 ($F_{1,59} = 00.832$, $P = 0.365$), 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

468 suggest that for our fish, the appetitive effect of the added olfactory cue,
469 combined with the availability of short-range visual cues, allowed for increased
470 consumption of prey.
471
472 It is possible that the clay used to create turbidity in our experiments may
473 have affected the availability of olfactory cues, which we did not control for in our
474 trials. However, if the clay had a strong negative effect on the availability or
475 perception of olfactory cues, we would expect to see a decrease in the
476 availability/use of olfactory cues in the medium and high turbidity treatments, and
477 a reduced effect of the added olfactory cue in the foraging experiment in turbid
478 water treatments, and this is not reflected in our results. Therefore, while the clay
479 used in our trials may have had some effect on olfactory cue, the primary effect
480 seems to be in limiting visual cues.
481
482 Olfaction is known to play a key role in a number of other behaviours in
483 sticklebacks specifically, and in other fish species. Olfactory cues are an
484 important component of social decision-making (Ward et al. 2004; Ward et al.
485 2005) and mate choice (Rafferty & Boughman 2006; Heuschele & Candolin
486 2007; Heuschele et al. 2009). In sticklebacks, increased algal turbidity leads to
487 an increased reliance on olfactory cues in mate choice in comparison to clear
488 water, where visual cues are of primary importance, with knock-on implications
489 for mate selection and the direction of sexual selection (Heuschele et al. 2009).
490 Roach (*Rutilus rutilus*), when exposed to olfactory predator cues from either pike
491 (*Esox lucius*) or perch (*Perca fluviatilis*), are able to successfully identify the
492 predator species and take suitable species dependent evasive action (Martin et
493 al. 2010). Together with previous studies, our results suggest that sticklebacks
494 are able to flexibly rely on olfactory cues, although this may not always
495 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

511 **Abrahams, M. V., & Kattenfeld, M.** 1997. The role of turbidity as a constraint on
512 predator-prey interactions in aquatic environments. *Behavioral Ecology and*
513 *Sociobiology*, **40**, 169-174.

514 **Aitchison, J.** 1982. The Statistical Analysis of Compositional Data. *Journal of the*
515 *Royal Statistical Society. Series B (Methodological)*, **44**, 139-177.

516 **Andersen, M., Jacobsen, L., & Skov, P.** 2008. Turbidity increases behavioural
517 diversity in northern pike, *Esox lucius* L., during early summer. *Fisheries*
518 *Management and Ecology*, **15**, 377-383.

519 **Bates, D., Maechler, M., & Bolker, B. M.** 2011. lme4: Linear mixed-effects
520 models using S4 classes. R package version 0.999375-42. http://cran.r-
521 project.org/package=lme4

522 **Behrmann-Godel, J., Gerlach, G., & Eckmann, R.** 2005. Kin and population
523 recognition in sympatric Lake Constance perch (*Perca fluviatilis* L.): can
524 assortative shoaling drive population divergence? *Behavioral Ecology and*
525 *Sociobiology*, **59**, 461-468.

526 **Berg, L., & Northcote, T. G.** 1985. Changes in Territorial, Gill-flaring, and
527 Feeding Behavior in Juvenile Coho Salmon (*Oncorhynchus kisutch*)
528 following Short-term Pulses of Suspended Sediment. *Canadian Journal of*
529 *Fisheries and Aquatic Sciences*, **42**, 1410-1417.

530 **Boogaart, K. G. V. D.** 2008. Using the R package " compositions ."
531 http://www.stat.boogaart.de/compositions

532 **Boogaart, K. G. V. D., & Tolosana-Delgado, R.** 2006. Compositional data
533 analysis with "R" and the package "compositions."*Geological Society,*
534 *London, Special Publications*, **264**, 119-127.

535 **Boogaart, K. G. V. D., Tolosana, R., & Bren, M.** 2011a. compositions:
536 Compositional Data Analysis. R package version 1.10-2. http://cran.r-
537 project.org/package=compositions

538 **Boogaart, K. G. V. D., Tolosana, R., & Bren, M.** 2011b. Package " compositions
539 ." http://cran.r-project.org/web/packages/compositions/compositions.pdf

540 **Brown, G. E., Ferrari, M. C. O., Malka, P. H., Russo, S., Tressider, M., &**
541 **Chivers, D. P.** 2011. Generalization of predators and nonpredators by
542 juvenile rainbow trout: learning what is and is not a threat. *Animal*
543 *Behaviour*, **81**, 1249-1256.

544 **Cardé, R. T., & Willis, M. A.** 2008. Navigational strategies used by insects to find
545 distant, wind-borne sources of odor. *Journal of chemical ecology*, **34**, 854-
546 66.

547 **Carthey, A. J. R., Bytheway, J. P., & Banks, P. B.** 2011. Negotiating a noisy,
548 information-rich environment in search of cryptic prey: olfactory predators
549 need patchiness in prey cues. *Journal of Animal Ecology*, **80**, 742-52.

550 **Chapman, B. B., Morrell, L. J., Tosh, C. R., & Krause, J.** 2010. Behavioural
551 consequences of sensory plasticity in guppies. *Proceedings. Biological*
552 *sciences / The Royal Society*, **277**, 1395-401.

553 **Conover, M. R.** 2007. *Predator-prey dynamics: the role of olfaction.* CRC Press.

554 **Cripps, I. L., Munday, P. L., & McCormick, M. I.** 2011. Ocean Acidification
555 Affects Prey Detection by a Predatory Reef Fish. *PLoS ONE*, **6**, e22736.

556 **Davies-Colley, R. J., & Smith, D. G.** 2001. Turbidity, Suspended Sediment, and
557 Water Clarity: A Review. *Journal of the American Water Resources*
558 *Association*, **37**, 1085-1101.

559 **Donohue, I., & Molinos, J. G.** 2009. Impacts of increased sediment loads on the
560 ecology of lakes. *Biological reviews of the Cambridge Philosophical Society*,
561 **84**, 517-31.

562 **Engström-Öst, J., & Candolin, U.** 2006. Human-induced water turbidity alters
563 selection on sexual displays in sticklebacks. *Behavioral Ecology*, **18**, 393-
564 398.

565 **Ferrari, M. C. O., Lysak, K. R., & Chivers, D. P.** 2010. Turbidity as an ecological
566 constraint on learned predator recognition and generalization in a prey fish.
567 *Animal Behaviour*, **79**, 515-519.

568 **Goerlitz, H. R., Greif, S., & Siemers, B. M.** 2008. Cues for acoustic detection of
569 prey: insect rustling sounds and the influence of walking substrate. *The*
570 *Journal of experimental biology*, **211**, 2799-806.

571 **Gracheva, E. O., Ingolia, N. T., Kelly, Y. M., Cordero-Morales, J. F.,**
572 **Hollopeter, G., Chesler, A. T., Sánchez, E. E., Perez, J. C., Weissman, J.**
573 **S., & Julius, D.** 2010. Molecular basis of infrared detection by snakes.
574 *Nature*, **464**, 1006-11.

575 **Granqvist, M., & Mattila, J.** 2004. The effects of turbidity and light intensity on
576 the consumption of mysids by juvenile perch (*Perca fluviatilis* L.).
577 *Hydrobiologia*, **514**, 93-101.

578 **Grecay, P. A., & Targett, T. E.** 1996. Effects of turbidity, light level and prey
579 concentration on feeding of juvenile weakfish *Cynoscion regalis*. *Marine*
580 *Ecology Progress Series*, **131**, 11-16.

581 **Gregory, R. S.** 1993. Effect of Turbidity on the Predator Avoidance Behaviour of
582 Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*). *Canadian Journal*
583 *of Fisheries and Aquatic Sciences*, **50**, 241-246.

584 **Gregory, R. S., & Northcote, T. G.** 1993. Surface, Planktonic, and Benthic
585 Foraging by Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in

586 Turbid Laboratory Conditions. *Canadian Journal of Fisheries and Aquatic*
587 *Sciences*, **50**, 233-240.

588 **Henley, W. F., Patterson, M. A., Neves, R. J., & Lemly, A. D.** 2000. Effects of
589 Sedimentation and Turbidity on Lotic Food Webs: A Concise Review for
590 Natural Resource Managers. *Reviews in Fisheries Science*, **8**, 125-139.

591 **Heuschele, J., & Candolin, U.** 2007. An increase in pH boosts olfactory
592 communication in sticklebacks. *Biology letters*, **3**, 411-3.

593 **Heuschele, J., Mannerla, M., Gienapp, P., & Candolin, U.** 2009. Environment-
594 dependent use of mate choice cues in sticklebacks. *Behavioral Ecology*, **20**,
595 1223-1227.

596 **Løkkeborg, S.** 1998. Feeding behaviour of cod,*Gadus morhua*: activity rhythm
597 and chemically mediated food search. *Animal Behaviour*, **56**, 371-378.

598 **Martin, C. W., Fodrie, F. J., Heck, K. L., & Mattila, J.** 2010. Differential habitat
599 use and antipredator response of juvenile roach (*Rutilus rutilus*) to olfactory
600 and visual cues from multiple predators. *Oecologia*, **162**, 893-902.

601 **Mazur, M. M., & Beauchamp, D. A.** 2003. A comparison of Visual Prey
602 Detection Among Species of Piscivorous Salmonids: Effects of Light and
603 Low Turbidities. *Environmental Biology of Fishes*, **67**, 397-405.

604 **Miner, J. G., & Stein, R. A.** 1993. Interactive Influence of Turbidity and Light on
605 Larval Bluegill (*Lepomis-macrochirus*) Foraging. *Canadian Journal of*
606 *Fisheries and Aquatic Sciences*, **50**, 781-788.

607 **Moore, A.** 1994. An electrophysiological study on the effects of pH on olfaction in
608 mature male Atlantic salmon (*Salmo salar*) parr. *Journal of Fish Biology*, **45**,
609 493-502.

610 **Nakata, K.** 2010. Attention focusing in a sit-and-wait forager: a spider controls its
611 prey-detection ability in different web sectors by adjusting thread tension.
612 *Proceedings. Biological sciences / The Royal Society*, **277**, 29-33.

613 **Nurminen, L., Pekcan-Hekim, Z., & Horppila, J.** 2010a. Feeding efficiency of
614 planktivorous perch *Perca fluviatilis* and roach *Rutilus rutilus* in varying
615 turbidity: an individual-based approach. *Journal of Fish Biology*, **76**, 1848-
616 1855.

617 **Nurminen, L., Pekcan-Hekim, Z., Repka, S., & Horppila, J.** 2010b. Effect of
618 prey type and inorganic turbidity on littoral predator--prey interactions in a
619 shallow lake: an experimental approach. *Hydrobiologia*, **646**, 209-214.

620 **Patullo, B. W., & Macmillan, D. L.** 2010. Making sense of electrical sense in
621 crayfish. *The Journal of experimental biology*, **213**, 651-7.

622 **Pekcan-Hekim, Z., & Lappalainen, J.** 2006. Effects of clay turbidity and density
623 of pikeperch (*Sander lucioperca*) larvae on predation by perch (*Perca*
624 *fluviatilis*). *Die Naturwissenschaften*, **93**, 356-9.

Plenderleith, M., van Oosterhout, C., Robinson, R. L., & Turner, G. F. 2005. Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology letters*, **1**, 411-4.

Quesenberry, N. J., Allen, P. J., & Cech, J. J. 2007. The influence of turbidity on three-spined stickleback foraging. *Journal of Fish Biology*, **70**, 965-972.

R Developement Core Team. 2011. R: A language and environment for statistical computing. Version 2.13.0. R Foundation for Statistical Computing. http://www.r-project.org/

Rafferty, N. E., & Boughman, J. W. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. *Behavioral Ecology*, **17**, 965-970.

Reusch, T. B., Häberli, M. A., Aeschlimann, P. B., & Milinski, M. 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, **414**, 300-2.

Richter, B. D., Braun, D. P., Mendelson, M. A., & Master, L. L. 1997. Contributed Papers Threats to Imperiled Freshwater Fauna. *Conservation Biology*, **11**, 1081-1093.

Sweka, J. A., & Hartman, K. J. 2003. Reduction of Reactive Distance and Foraging Success in Smallmouth Bass, *Micropterus dolomieu*, Exposed to Elevated Turbidity Levels. *Environmental Biology of Fishes*, **67**, 341-347.

Therneau, T. 2011. coxme: Mixed Effects Cox Models. R package version 2.1-3. http://cran.r-project.org/package=coxme

Therneau, T., & Lumley, T. 2011. survival: Survival analysis, including penalised likelihood. R package version 2.36-5. http://cran.r-project.org/package=survival

Turesson, H., & Brönmark, C. 2007. Predator-prey encounter rates in freshwater piscivores: effects of prey density and water transparency. *Oecologia*, **153**, 281-90.

Utne, A. C. W. 1997. The effect of turbidity and illumination on the reaction distance and search time of the marine planktivore *Gobiusculus flavescens*. *Journal of Fish Biology*, **50**, 926-938.

Utne-Palm, A. C. 1999. The effect of prey mobility, prey contrast, turbidity and spectral composition on the reaction distance of *Gobiusculus flavescens* to its planktonic prey. *Journal of Fish Biology*, **54**, 1244-1258.

Utne-Palm, A. C. 2002. Visual feeding of fish in a turbid environment: Physical and behavioural aspects. *Marine and Freshwater Behaviour and Physiology*, **35**, 111-128.

Ward, A. J. W., Hart, P. J. B., & Krause, J. 2004. The effects of habitat- and diet-based cues on association preferences in three-spined sticklebacks. *Behavioral Ecology*, **15**, 925-929.

665 **Ward, A. J. W., Holbrook, R. I., Krause, J., & Hart, P. J. B.** 2005. Social
666 recognition in sticklebacks: the role of direct experience and habitat cues.
667 *Behavioral Ecology and Sociobiology*, **57**, 575-583.

668 **Webster, M. M., Atton, N., Ward, A. J. W., & Hart, P. J. B.** 2007a. Turbidity and
669 foraging rate in threespine sticklebacks: the importance of visual and
670 chemical prey cues. *Behaviour*, **144**, 1347-1360.

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

674 **Weissburg, M. J., Ferner, M., Pisut, D. P., & Smee, D. L.** 2002. Ecological
675 Consequences of Chemically Mediated Prey Perception. *Journal of*
676 *Chemical Ecology*, **28**, 1953-1970.

677 **Wootton, R. J.** 1976. *The biology of the sticklebacks.* Academic Press.

678 **Zimmer-Faust, R. K., Finelli, C. M., Pentcheff, N. D., & Wethey, D. S.** 1995.
679 Odor Plumes and Animal Navigation in Turbulent Water Flow: A Field Study.
680 *Biological Bulletin*, **188**, 111-116.

Tables

Table 1. Behaviours recorded in the foraging trials.

Behaviour	**Description**
Swimming	Moving around in the arena including saltatory and steady movement, but not along the edges of the arena.
Hiding	The fish is under the shelter and invisible to the observer
Edge	Continuous swimming along the edge of the arena
Inactive	Time spent immobile for at least 5 seconds in one bout

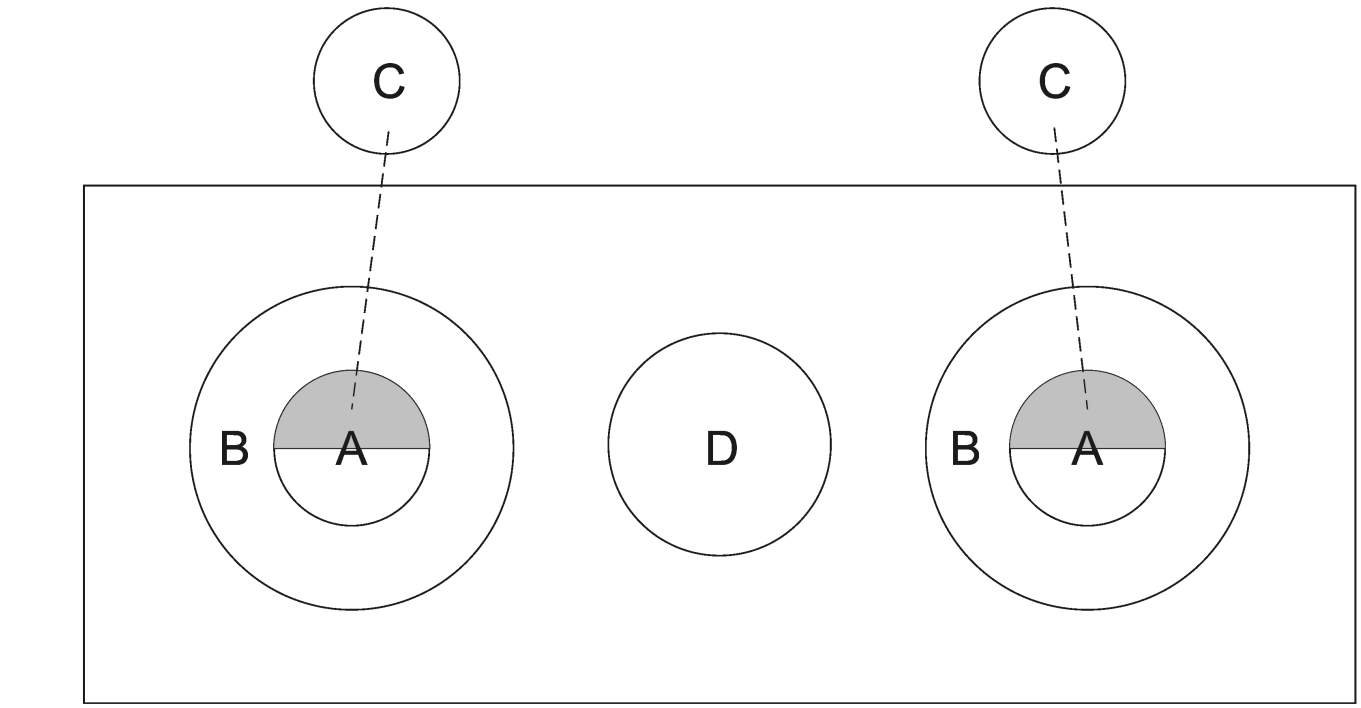
Figure legends

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.

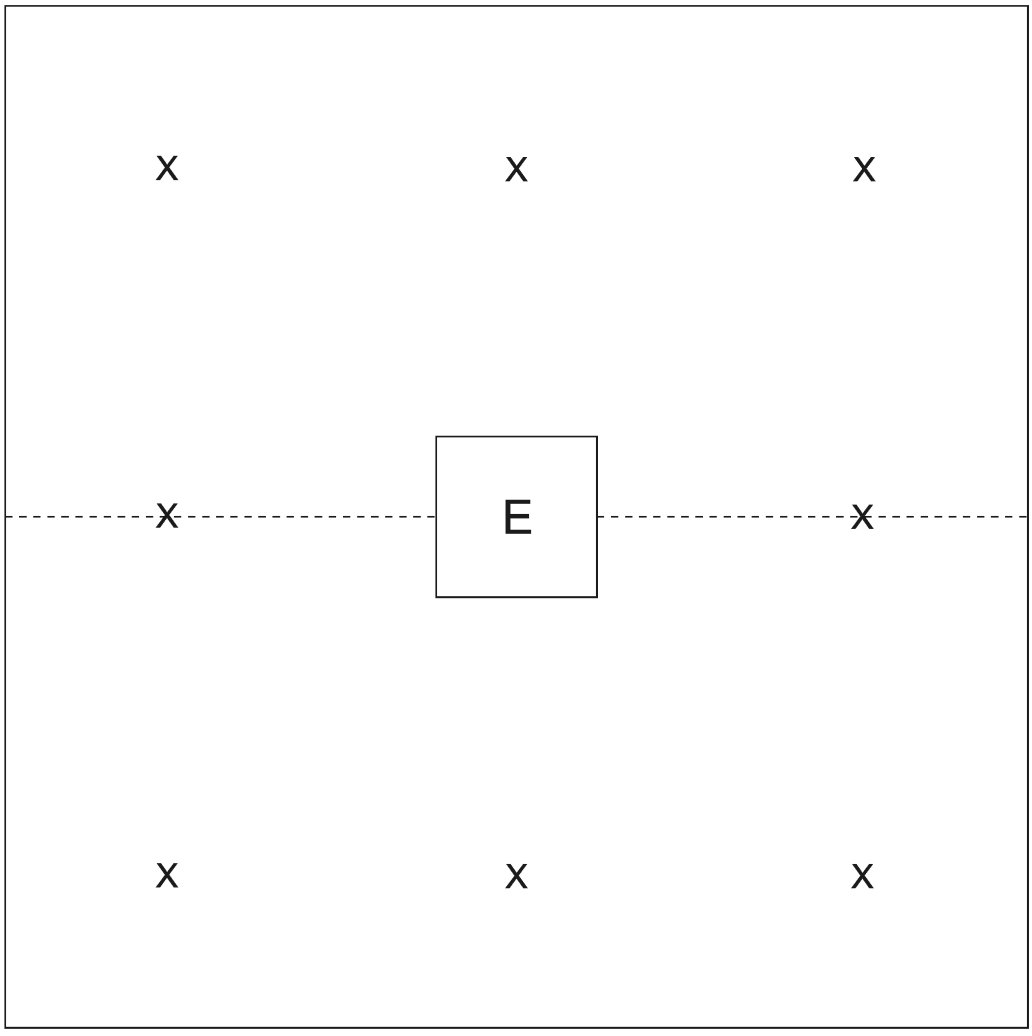
Fig. 2 Mean proportion of time spent with the food container with error bars of two standard errors. Grey is olfactory cue only, white is combined cues and black is visual cue only. Significant effects are marked with an asterisk.

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).

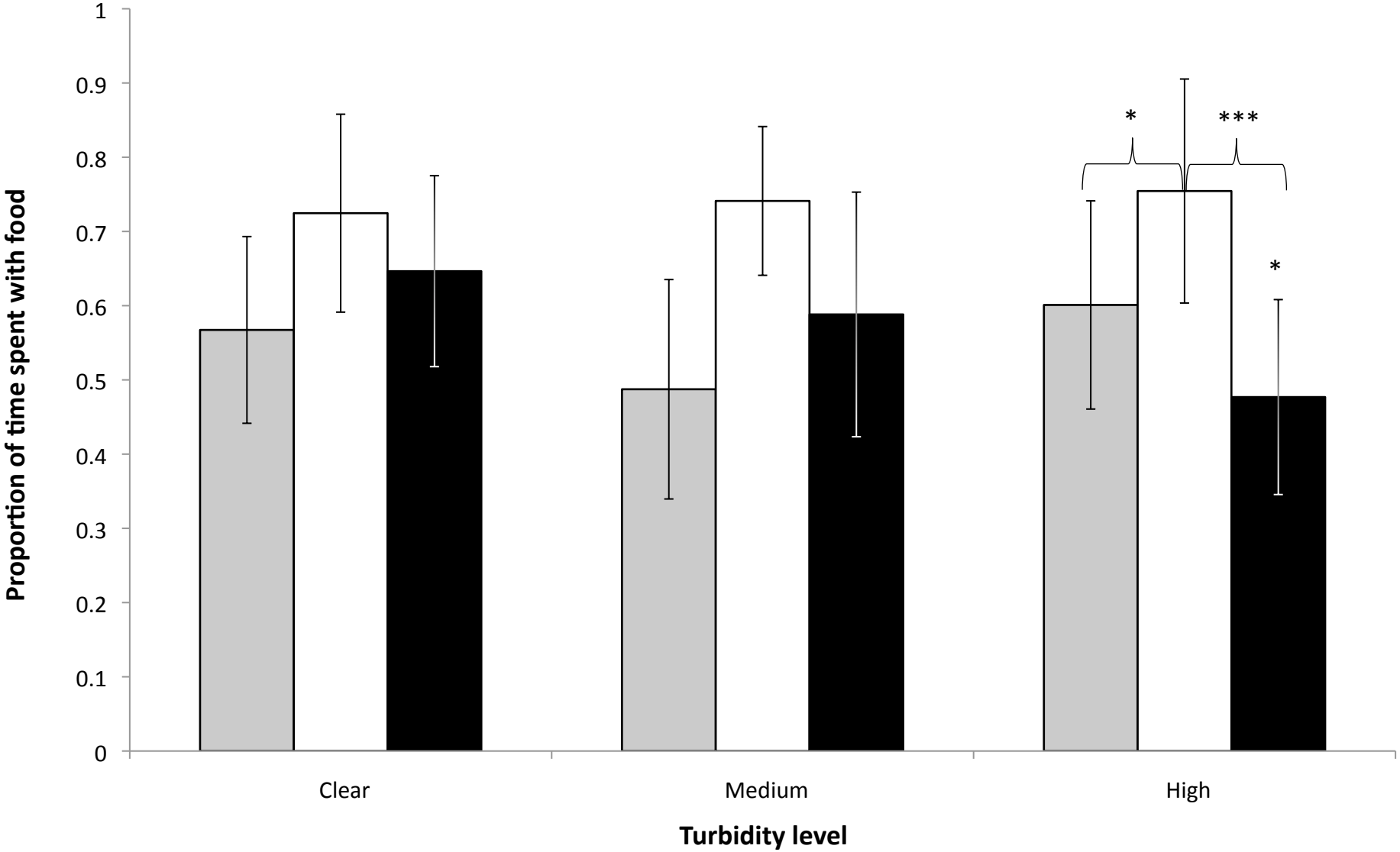
Fig. 4 The proportion of time spent swimming (A) and hiding (B) in the six treatment groups. Black bars are no added cue, and white bars are added olfactory cue. The error bars are two standard errors. Level of turbidity had an effect on behaviours whereas olfactory cue did not.

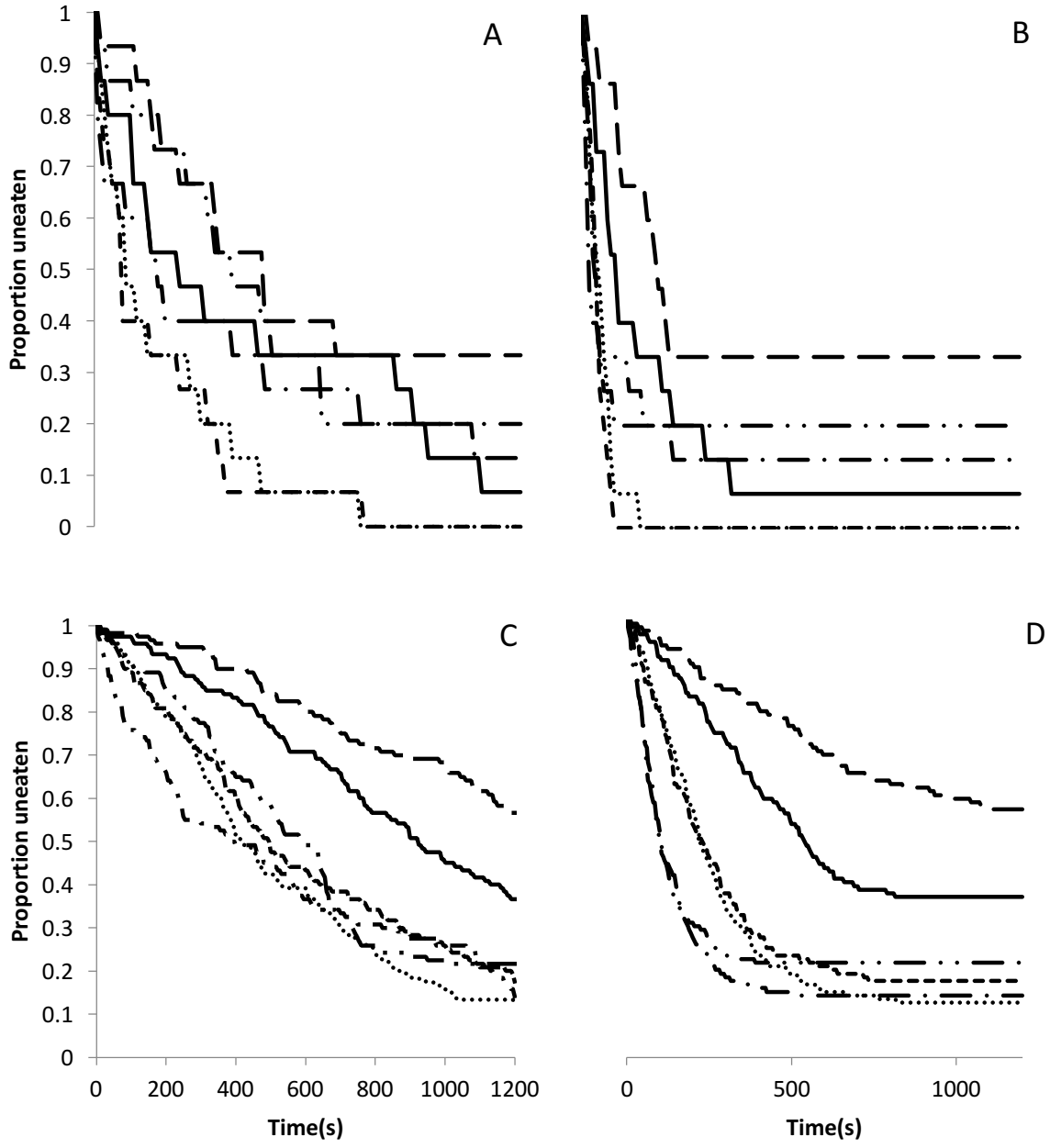


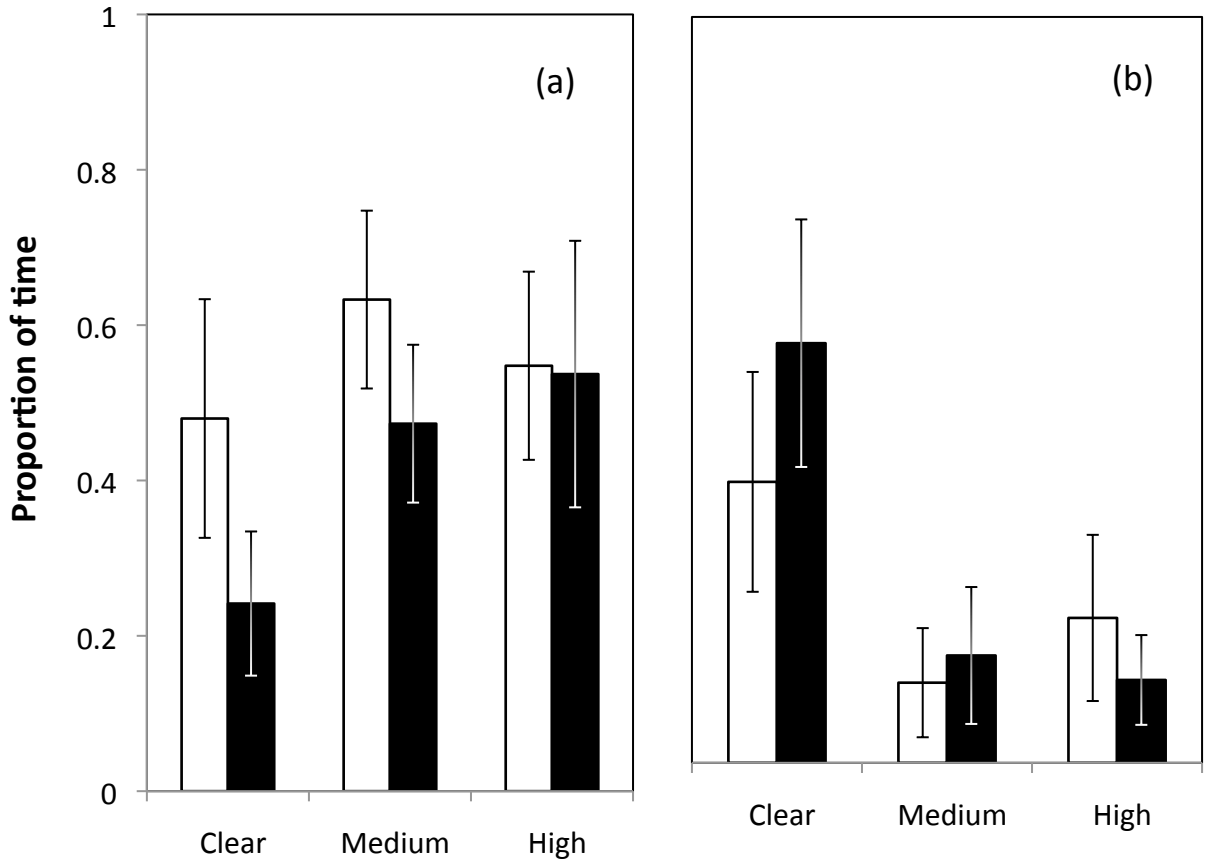
(a)



(b)


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion of time spent with food
*
***
*
Clear
Medium
High
Turbidity level


A
B
C
D
Proportion uneaten
Proportion uneaten
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
0
500
1000
Time(s)
Time(s)


Proportion of time
1
0.8
0.6
0.4
0.2
0
(a)
Clear
Medium
High
(b)
Clear
Medium
High