

Swimming kinematics and hydrodynamic imaging in the blind Mexican cave fish (*Astyanax fasciatus*)

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Accepted 23 June 2008

SUMMARY

Blind Mexican cave fish (*Astyanax fasciatus*) lack a functioning visual system, and are known to use self-generated water motion to sense their surroundings; an ability termed hydrodynamic imaging. Nearby objects distort the flow field created by the motion of the fish. These flow distortions are sensed by the mechanosensory lateral line. Here we used image processing to measure detailed kinematics, along with a new behavioural technique, to investigate the effectiveness of hydrodynamic imaging. In a head-on approach to a wall, fish reacted to avoid collision with the wall at an average distance of only 4.0 ± 0.2 mm. Contrary to previous expectation, there was no significant correlation between the swimming velocity of the fish and the distance at which they reacted to the wall. Hydrodynamic imaging appeared to be most effective when the fish were gliding with their bodies held straight, with the proportion of approaches to the wall that resulted in collision increasing from 11% to 73% if the fish were beating their tails rather than gliding as they neared the wall. The swimming kinematics of the fish were significantly different when swimming beside a wall compared with when swimming away from any walls. Blind cave fish frequently touched walls when swimming alongside them, indicating that they use both tactile and hydrodynamic information in this situation. We conclude that although hydrodynamic imaging can provide effective collision avoidance, it is a short-range sense that may often be used synergistically with direct touch.

Key words: *Astyanax fasciatus*, biomechanics, blind cave fish, hydrodynamic imaging, kinematics, lateral line.

INTRODUCTION

The hypogean (cave-dwelling) form of *Astyanax fasciatus*, commonly known as the blind Mexican cave fish, lacks a functional visual system and uses hydrodynamic cues to gather information about its surroundings (von Campenhausen et al., 1981). This behaviour has been termed ‘hydrodynamic imaging’ (Hassan, 1989). As the fish swims, it displaces the water in front of itself, creating a flow field around its body. When the fish approaches an obstacle, distortion of this flow field is sensed by the fish using its mechanosensory lateral line. The lateral line sensory system is found in all fish species and senses properties of the motion of the water around the fish. The individual sensory organs of the lateral line are neuromasts. Each neuromast is composed of hair cells that are covered by a gelatinous cupula. When the cupula is displaced by water motion, the hair cells are activated and send sensory information to the fish’s nervous system. There are two subsystems of neuromasts found in most fish: superficial neuromasts are distributed over the surface of the fish’s body and are thought to encode the flow velocity over the skin surface, whereas canal neuromasts are found in canals under the skin, located between pores, which open to the surrounding fluid and are thought to encode pressure gradient information (for a review, see Coombs and Montgomery, 1999).

Previous studies have explored the ability of blind cave fish to sense their surroundings and specifically to discriminate the spacings in a wall grating (Hassan, 1986; von Campenhausen et al., 1981; Weissert and von Campenhausen, 1981). It has been found that when blind cave fish explore an unfamiliar environment they increase their swimming velocity, yet over the course of the following hours their swimming velocity gradually decreases back to its normal value

(Teyke, 1985; Teyke, 1988; Teyke, 1989). The suggestion was made that faster swimming speed increases the stimulus to the lateral line, enhancing the fish’s ability to sense its surroundings (Hassan, 1985; Hassan et al., 1992; Teyke, 1985; Teyke, 1988; Teyke, 1989). One prediction of this hypothesis is that at higher velocities the fish should be able to detect objects at greater distances. However, to date there has not been a clear quantitative measure of the effective range of hydrodynamic imaging.

The aim of this study was to examine the effect of swimming kinematics on the effective range of hydrodynamic imaging. We developed a technique to induce fish to swim directly at a wall and studied swimming kinematics in this ‘head-on’ situation, as well as when the fish swam parallel to the wall. In the head-on approach we were able to define an objective measure of the effective working distance of hydrodynamic imaging. In this regard, our hypothesis was that if increased swimming velocity enhances the fish’s ability to sense its surroundings, then fish swimming at higher speeds will react to the presence of objects at greater distances.

MATERIALS AND METHODS

Fish

Blind Mexican cave fish (*Astyanax fasciatus* Cuvier 1819) were purchased from a commercial aquarium supplier. Adult blind cave fish, ranging in size from 40 to 60 mm in total length, with a mean length of 44 ± 4 mm, were housed in glass aquaria (250 l and 75 l) and maintained at a constant temperature of 25°C. Water in the aquaria was standardised by adding CaCl_2 and synthetic sea salt (Instant Ocean, Aquarium Systems, OH, USA) to deionised water in order to maintain a Ca^{2+} concentration of slightly above 1.0 mmol l^{-1} , at a conductance of approximately $750 \mu\text{S}$. The Ca^{2+}

concentration was regularly checked using a mass spectrometer, as it has been shown that Ca^{2+} concentrations below this level can decrease the sensitivity of the lateral line system (Sand, 1975). Water pH was maintained at 7.0 by the addition of NaHCO_3 as required. Water quality was maintained with weekly water changes of 10% of the water volume. All experiments were conducted in a separate experimental tank filled daily with water from the holding tanks, except where stated. All experiments were carried out in accordance with the animal care policy of the University of Auckland.

Experimental procedure

Trials were conducted in a 400 mm × 300 mm × 80 mm acrylic experimental tank as shown in Fig. 1. The tank was partitioned with acrylic dividers. The tops of the dividers did not break the surface of the water, preventing distortions in the recorded video images due to any meniscus. Two different setups of the tank were used to measure the effective range of hydrodynamic imaging in two different orientations. In the setup shown in Fig. 1 a divider was placed in the middle of the tank to direct the fish towards the centre of the opposite wall, so as to record the fish's reaction as it approached the wall head-on. These trials shall be referred to as head-on trials. The other setup used was identical, but lacked the divider in the middle of the tank. These trials were used to measure the distance between the fish and the wall when the fish was swimming parallel to the wall, and shall be referred to as parallel trials.

It is not possible by observation alone to measure when a blind cave fish first detects the presence of an object; it is only possible to measure when the fish first reacts to the presence of the object by changing its behaviour. The difference between these two time points is the reaction time of the fish. Assuming that the fish reacts as quickly as possible after it detects an object, then the distance the fish is from the object when it first reacts to the object is the effective range of hydrodynamic imaging.

To establish that the fish was using its lateral line to sense its surroundings and not some other sense, the head-on trials were repeated after exposing the fish to a solution with a Co^{2+} concentration of 0.1 mmol l^{-1} and a Ca^{2+} concentration of 0.05 mmol l^{-1} for 24 h. This cobalt/calcium concentration has been shown to completely block the mechanosensitivity of the entire lateral line (Karlsen and Sand, 1987).

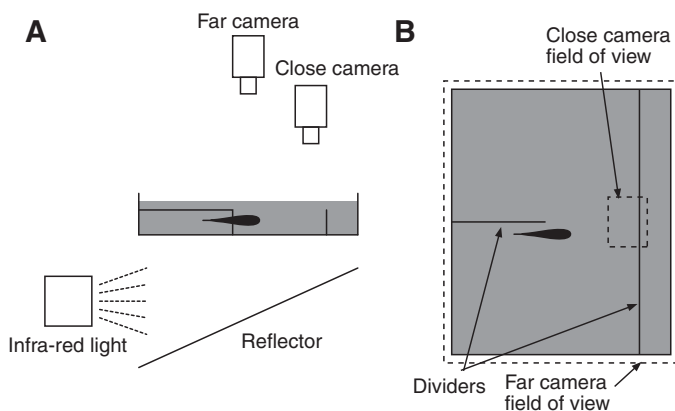


Fig. 1. Experimental setup to record swimming kinematics of blind cave fish using digital video cameras. Setup shown is for head-on trials. Setup for parallel trials was similar except the divider in the centre of the tank was not present. (A) Side view. (B) Top view.

The swimming kinematics of the fish were recorded using two digital scientific video cameras (Marlin F131B, AVT, Stadroda Germany). The cameras were equipped with complementary metal-oxide semiconductor (CMOS) chips that allowed a sub-sample of the full imaging area (1280 pixels × 1024 pixels, 8-bit greyscale) to be sampled at an increased frame rate. One camera, referred to as the far camera, imaged the entire tank from above at 50 frames s^{-1} , with $900 \text{ pixel} \times 680 \text{ pixel}$ resolution using a 10 mm focal length lens. The second camera, referred to as the close camera, imaged a small section along the top wall of the tank (Fig. 1) at an increased magnification using a 50 mm macro-lens. The settings for the close camera were altered to suit the orientation of the fish for the two types of trial. For the head-on trials an imaging area of $752 \text{ pixels} \times 750 \text{ pixels}$ was captured at 50 frames s^{-1} . For the parallel trials an imaging area of $1280 \text{ pixels} \times 500 \text{ pixels}$ was captured at 50 frames s^{-1} . The cameras were synchronised to capture frames simultaneously using a 50 Hz signal from a function generator (CFG-8020H, Instek, Tucheng City, Taiwan). The tank was back-lit with infra-red LED floodlights (TF-30M80/IR, Ta-Fu Electronics, Kaohsiung Hsien, Taiwan) and a system of reflectors and diffusers.

Individual fish were placed into the experimental tank and their behaviour recorded for 30 min. Fish were transferred in a plastic container with a small volume of water to prevent any damage to their superficial neuromasts. The water in the experimental tank was completely still apart from the motion generated by the fish's movement. Between trials an aerator and heater were placed in the experimental tank to maintain the water temperature and oxygen level. The head-on trials and the parallel trials were conducted with the same group of fish. To prevent any possible effects that might be caused by learning, all trials were conducted at least 1 month apart. It has been shown that cave fish will react as if an environment is unfamiliar if they are removed from it for a period of longer than 2 days (Teyke, 1989).

Image processing

All image processing and the extraction of kinematic parameters were done using custom-written software in MATLAB (The Mathworks Inc., Natick, MA, USA). The close camera footage of the head-on trials was manually digitised using a graphical user interface custom-written in MATLAB. For each head-on approach to the wall where the fish was at an angle of less than 60 deg. to perpendicular, the position of the nose of the fish was manually tracked. Approaches were categorised as avoidances or collisions depending on whether the fish altered its course in time to avoid impending contact with the wall. For avoidances, the frame in which the fish first reacted to the presence of the wall was recorded. The first sign that the fish had detected the wall was normally the rapid extension of the pectoral fins away from the body. In the case of collisions, the frame in which the fish made contact with the wall was recorded. In both cases the motion of the fish immediately prior to reacting to the presence of the wall or colliding with the wall was categorised as gliding or tail beating depending on whether the body was held straight or was curved in the action of beating the tail.

The algorithm for the image processing of the far camera footage is outlined in Fig. 2. For each frame a background image taken before the fish was placed in the tank was subtracted. The resulting image was then segmented by intensity and the fish identified by selecting the object with the most appropriate location, total area and aspect ratio within a given set of limits. To find the midline of the fish the image was skeletonised and the branching structure of the resulting skeleton was pruned by removing any short arms branching off the

longest segment. Next the head and tail of the fish were identified by measuring the width of the fish one-sixth and five-sixths of the way down the midline. The wider end was taken to be the head of the fish. A straight line was fitted to the first third of the midline and a fifth order polynomial was fitted to the remaining two thirds

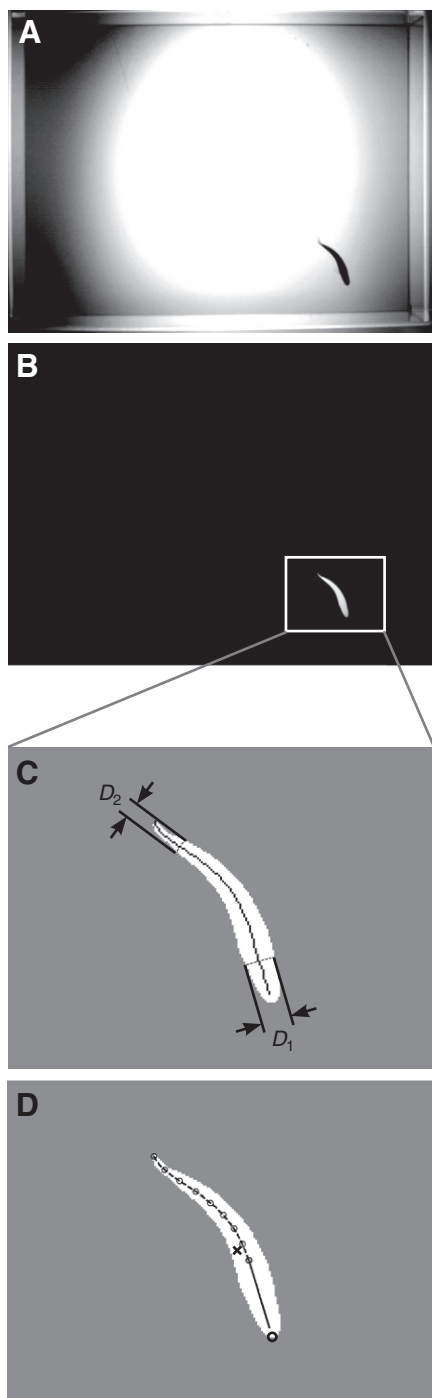


Fig. 2. Image processing algorithm to extract kinematic parameters from far camera footage. (A) Original image. (B) Image with background subtracted. (C) Result of thresholding by intensity. Skeletonised midline shown with measurements D_1 and D_2 of body width one-sixth of the way down the midline from each end. The wider end was taken to be the head. (D) Fitted midline approximation with the first third linear and the remaining two thirds represented by a fifth order polynomial. The centre of area is marked by X and the nose of the fish by O.

of the midline. The nose of the fish was taken as the intercept of the linear portion of the midline and the outline of the head of the fish. The back two-thirds of the midline were characterised by nine evenly spaced points placed along the polynomial representation of the midline. This was found to be a robust way of characterising the midline, matching the increasing flexibility along the length of the fish's body. The far camera footage of the head-on trials was not analysed as initial analysis showed the fish's behaviour to be very similar to that seen in the parallel trials. An image calibration algorithm implemented in MATLAB (Bouguet, 2007) was used to correct for optical distortions of the far camera footage created by the wide-angle lens. This was found to be unnecessary for the close camera footage as there was minimal lens distortion. The close camera footage of the parallel trials was processed using a similar algorithm to that already described for the far camera footage. As the far camera footage recorded the same behaviour, the close camera footage was only used to measure the distance at which the fish glided parallel to the wall. The close camera was looking straight down the wall of the tank, which eliminated the problem of perspective, which affected the far camera footage. For each fish, 10 passes were analysed where the fish was gliding parallel to the wall ($\pm 15^\circ$) with its body held straight and making no contact with the wall.

Kinematic analysis

Following image processing and the extraction of the parameters described above, the data were processed to measure additional kinematic parameters. Each head-on approach to the wall was characterised by the parameters shown in Fig. 3. The velocity of the nose of the fish in the frame immediately before reaction to, or collision with, the wall was calculated using B-spline fitting by generalised cross-validation and taking the first derivative (Woltring, 1986). This has been shown to be a robust method for calculating velocity from video data (Walker, 1998). The nose position was used rather than the centre of area as the entire fish was often not in the field of view in the close camera footage. The orientation of the fish just before first reacting to, or colliding with, the wall was calculated by fitting a straight line to the nose position in the four frames before reaction or collision and measuring the angle of this line to the wall. The distance to the wall at the first response was measured as the distance between the fish's nose and the wall along this line.

For the far camera footage of the parallel trials, the velocity of the centre of area of the fish was measured using the B-spline fitting method described above. In addition to this each frame was categorised by the location of the fish and its orientation to the wall (Fig. 4). A fish was categorised as being beside the wall if its nose was within 0.5 body lengths (BL) of the wall and its body was oriented at an angle of less than 45° to the wall. The side of the fish that was closest to the wall was also recorded (i.e. left or right). A fish was categorised as being in the middle of the tank if its nose was more than 0.5 BL away from any wall. Mathematical modelling studies indicate that the stimulus to the lateral line only changes within 0.25 BL of a wall (Hassan, 1992), so at twice this distance it should be safe to assume that the fish cannot detect the wall. The nose position was used rather than the centre of area, as the head is where most of the important sensory information is going to be collected with regard to approaching a wall (Hassan, 1992). Any frame that did not meet these criteria or where the nose was within 0.5 BL of a corner of the tank was classified as being in transition and was not included in the final analysis. For each frame the tail angle, as defined by the angle between the final point along the

midline and the body axis down the first third of the fish, was calculated. The rate of change of the tail angle was calculated using B-spline fitting by generalised cross-validation and taking the first derivative. Each frame was then categorised as tail beating or gliding based on these measurements. If the absolute tail angle was less than 5 deg. or the rate of change of the tail angle was less than 100 deg. s^{-1} then the frame was classified as a glide; otherwise it was classified as a tail beat. To account for frames where the tail was passing across the body axis on the way to beating on the opposite side of the fish, any glide series less than three frames in length was reclassified as tail beating. These parameters were found to give the closest results to categorisations based on observation.

Cave fish swim using an intermittent tail beat and glide mode. To simplify kinematic analysis the fish's motion was broken down into sequences of tail beat and glide pairs. The kinematic parameters for the tail beat and glide phases were then summarised as shown in Fig. 4. Each tail beat phase was categorised according to the motion of the tail. Sometimes the fish would execute a two-sided tail beat where the tail moved to both sides of the body axis, and sometimes the tail would only move to one side of the body axis before the body was held straight and the fish went into a glide. The latter one-sided tail beats were categorised as single beats to the left or single beats to the right depending on which side of the body the tail moved to. The two-sided tail beats were categorised as double tail beats. Each tail beat–glide sequence was also categorised by location as described above. If not all the frames in the sequence were categorised as being in the same location then the sequence was not analysed.

For the selected frames of the close camera footage from the parallel trials the horizontal distance between the wall and the body of the fish one quarter of the way down its midline was measured.

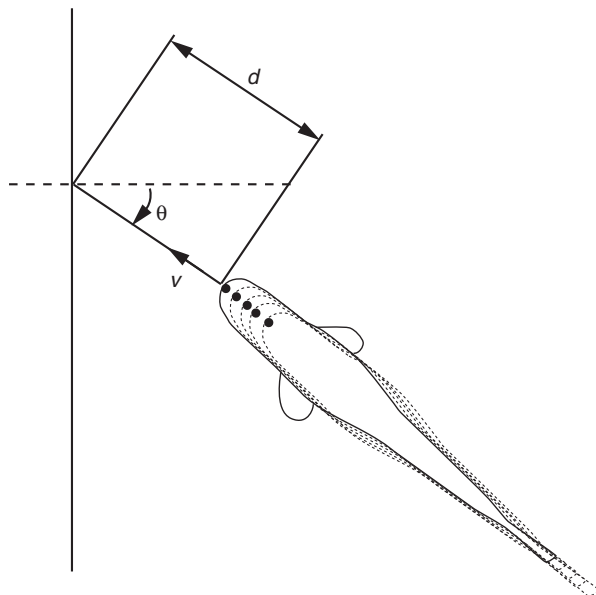


Fig. 3. Parameters measured in head-on approaches where fish avoided the wall. The first sign that a fish had detected the wall was the rapid extension of the pectoral fins (solid outline). At this point the orientation of the fish relative to the wall (θ) was measured by fitting a straight line to the position of the fish's nose in the previous four video frames (dashed outlines). The distance between the wall and the nose of the fish (d) was also measured down this line. The velocity of the fish (v) was calculated from the nose position using B-spline fitting by generalised cross-validation and taking the first derivative.

This was approximately the widest part of the fish. The mean distance was then calculated for each pass.

Statistics

Linear mixed-effect models were used to test for correlations between the swimming kinematics of the fish and the effective range of hydrodynamic imaging in both the head-on and parallel trials. Generalised additive models were first used to confirm that there were no significant non-linear interactions between variables. For the analysis of the head-on trials, the mean parameters of the approaches resulting in avoidance and the approaches resulting in collision, for an individual fish, were compared using Student's paired t -tests. For the analysis of the data collected in the parallel trials, the median values of the parameters measured beside the wall and in the middle of the tank, for each individual fish, were compared using Student's paired t -tests. The median was used as the test statistic for the parallel trials as it gave a better representation of the central tendency of the data than the mean, given the positive skew that was present. Parameters measured as proportions were first transformed using an arc-sine transformation. All tests were considered significant at the 0.05 level. Values are given as means \pm s.e.m. unless specified otherwise; N is the number of fish.

RESULTS

Head-on results

In the head-on trials the first sign that the fish was responding to the presence of the wall was normally the rapid extension of the pectoral fins away from the body. This was then normally followed by the fish curving its body away from the wall, so as to execute a tight turn that left the fish on a course approximately parallel with the wall (Fig. 5). Although the pectoral fins were also extended at the beginning of each tail beat during routine swimming, the distribution of these events indicates that they were in response to the fish sensing the wall (Fig. 6). If the extension of the pectoral fins was not in response to the wall, then it would be expected that this distribution would be flat, as was seen beyond 10 mm from the wall.

Each individual fish approached the wall head-on repeatedly during each 30 min trial at differing orientations and velocities. The effective range of hydrodynamic imaging varied both within and between individuals. Looking at all of the approaches by all fish using mixed-effect modelling, there was no significant correlation found between effective range and fish body length (Fig. 7A), or between effective range and swimming velocity (Fig. 7B), or effective range and orientation. Although there was variation between approaches, the mean effective distance of hydrodynamic imaging for each fish was relatively constant. The mean effective range of hydrodynamic imaging calculated for all fish was $4.0 \pm 0.2 \text{ mm}$, at a mean velocity of $65 \pm 4 \text{ mm s}^{-1}$, giving a fish on average $71 \pm 6 \text{ ms}$ to change course before it would have collided with the wall (Table 1).

In 128 of the 172 approaches recorded the fish successfully avoided collision with the wall (Table 2); in the other 44 approaches the fish collided with the wall. In some of these cases the fish would extend its pectoral fins away from its body and begin to turn but not in time to avoid collision. In other cases, the fish showed no indication that it had detected the wall before collision (Fig. 8). There was a clear correlation between whether the fish was gliding or beating its tail as it approached the wall and whether it was able to avoid the wall (Table 2). Of the approaches where the fish was gliding when nearing the wall, 11% resulted in collision with the wall. In comparison, if the fish was tail beating as it approached the wall, 73% of the approaches ended in collision. There was a

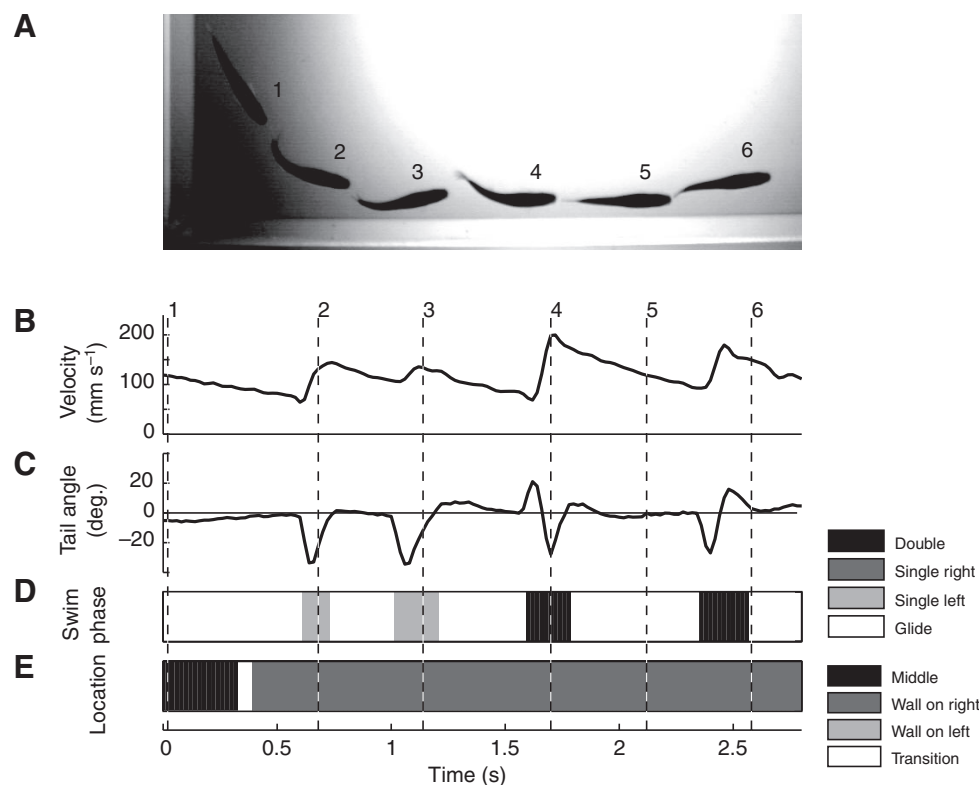


Fig. 4. Kinematic parameters measured from far footage of parallel trials. (A) Image series of a blind cave fish swimming along a wall showing the corresponding kinematic parameters. (B) Velocity of the centre of area of the fish. (C) Tail angle of the fish; tail beats to the left correspond to negative angles, those to the right to positive angles. (D) Swimming phase classification based on tail angle and the rate of change of tail angle (not shown). Double refers to a tail beat on both the right and left sides of the fish, single left and single right refer to a tail beat on one side of the fish only. (E) Classification by location in tank.

significant difference in the mean velocity ($t_{12}=-2.2$, $P=0.045$) and orientation ($t_{12}=4.3$, $P=0.001$) of the avoidance and collision events for each fish as shown by paired t -tests. Within the approaches of each individual fish, collisions occurred at a higher velocity ($73\pm 6\text{ mm s}^{-1}$) than avoidances ($65\pm 4\text{ mm s}^{-1}$) and at a more perpendicular angle ($18\pm 2\text{ deg.}$) than avoidances ($33\pm 2\text{ deg.}$). However, these appeared to be secondary factors in comparison to the effect of tail beating. Overall, a fish was much more likely to collide with the wall if it was in the process of beating its tail as it approached the wall.

When the head-on trials were repeated with the lateral line blocked by exposure to Co^{2+} , 198 of the 199 approaches resulted in the fish colliding with the wall (Table 3). The single case of the fish avoiding the wall was likely to be a random turn, rather than the fish reacting to the presence of the wall. The swimming behaviour of the fish was greatly altered after exposure to Co^{2+} ; the fish swam with their heads at the surface or pressed into the bottom of the tank or into the walls of the tank. The fish glided only occasionally for very brief periods, with most time spent tail beating.

Parallel results

Inspection of the close camera footage of the parallel trials revealed that the fish would often make contact with the wall with their pectoral fins. As shown in the representative image series in Fig. 9, a fish would routinely finish a glide and then extend its pectoral fins, making contact with the wall with one fin as it was going into a tail beat. During the tail beat the fin would normally lose contact with the wall as the tail beat progressed, due to the fin being retracted or the fish angling away from the wall slightly. On some occasions, especially when the fish's tail beat was directed to the side facing the wall, the pectoral fin would remain in contact with the wall for the duration of the tail beat and sometimes into the following glide. There was no significant correlation between the swimming velocity of the fish and the distance that the fish glided parallel to the wall

as tested using mixed-effect modelling. The fish glided at a mean distance of $4.7\pm 0.5\text{ mm}$ or $0.10\pm 0.01\text{ BL}$ ($N=9$) from the wall. The mean length of the leading edge of the pectoral fins when extended was $0.128\pm 0.003\text{ BL}$ ($N=10$) as measured from three frames from each fish where the fins were extended away from the body. In 2 of the 11 trials the fish swam with their pectoral fins touching the wall the majority of the time when they were near the wall. These trials were not included in the close camera analysis. The close camera footage also revealed that fish would sometimes swim with their bodies rolled relative to the wall, with the dorsal surface of the body being rolled slightly away from the wall at an angle of approximately 25 deg. This rolling behaviour was very consistent in the two fish that kept constant pectoral fin contact with the wall but was more variable in occurrence in the other nine trials.

Inspection of the far camera footage of the parallel trials showed that the blind cave fish would start swimming as soon as they were released into the experimental tank. In all trials the fish showed a clear preference for staying close to the wall, on average spending $84\pm 3\%$ of their time within 0.5 BL of the wall. The fish would normally swim around the walls of the tank in one direction for a short period, of the order of about 5 to 10 s, before changing direction and swimming around the walls in the opposite way. When approaching a corner, a fish would normally either turn to follow the intersecting wall or nose into the corner, often swimming up and down with its nose pressed into the corner. This would normally last 0.5 to 1 s before the fish would return to swimming along the wall. All footage where the fish's nose was within 0.5 BL of the corner was excluded from the kinematic analysis. The fish tended to swim close to the bottom of the tank, but did show some changes in swimming depth as they explored the experimental tank. The depth of the water was kept at 80 mm to minimise the effects of the fish being pitched up or down in the measurement of the kinematic parameters. The fish would occasionally swim away from the wall and across the middle of the tank. The path of these swims was

normally reasonably straight until the fish reached another wall of the tank whereupon they would routinely resume wall following.

The analysis of the swimming kinematics measured in the far footage of the parallel trials showed that there was a distinct difference in the swimming kinematics of the fish when they were swimming parallel to a wall compared with when they were in the middle of the tank. The fish swam faster when they were beside a wall by $39 \pm 7\%$ ($t_{10}=5.54$, $P=0.0002$) as shown in Table 4. The glide duration was significantly shorter ($-32 \pm 7\%$, $t_{10}=-4.82$, $P=0.0007$) when beside a wall than when in the middle of the tank. There was also a significant increase in the proportion of double tail beats to

single tail beats (0.30 ± 0.04 , $t_{10}=7.39$, $P<0.0001$), indicating that the fish were using relatively more double tail beats when they were alongside the wall. Studying the single tail beats, it can be seen that the fish had a significant preference for beating their tails on the side away from the wall. During single tail beats the fish's head was turned to the same side as the tail at the start of the beat and then returned to the centre as the fish passed a wave of bending down the body, which ended with the tail straightening as the fish went into a glide as shown in Fig. 9. The motion of a double tail beat was similar except that the tail would move to both sides of the fish's body. Examination of the tail beat phase of the tail beat–glide sequence showed that double tail beats were significantly longer ($46 \pm 4\%$, $t_{10}=-11.12$, $P<0.0001$) and propelled the fish at a faster velocity ($55 \pm 4\%$, $t_{10}=-16.41$, $P<0.0001$) than single tail beats (Table 5). The orientation of the fish changed by a slightly smaller angle on average during a double tail beat than during a single tail beat (1.9 ± 0.06 deg., $t_{10}=3.32$, $P=0.008$). Analysis of the two trials where the fish kept their pectoral fins in contact with the wall showed no major differences in the fish's swimming kinematics from the other trials.

DISCUSSION

Effective range and velocity effects

Previous studies (Teyke, 1985; Teyke, 1988; Teyke, 1989) have found that when blind cave fish explore an unfamiliar environment they increase their swimming velocity, and it has been suggested that this increases the stimulus to the lateral line and enhances the fish's ability to sense its surroundings (Hassan, 1985; Hassan, 1992; Teyke, 1985; Teyke, 1988; Teyke, 1989). This hypothesis is based on potential flow modelling work by Hassan (Hassan, 1985), which found that the magnitude of the velocity stimulus scaled with the velocity of the fish, and the magnitude of the pressure gradient stimulus scaled with the square of the velocity. It was also found that the shape of the spatial distribution of the flow field on the body of the fish was not altered by changes in velocity; only the amplitude and time duration were changed.

This study found no significant systematic correlation between the swimming velocity of the fish and the distance at which the fish first visibly responded to the presence of the wall when approaching head-on. The effective range was relatively constant across all fish regardless of their swimming velocity. On average the fish responded to the wall when 4.0 ± 0.2 mm (0.086 ± 0.006 BL) away when

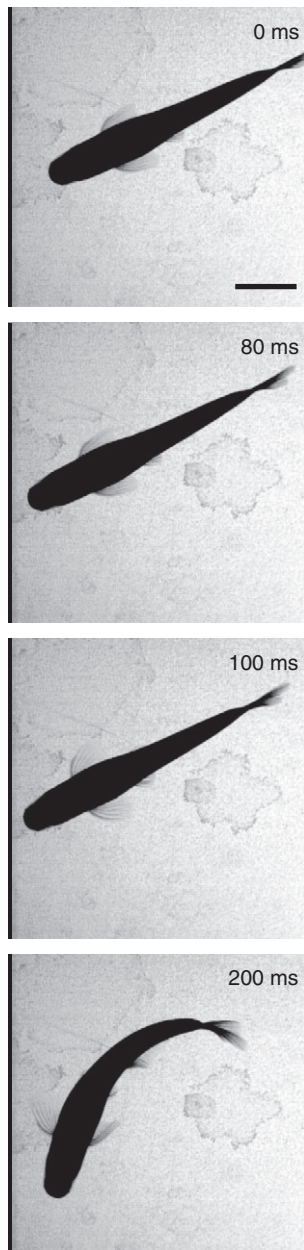


Fig. 5. Series of images from close camera footage of a head-on trial with a fish approaching the wall and avoiding collision. The fish glides towards the wall, then at 100 ms extends the pectoral fins away from the body; at this point the nose is 2.7 mm away from the wall. The fish then curves its body to the left, turning to follow along the wall, without making any contact. Length of scale bar is 1 cm.

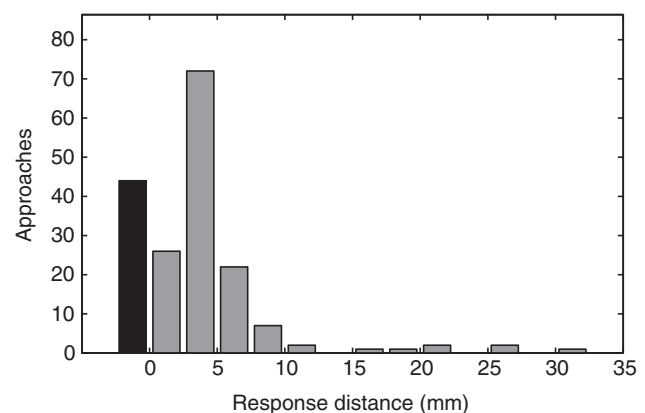


Fig. 6. Histogram of the distances at which the fish appeared to respond to the wall by initiating a tight turn when approaching head-on. Grey bars represent avoidances, black bar represents number of collisions with the wall. Approaches of all fish pooled ($N=12$).

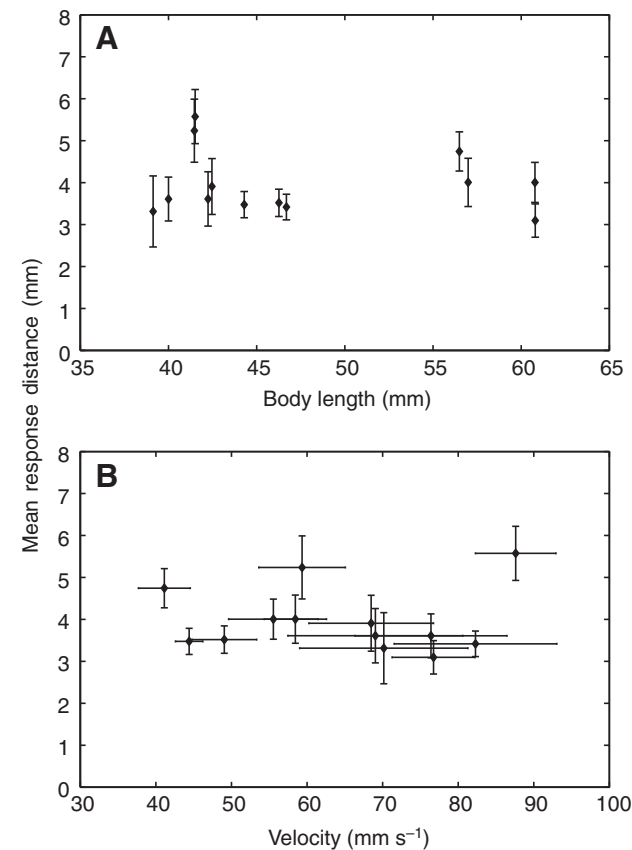


Fig. 7. (A) Mean distance at which fish reacted to the wall plotted against body length. (B) Mean distance at which fish reacted to the wall plotted against the mean velocity that the fish was swimming at. Error bars show s.e.m. Number of approaches per fish ranged from 6 to 21 with a mean of 13.

approaching head-on. There was no clear change in the ability of the fish to sense their surroundings with increased swimming velocity over the range of swimming velocities recorded. If a fish could detect a certain fixed magnitude of change in the flow field, for example a change of 10 mm s⁻¹, then it would be expected that the detection distance would increase linearly with velocity if the fish was using its superficial neuromasts, and increase in proportion to the velocity squared if it was using its canal lateral line. However, if the fish was detecting a certain relative change in the stimulus to the lateral line, for example a 25% change in the stimulus relative to that when the fish was away from any objects, then this would no longer hold. If the flow field scaled with velocity as the potential models indicate, then the distance at which a certain relative change occurred would remain constant. However, as potential models

Table 2. Occurrence of collisions based on swimming phase

	Tail beat	Glide
Avoidance	11	117
Collision	29	15
Percentage of collisions	73	11

Occurrence of collisions in relation to the swimming phase as the fish approached the wall head-on. Data for approaches from all fish pooled (N=13).

assume an inviscid fluid they may not represent the flow well at the low speeds and small scale of cave fish, where viscous effects are likely to be important. Further studies of how the flow fields change with swimming velocity are required to explore this further.

To take into account the time that the fish took to react to the wall, estimates of minimum possible reaction time were made. Weiss and colleagues measured the startle response time of goldfish to a 300 Hz sound pulse as 15.7±0.45 ms (Weiss et al., 2006). Using this figure as an estimate for the fish's minimum possible reaction time, the minimum mean distance at which the fish could have detected the wall was 5.0±0.2 mm (0.108±0.007 BL, Table 1). Again, there was no significant correlation between swimming velocity and estimated detection distance. The actual reaction time of blind cave fish is likely to be greater than this assumed reaction time as the startle response measured by Weiss and colleagues (Weiss et al., 2006) is mediated by the Mauthner cells, which are adapted for extremely fast reactions and represent an extreme for the minimum possible reaction time. However, the reaction times of fish for responses not mediated by the Mauthner cells are of the same order (Eaton et al., 1984; Lefrancois and Domenici, 2006) and would lead to an extension of the detection distance of the order of a few millimetres. For example, a reaction time of 40 ms would lead to a detection distance of 6.5 mm. It is possible that the fish detect the wall from further away as they approach and then delay changing course until they get closer, but this seems unlikely given the number of collisions that were observed. Some of the collisions observed were somewhat violent, suggesting that there is a significant motivation for the fish to change course as soon as an obstacle is detected.

As there appears to be no increase in the effective range of hydrodynamic imaging with increased swimming velocity, it is not clear why blind cave fish increase their velocity in unfamiliar environments. By swimming faster the fish have less time to react when approaching an object, although it appears that reaction speed is not the limiting factor in whether the fish collide with a wall. Swimming faster in an unfamiliar environment may simply enable the fish to explore their environment in a shorter period of time.

The distribution of the sudden changes in direction as the fish approached the wall indicates that these turns were reactions to the fish detecting the wall and not just routine turns. The results of the

Table 1. Kinematic parameters of cave fish approaches in head-on trials

	Response distance (mm)	Velocity (mm s ⁻¹)	Orientation (deg.)	Min. detection distance (mm)	Time to collision (ms)
Avoidance	4.0±0.2	65±4	33±2	5.0±0.2	71±6
Collision		73±6	18±2		
Difference		-9±4	14±3		
P value		0.045 (-2.2)	0.001 (4.3)		

Summary kinetic parameters of cave fish approaches in head-on trials (N=13) by outcome. Minimum detection distance calculated on assumed reaction time of 15.7 ms (Weiss et al., 2006). Values are overall means of the mean value for each trial ±s.e.m. P values are based on two-tailed paired t-tests for difference of means. Values in parentheses are t values. Number of approaches per fish ranged from 6 to 21 with a mean of 13.

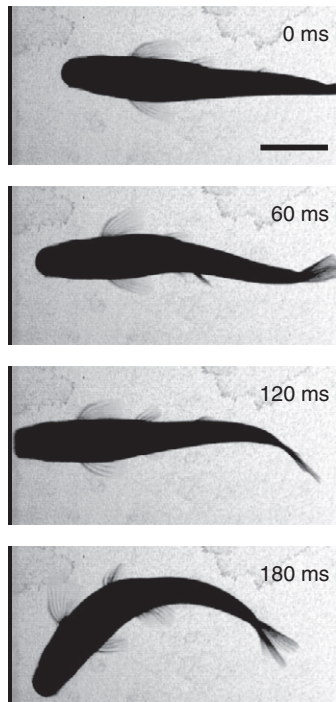


Fig. 8. Series of images from close camera footage of a head-on trial with a fish colliding with the wall. The fish starts a tail beat as it approaches the wall and shows no sign of detecting the wall before it collides with it. After colliding with the wall the fish turns to the left and swims along the wall. Length of scale bar is 1 cm.

cobalt trials also indicate that the turns were mediated by the lateral line as they were virtually absent when the entire lateral line of the fish was blocked. Previous studies have shown that blind cave fish can still navigate successfully without functioning superficial neuromasts but that this ability is lost when the canal neuromasts are disabled (Abdellatif et al., 1990; Montgomery et al., 2001).

The response distance shown by blind cave fish was surprisingly short given the lateral line is normally thought to be able to detect prey 1–2 *BL* away (Coombs and Montgomery, 1999). However, John used cinematography to record the occurrence of contact and avoidance by blind cave fish as they approached a glass surface placed in the aquarium and noted that all avoidances were initiated at distances of less than 4 mm (John, 1957), which is in line with our measurements. This reduced effective range is in line with the differences in the hydrodynamic signals. For prey detection the fish is detecting an external signal quite different from the signal created by its own motion, whereas in hydrodynamic imaging the fish is detecting a subtle change in the signal that is normally present while swimming. The relationship between the relative strength of the

Table 3. Cobalt trial collisions based on swimming phase in head-on trials

	Tail beat	Glide
Avoidance	0	1
Collision	161	37
Percentage of collisions	100	97

Occurrence of collisions in relation to the swimming phase as the fish approached the wall head-on with the lateral line disabled by cobalt. Data for approaches from all fish pooled ($N=10$).

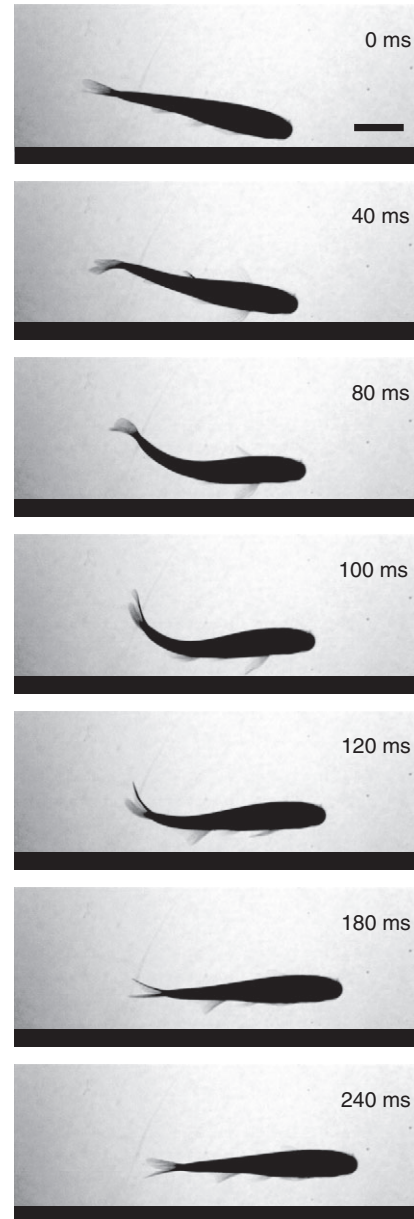


Fig. 9. Series of images from close camera footage of a parallel trial showing pectoral fin contact with the wall. The fish is gliding along the wall (line near bottom of images) then beats its tail to the left side of its body. At the start of the tail beat the pectoral fins are extended and the right fin makes contact with the wall. This lasts for 60 ms before the fins are retracted as the fish finishes the tail beat and resumes gliding. Length of scale bar is 1 cm.

signal and the distance from the source may also differ in these two situations.

Collision factors

The major factor that impacted on whether the fish collided with the wall was whether the fish was tail beating as it approached the wall (Table 2). This result supports those of Teyke, who found that fish that made a tail movement when their noses were closer to the wall than 25 mm always collided with the wall (Teyke, 1985). There are a number of possible reasons for an increased likelihood of collision if the fish is beating its tail as it approaches the wall. One

Table 4. Kinematic parameters of parallel trials by location

Location	Tail beat velocity (mm s ⁻¹)	Glide velocity (mm s ⁻¹)	Tail beat duration (s)	Glide duration (s)	Double tail beat proportion	Tail beat proportion wall side
Wall	104±7	88±7	0.16±0.01	0.30±0.03	0.51±0.05	0.31±0.03
Middle	75±4	63±4	0.15±0.01	0.44±0.05	0.21±0.03	
Difference	29±5	25±4	0.009±0.003	-0.14±0.03	0.30±0.04	
P value	0.0002 (5.54)	0.0002 (5.67)	0.02 (2.89)	0.0007 (-4.82)	<0.0001 (7.39)	

Summary kinematic parameters of swimming behaviour of blind cave fish from all parallel trials (*N*=11) by location in tank. Values are means of medians for each trial ±s.e.m. *P* values are based on two-tailed paired *t*-tests for difference of means. Values in parentheses are *t* values. Proportions were transformed using an arcsine transform for *t*-tests. Number of tail beat–glide sequences measured for each individual fish ranged from 1143 to 2433 measurements with a mean of 1777.

possibility is that by being in the process of moving its tail the fish is less able to change its body posture in order to turn. Executing the motor pattern of tail beating may delay or preclude the fish's ability to turn. Another possible reason for the fish colliding with the wall when tail beating may be that the fish's ability to sense the wall was reduced. It has been suggested (Teyke, 1985; von Campenhausen et al., 1981) that the lateral line of the cave fish may be inhibited by activation of the efferent system by motor activity. Motor activity has been shown to inhibit lateral line afferents in other fish species (Roberts and Russell, 1972). If this is the case, then the sensitivity of the lateral line to relative changes in the flow field would be reduced while the fish is tail beating. The action of tail beating may also reduce the fish's ability to sense its surroundings by generating hydrodynamic noise. This self-generated noise would greatly increase the complexity of the flow field around the body of the fish as it goes through a tail beat as shown by flow imaging studies (Anderson et al., 2001; Wolfgang et al., 1999). With this added complexity it is likely to be more challenging to measure distortions created by the presence of nearby objects. In comparison, the flow field is closer to a steady state when the fish is gliding, which is likely to make it easier to detect any changes.

Tactile wall following

Contact with the wall was commonly observed as the fish swam parallel to the wall. Contact was often made with the pectoral fin but occasionally also with the side of the nose or the caudal fin. This has not been described in previous studies but is likely to be due to the difficulty in observing the contact rather than its absence. Blind cave fish swim rapidly in close proximity to a wall. It requires imaging with high spatial and temporal resolution with good contrast to be able to see the brief contact between the edge of the transparent pectoral fin and the surface of the wall. It has been noted

in a number of previous studies (John, 1957; Teyke, 1985; von Campenhausen et al., 1981) that it is difficult to see whether the fish make contact with the wall, even in the more obvious case of a head-on approach to the wall. Frequent tactile contact with surfaces was noted by Baker and Montgomery while studying the rheotactic behaviour of blind cave fish (Baker and Montgomery, 1999). Fish were observed to make tactile contact with a surface at least once every 5 s with either a fin or part of the head. John noted that fish make frequent contact with tank walls as part of their normal behaviour and do not seem averse to this contact (John, 1957). Tactile contact does not appear to be limited to the exploration of novel environments as it is also commonly seen in the fish's normal holding tanks (S.P.W., personal observation).

The frequent occurrence of tactile contact between the fish's pectoral fin and the wall makes it difficult to attribute the fish's ability to follow parallel to a surface to information gathered through a single sensory system. It is highly likely that the fish are using both tactile and hydrodynamic information to follow along the wall. As such it would be misleading to measure the distance that the fish maintains parallel to the wall and use this as a measure of the effective range of hydrodynamic imaging in this situation. There were two trials where the fish could have been relying solely on tactile contact with the wall for guidance, but it was common for the other nine fish to complete a tail beat and glide cycle and not make any contact with the wall, yet still follow alongside the wall at a relatively constant distance. The fish maintained a mean distance of 4.7±0.5 mm when gliding parallel to the wall without tactile contact. However, the pectoral fin of the fish is long enough that the fish could touch the wall with its fin at this distance when extended, as often happened at the beginning of a tail beat. Overall, it seems likely that the fish use a combination of inputs from their different sensory systems to follow along a wall. In the head-on trials the fish responded to the presence of the wall before contact was made, so here it seems reasonable to use response distance as a measure of the effective range of hydrodynamic imaging. Additional experiments to test the relative contributions of tactile and hydrodynamic information to wall-following behaviour are necessary to further clarify the contribution of the different sensory systems.

Sensory implications of kinematics

The swimming kinematics of blind cave fish should have a direct impact on the information they will be able to collect about their surroundings. The motion of the fish is what sets up the flow field around the fish's body and it is the distortion of this flow field by nearby objects that is measured by the lateral line to build up an image of the fish's surroundings. The fish showed a distinct tail beat and glide mode of swimming in all trials. This mode of swimming is not unusual in fish but has some interesting

Table 5. Tail beat parameters of parallel trials

Tail beat type	Beat duration (s)	Fish velocity (mm s ⁻¹)	Turn angle (deg.)
Single	0.13±0.004	82±6	8.5±0.7
Double	0.19±0.008	126±5	6.5±0.3
Difference	0.06±0.005	-45±3	1.9±0.6
P value	<0.0001 (-11.12)	<0.0001 (-16.41)	0.008 (3.32)

Tail beat parameters of swimming behaviour of blind cave fish from all parallel trials (*N*=11). In single tail beats the tail only moved to one side of the fish's body; in double tail beats the tail moved to both sides. Turn angle is the angle by which the orientation of the fish changed over the tail beat. Values are means of medians for each trial ±s.e.m. *P* values are based on two-tailed paired *t*-tests for difference of means. Values in parentheses are *t* values. Number of tail beat–glide sequences measured for each individual fish ranged from 1143 to 2433 measurements with a mean of 1777.

implications when considered in the case of blind cave fish. There have been a number of advantages suggested for intermittent swimming in sighted fish, including energetic savings and perceptual benefits from reducing the complexity of the motion of the visual field (for a review, see Kramer and McLaughlin, 2001). It has been found that intermittent swimming could considerably reduce energy costs when forward motion continues during pauses in locomotion (Weihs, 1974; Wu et al., 2007). This could be important in the case of blind cave fish, which normally remain in constant motion. Ceasing swimming would prevent blind cave fish using hydrodynamic imaging to sense their surroundings. A second potential benefit of this mode of swimming is the reduction of self-generated noise created by the fish's own tail beating as mentioned above. It is also likely that there will be no efferent inhibition of the lateral line during gliding as noted previously. Therefore the optimal conditions for hydrodynamic imaging are likely to occur when the fish is gliding with its body held straight.

Kinematics of wall following

Blind cave fish clearly showed different swimming kinematics when swimming parallel to a wall when compared with swimming in the middle of the tank. When swimming parallel to a wall the fish swam and glided significantly faster. Looking at the other kinematic parameters measured, the increased velocity appears to be due to the increased proportion of double tail beats and the decreased duration of the glides between tail beats. Double tail beats produced a faster swimming velocity than single tail beats, therefore the average velocity of the fish increased as the relative proportion of double to single tail beats increased. The fish also glided for a shorter time, reducing the velocity decrease between tail beats. The use of incomplete cycles of tail beating during intermittent swimming has also been recorded by Wu and colleagues in koi carp (Wu et al., 2007). They found that single tail beats correlated with a substantial change in orientation of the fish ($15.3 \pm 7.8^\circ$) while the double tail beats did not change the direction of movement visibly ($3.0 \pm 1.8^\circ$). They also found that there was no significant difference in the velocity of the fish when using the two different tail beat modes. This is in contrast with our results, where on average the fish's direction of movement changed by $8.5 \pm 0.7^\circ$ for single tail beats and $6.5 \pm 0.3^\circ$ for double tail beats. There was also a highly significant difference ($t_{10} = -16.41$, $P < 0.0001$) in the velocity of the fish when using the different swimming modes, with the fish swimming 55% faster when using double tail beats. This suggests that the blind cave fish are using these two swimming modes in a different way from the koi carp.

The differences in the cave fish's swimming kinematics when swimming beside a wall and when in the middle of the tank could be interpreted in a number of ways. In addition to the increase in velocity, reduced glide duration and an increase in the proportion of double tail beats when beside the wall, the cave fish also showed a number of other changes to their swimming kinematics. These included a clear preference to beat their tails away from the wall when using single tail beats and sometimes rolling their dorsal surfaces away from the wall. The increased swimming velocity could serve to increase the magnitude of the stimulus to the lateral line or could simply be an effect of the fish tail beating more frequently in order to have more frequent tactile contact with the wall. Investigation of the hydrodynamic signal available to the fish and how this scales with velocity is required to explore this further.

This study measured the effective range of hydrodynamic imaging and tested whether increased swimming velocity enhanced this range. Our results do not support the hypothesis that increased

velocity increases the effective range of hydrodynamic imaging and show that hydrodynamic imaging is a short-range sense and that blind cave fish could also use tactile information to sense their surroundings. It was also shown that blind cave fish systematically change their swimming kinematics when swimming parallel to surfaces.

The authors would like to thank Dr Mat Pawley for assistance with statistics. This work was funded by a Tertiary Education Commission Top Achiever Doctoral Scholarship and a University of Auckland Doctoral Scholarship to S.P.W.

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