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RESEARCH ARTICLE

Feeding in the dark: lateral-line-mediated prey detection in the peacock cichlid *Aulonocara stuartgranti*

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SUMMARY

The cranial lateral line canal system of teleost fishes is morphologically diverse and is characterized by four patterns. One of these, widened lateral line canals, has evolved convergently in a wide range of teleosts, including the Lake Malawi peacock cichlids (*Aulonocara*), and has been attributed to its role in prey detection. The ability to study *Aulonocara* in the laboratory provides an opportunity to test the hypothesis that their reported ability to feed on invertebrate prey living in sandy substrates in their natural habitat is the result of lateral-line-mediated prey detection. The goal of this study was to determine whether *Aulonocara stuartgranti* could detect hydrodynamic stimuli generated by tethered brine shrimp (visualized using digital particle image velocimetry) under light and dark conditions, with and without treatment with cobalt chloride, which is known to temporarily inactivate the lateral line system. Fish were presented with six pairs of tethered live and dead adult brine shrimp and feeding behavior was recorded with HD digital video. Results demonstrate that *A. stuartgranti*: (1) uses the same swimming/feeding strategy as they do in the field; (2) detects and consumes invertebrate prey in the dark using its lateral line system; (3) alters prey detection behavior when feeding on the same prey under light and dark conditions, suggesting the involvement of multiple sensory modalities; and (4) after treatment with cobalt chloride, exhibits a reduction in their ability to detect hydrodynamic stimuli produced by prey, especially in the dark, thus demonstrating the role of the lateral line system in prey detection.

Key words: cichlid, lateral line, neuromast, prey detection.

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INTRODUCTION

Vision mediates prey detection in most fishes (Blaxter, 1988; Guthrie and Muntz, 1993; Evans, 2004), but the non-visual modalities, including the lateral line system, also play a crucial role. This is especially important in fishes feeding in light-limited environments (Holland, 1978; Hara, 1993; MacIver et al., 2001; Bergstrom and Mensinger, 2009; reviewed by Webb et al., 2008). However, fishes rarely rely on input from only one sensory system and can modulate their responses to cues using several sensory modalities depending on behavioral demands and the sensory environment in which they are foraging (von der Emde and Bleckmann, 1998; New, 2002; Gardiner and Atema, 2007; Bassett and Montgomery, 2011). Nevertheless, the presence of morphological and physiological specializations of individual sensory systems (e.g. Livingston, 1987; Schwarz et al., 2011) is traditionally used as an indication of the enhancement of behavioral capabilities. This correlation provides evidence for adaptive evolution, but the link between sensory morphology and prey detection behavior is not always clear.

The mechanosensory lateral line system of fishes is composed of a spatial array of neuromast receptor organs that is used to detect unidirectional and oscillatory water flows in a diversity of behavioral contexts, including prey detection (Coombs and Montgomery, 1999; Coombs and Van Netten, 2006; reviewed in Webb et al., 2008). In addition to having superficial neuromasts on the skin of the head, trunk and tail, bony fishes have a series of pored cranial lateral line canals associated with dermal bones on the head. They also tend to have one trunk canal contained in the lateral line scales that travels down the body to the caudal fin (Webb, 1989). Among teleost fishes, there are four cranial lateral line canal patterns: narrow, branched, widened and reduced (Webb, 1989). Most teleosts have narrow canals (which are well ossified with small pores and canal neuromasts), but widened canals (characterized by weak ossification of the canal roof, large pores and large canal neuromasts) are found only in a small number of fish families (Webb, 1989).

The convergent evolution of widened lateral line canals in diverse teleost taxa that tend to be benthic and/or feed on active prey in hydrodynamically quiet or light-limited environments (Coombs et al., 1988; Janssen, 1997) has been used to suggest that widened canals are an adaptation for prey detection. Several fishes are thought to use their widened canals to sense prey in the water column [e.g. melamphaeids and other mesopelagic species (Marshall, 1996)] and either on or in a sandy or muddy substrate [e.g. witch flounder, *Glyptocephalus zachirus* (Webb, 1995), silverjaw minnow, *Notropis buccatus* (Reno, 1966; Reno, 1971; Wallace, 1976), Eurasian ruffe, *Gymnocephalus cernuus* (Janssen, 1997)]. The ruffe, a freshwater percid with widened lateral line canals (Denton and Gray, 1989; Gray and Best, 1989; Janssen, 1997; Ćurčić-Blake and van Netten, 2006), is an invasive species in North American waters (Ogle et al., 1995) that has been used to explore the role of the lateral line canal system in prey detection. In the laboratory, the ruffe has been shown to detect free-swimming prey (*Daphnia*) and tethered tube-dwelling prey (mayfly larvae) in the

dark, during the glide phase of a thrust and glide swimming strategy at distances of <1 body length (Janssen, 1997). Behavioral and ecological comparisons of the exotic ruffe (widened canals) and native yellow perch (narrow canals) in the field have shown that these two species feed on similar crustacean prey throughout their lives (Bergman, 1991). However, ruffe tend to feed at night [reducing competitive interference from perch (Schleuter and Eckmann, 2006)], and are able to increase in abundance in reduced light conditions (Bergman, 1991). Theoretical and experimental work has shown that widened canals are most sensitive to lower frequencies (<60 Hz), like those produced by crustacean prey, and tend to be more sensitive, but respond more slowly (due to canal resonance), than narrow canals (Denton and Grey, 1988; Denton and Grey, 1989; van Netten and van Maarseveen, 1994; reviewed by Coombs et al., 1992). Thus, it is reasonable to hypothesize that the evolution of widened canals among diverse teleost taxa is considered to be an adaptation for prey detection, especially in low-noise environments in which fish may successfully compete or exploit novel or underappreciated trophic niches.

Cichlid fishes (Perciformes, Cichlidae) possess an impressive range of feeding morphologies and feeding strategies, a hallmark of the process of explosive adaptive radiation for which they are so well known (Fryer and Iles, 1972; Albertson et al., 2005). Their visual system is well studied (Carleton et al., 2006; O'Quin et al., 2011) and is considered to be critical for communication and sexual selection (Fernald and Liebman, 1980; Seehausen and van Alphen, 1998; Couldridge and Alexander, 2002; Seehausen et al., 2008). The sensory basis for prey search and detection behavior has not been well established in cichlids, but given the importance of vision in other aspects of their behavior, it is not surprising that cichlids are generally considered to be visual feeders (Fryer and Iles, 1972).

Like most teleost fishes, the vast majority of cichlids have narrow lateral line canals [as illustrated in the classic studies of Greenwood (Greenwood, 1981) and Trewavas (Trewavas, 1983)], but a few genera endemic to Lake Tanganyika (e.g. *Trematocara* and *Aulonocranus*) and Lake Malawi (*Aulonocara*, *Alticorpus* and *Trematocranus*) have widened canal systems (Konings, 2007). The 16–21 species of peacock cichlids of the genus *Aulonocara* [one of 56 endemic cichlid genera in Lake Malawi (Meyer et al., 1987; Konings, 1990; Konings, 2007)] have been described as 'sonar feeders' (Konings, 2007), that have 'deep pits' (Fryer, 1959), 'an acoustic system on the enlarged suborbital bones which consists of swollen pit organs' (Meyer et al., 1987), 'enlarged cephalic pores' (Konings, 1990), or an 'enlarged lateral line system…visible externally as pits and grooves especially on the lower part of the head' (Konings, 2007) (Fig. 1). *Aulonocara* is also reported to employ an unusual feeding strategy in the field in which they swim just a few millimeters above the sandy sediment and strike at invertebrate prey buried in the substrate. Observations of this behavior have been the basis for the assertion that the obvious widened lateral line canals on the lower jaw are used for prey detection by these fishes (Fryer, 1959; Konings, 1990; Konings, 2007). However, experimental evaluation of this assertion has been lacking.

The goal of this study was to test the hypothesis that *A. stuartgranti* uses the lateral line system to detect hydrodynamic stimuli generated by live, benthic invertebrate prey. Behavioral trials were carried out in which fish were presented with live (mobile) and dead (immobile) prey under light and dark conditions (Experiment I). The role of the lateral line system in prey detection was then determined by treating fish with cobalt chloride, which is known to temporarily inactivate the lateral line system (Experiment II). Knowledge of how the widened lateral line canal system is used in prey detection and its role in crepuscular or nocturnal feeding (not currently known in these fishes) would add a new dimension to our understanding of the ecology and evolution of this genus and of cichlid fishes more generally.

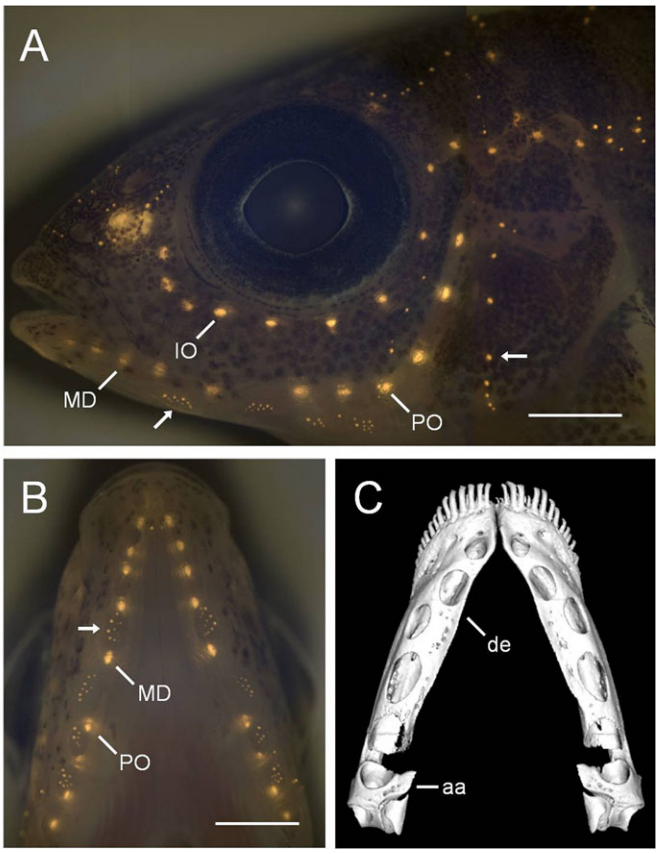

Fig. 1. The mechanosensory lateral line system of *Aulonocara* spp. (A) Juvenile *A. stuartgranti* [standard length (SL)=28 mm] stained with DASPEI (0.01%, for 30 min) to reveal lines and clusters (arrows) of small superficial neuromasts and lines of larger canal neuromasts, which are enclosed in canals: the infraorbital canal (below the eye; IO), the mandibular canal (in the lower jaw; MD), which continues as the curved preopercular canal (PO), which extends dorsally to its junction with the infraorbital and otic canals, which then continue caudally. The supraorbital canal (above the eye) is not visible in this image. The olfactory organ (rostral to the eye) is stained intensely by DASPEI and is seen through the skin and single naris. (B) Ventral view of the fish shown in A showing large canal neuromasts in the mandibular canal (five neuromasts) and in the ventral portion of the preopercular canal (four neuromasts), and clusters (arrow) of small superficial neuromasts in the skin (pierced by small pores, not visible) overlying the large bony pores of the canal. (C) MicroCT image of the isolated mandible [dentary (de) and anguloarticular (aa) bones] showing the large bony pores in the mandibular canal of an adult fish (*A. baenschi*, SL=87 mm). Scale bars, 2.0 mm.

MATERIALS AND METHODS

Adult *Aulonocara stuartgranti* Meyer and Riehl 1985 were acquired from commercial suppliers (Bluegrass Aquatics, Louisville, KY, USA) and housed in 1901 aquaria at 26±1°C and 1.0±0.2 p.p.t. salinity (using Cichlid Lake Salt, Seachem Laboratories, Inc., Madison, GA, USA) with appropriate mechanical and biological filtration. Fish were fed cichlid pellets (New Life Spectrum Cichlid Formula, New Life International, Inc., Homestead, FL, USA) one

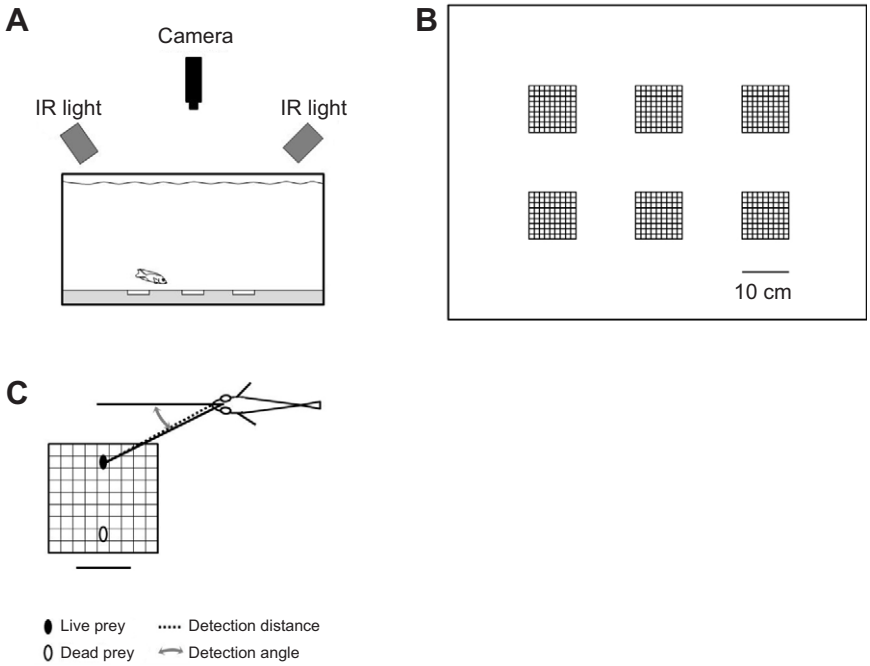

Fig. 2. Quantification of feeding behavior of *Aulonocara stuartgranti*. (A) Diagram showing the experimental setup used to record feeding behavior on tethered brine shrimp. (B) Camera view of experimental arena. (C) Illustration of tethering dish indicating positions of live (black oval) and dead (white oval) prey. The lines connecting the fish to the live prey represent detection distance (dashed) and angle (solid).

to two times daily and supplemented with live adult brine shrimp. Fish were provided with standard white fluorescent light on a 12 h:12 h diurnal cycle (lights on 07:00–19:00 h). Individual fish were not used in feeding experiments if breeding behavior was observed. Animal care and all experimental procedures followed an approved University of Rhode Island IACUC protocol.

Behavioral experiments

Behavioral trials (in Experiments I and II) were conducted in an experimental tank (120×90×60 cm; 375 l) lined with light colored sand (Aragamax Sand, CaribSea, Fort Pierce, FL, USA) over quartz gravel, intended to mimic the sandy substrate of the fishes' natural habitat in which they feed in Lake Malawi (Fig. 2A). Two methods were used to present live and dead (freshly frozen) adult brine shrimp (*Artemia*) to individual fish. Brine shrimp were attached with aquarium-grade silicone to the back of 8.5 cm diameter glass Petri dishes. Alternatively, brine shrimp were attached to square platforms (10×10 cm) made of plastic egg crate louver covered with a fine plastic mesh with elastic thread (1 mm diameter) woven through the mesh. The first three fish in Experiment I were presented with prey tethered with silicone to glass Petri dishes, whereas all other fish in Experiments I and II were presented with prey tethered to mesh platforms. Brine shrimp were secured to the platform by positioning them ventral side up and placing the elastic thread over their abdomen, allowing the brine shrimp to freely move their appendages, which generated a hydrodynamic stimulus that was visualized using digital particle image velocimetry (DPIV; see below). To measure the frequency of hydrodynamic stimuli generated by brine shrimp tethered to platforms, movements of brine shrimp appendages were recorded using an HD digital video camera (Sony HDR-CX550V; 30 frames s^{-1}) under light (N=3) and dark (N=3) conditions. Beat rate (beats s^{-1}) was calculated at 0, 10, 20 and 30 min for each individual, and parametric statistics (data were normally distributed) were used to compare beat frequency under light *vs* dark conditions (Student's t-test) at the beginning and end of a 30 min period (paired t-test), and at 0, 10, 20 and 30 min (ANOVA).

One or two fish were allowed to acclimate to the experimental tank for at least 24 h and food was withheld for 24 h before a behavioral trial. When two fish were in the experimental tank, they were separated from one another at all times. Fish were placed behind opaque dividers during the setup of a trial and the process of tethering the brine shrimp was carried out in a separate water-filled container. One live and one dead brine shrimp were positioned on opposite sides of each dish or platform, approximately 7 cm apart. Six dishes or platforms were then gently lowered into the experimental tank and arranged in a 2×3 grid flush with the surface of the sandy substrate (Fig. 2B). The relative placement of the live and dead prey on all six dishes or platforms was the same in a trial. To avoid spatial learning, all dishes or platforms were rotated 90 deg in sequential trials in Experiment I, but this was not done in Experiment II, after it became apparent that spatial learning was not an issue. Observations confirmed that brine shrimp remained tethered (and alive) for more than 30 min when fish were not present.

Immediately following the placement of the six dishes or platforms, one fish was released into the experimental arena from behind an opaque divider. Feeding behavior was recorded for 30 min using either a standard (Sony Handycam DCR-HC65-NTSC, 30 frames s^{-1}) or an HD (Sony HDR-CX550V, 30 frames s^{-1}) digital video camera mounted directly above the tank with a vertical view of the entire experimental arena. Light trials were carried out under standard white fluorescent illumination and dark trials were carried out in complete darkness with infrared illumination (840 nm; Speco Provideo, IR-200/24, Amityville, NY, USA), which is out of the visible range of these fishes (Carleton, 2009). Day trials were carried out between 10:00 and 18:00 h and dark trials began shortly after lights went off at 19:00 h. All water pumps and filtration systems in the experimental tank were turned off prior to the start of a trial to eliminate acoustic and hydrodynamic noise.

Two experiments, involving a total of 39 light and 39 dark trials, were carried out over a period of 20 months using 13 different fish [total length (TL)=6.2–12.5 cm; only one fish was used in Experiment I and then in Experiment II]. In Experiment I, normal feeding behavior was recorded for each of six fish (TL=6.2–10.1 cm; two females and four males) in three light and three dark trials (six trials per fish). All trials were carried out in the same sequence (three light trials then three dark trials), all on separate days; the mean

time between the first light trial and last dark trial was 47 days. At the end of each trial, all prey remaining on the tethering dishes or platforms were counted and live prey were confirmed to be alive. Strike success was confirmed in video recordings. One additional light and one additional dark trial were carried out using each of two fish and recorded in lateral view to examine the vertical position of the fish in the water column relative to the substrate during the course of a 30 min trial.

Video sequences leading to individual prey strikes were cut from each 30 min video using Adobe Premier Pro (v.2.0 or CS5, Adobe Systems, San Jose, CA, USA). All sequences were viewed to identify when detections occurred relative to the start of the trial and during which phase of swimming behavior (thrust, glide or pause) prey was detected. These phases were defined as: thrusts (quick accelerations generated by the beating of the caudal fin), glides (characterized by a decrease in swimming velocity, varied in duration, and may have included a left or right maneuver), and pauses (a lack of forward movement when a fish was stationary, with pectoral fins extended). No strikes occurred during a thrust, so data are recorded as a percentage of total strikes occurring during either a glide or a pause. Detection distance and detection angle were measured in still images exported from behavioral sequences using ImageJ (v.1.41o, National Institutes of Health, Bethesda, MD, USA). Detection distance was defined as the distance from the tip of a fish's mouth to the prey, in the frame immediately before the fish oriented towards it (e.g. before a turn or swimming reversal; Fig. 2C). Detection angle was defined as the angle between the prey and the midpoint between the fish's eyes, with reference to the long axis of the fish's body, in the same captured frame in which detection distance was determined.

In Experiment II, the role of the lateral line system in prey detection was demonstrated by treating the fish with cobalt (II) chloride heptahydrate (cobalt chloride; Sigma-Aldrich, St Louis, MO, USA) to temporarily inactivate the lateral line system (Karlsen and Sand, 1987). At the time of the planning of these experiments, it was still thought that aminoglycoside antibiotics deactivated only canal neuromasts but not superficial neuromasts (e.g. Song et al., 1995); however, recent studies have now demonstrated that these antibiotics ablate all neuromasts (Van Trump et al., 2010; Brown et al., 2011). Cobalt chloride was chosen for the present study because it was known to deactivate both superficial and canal neuromasts, and shorter exposures and lower doses of cobalt chloride have been shown to have little or no side effects (Karlsen and Sand, 1987). Each of seven fish (TL=8.2–12.5 cm; five females and two males) was run through one light and one dark 'pre-cobalt' trial (the same protocol as Experiment I) on the same day. Then, within 2–7 days, each fish was treated with cobalt chloride (0.1 mmol l^{-1} in conditioned tap water) for 3 h, after which it was returned to the experimental tank. A light trial with cobalt treatment ('cobalt trial') commenced only after a fish appeared to be behaving normally, which was indicated by normal respiration rate and swimming (this occurred 2–3 h after cobalt treatment). A dark trial was then carried out 3–4 h later, shortly after the overhead lights went off. All fish resumed feeding on commercial pellets and/or brine shrimp immediately following cobalt dark trials. After 21–28 days, each fish was then run through one light and one dark 'post-cobalt' trial to assess recovery. All light trials were carried out during the day (11:30–17:30 h) and all dark trials were started within 1 h after the overhead lights went off (19:00–20:00 h). The effect of cobalt chloride has been shown to begin wearing off within hours of a fish being placed in water containing calcium (Karlsen and Sand, 1987), so light and dark trials were completed within a few hours of each other. All fish were observed to eat more than 24 brine shrimp in one day during routine feeding, so fish in Experiment II could not have been satiated by the end of each light trial, in which only 12 brine shrimp were presented. In addition, fish were starved for 24 h before each set of trials. To determine whether feeding behavior was altered by handling during cobalt treatment, each of two fish were run through a light and dark trial (=normal trial) followed by 3 h immersion in conditioned tap water in the same type of container used for cobalt treatment. Then, a light and a dark trial (=cobalt sham trial) were carried out as in Experiment II. All video analysis was carried out as described for Experiment I.

Statistical analysis

The number of prey strikes, detection distance, detection angle, time to first detection and order of prey capture (live *vs* dead) were tested with various statistical tests to find significant differences among prey (live or dead) and trial type (light and dark; pre-cobalt, cobalt and post-cobalt) using SPSS (v.19, IBM, Armonk, NY, USA) or Oriana (v.3, Kovach Computing Services, Anglesey, UK; detection angles only). All data were tested for normality using the Kolmogorov–Smirnov test. A generalized linear mixed model (GLMM) was used to analyze the number of prey strikes and detection distance in Experiments I and II. This approach allowed the selection of random (individual) and fixed effects (light *vs* dark, live *vs* dead prey, treatment type) while addressing repeated measures for the same individual. However, a repeated-measures model (GLM repeated measures) was not appropriate because the data were not balanced (e.g. if prey were not consumed, detection distance could not be recorded). For analysis of detection distance in Experiment I, data were $\log_{10}$-transformed to achieve normality, which is appropriate for a GLMM analysis. Time to first detection was analyzed using univariate ANOVA in both Experiments I and II. Prey preference was calculated following a method described in Taplin (Taplin, 2007). Briefly, Taplin's analysis involves determining prey preference by ranking the prey by the order in which they were consumed, and then calculating a preference score by taking the mean of the order values for each prey type. Assumptions for this analysis include that multiple types of prey must be offered simultaneously (e.g. live and dead tethered brine shrimp) and prey consumed last cannot be distinguished from uneaten prey. Scores closer to one indicate a strong preference, whereas scores closer to 12 (=total number of prey offered) indicate no preference or rejection. Preference scores for live or dead prey in each trial type (light and dark; pre-cobalt, cobalt and post-cobalt) were compared using paired *t*-tests. In Experiment I, means of prey preference scores from the three replicate trials carried out for each fish were calculated prior to carrying out the paired *t*-test, so that the replicate variable was the fish (individual) and not the trial. All tests were considered significant at $P<0.05$. Values are given as means ± s.e.m. unless otherwise specified.

Digital particle image velocimetry

The hydrodynamic stimulus generated by adult brine shrimp (N=4, tethered to a mesh platform as described above) was visualized and quantified using DPIV. A tethered brine shrimp was placed in a 191 tank seeded with silver coated, near neutrally buoyant, reflective particles at a density of 0.1 g l^{-1} (12–14 μm diameter; Potters Industries, Inc., Parsipanny, NJ, USA). A light beam from a continuous 5 W argon-ion laser was focused into a 2-mm-thick and 10-cm-wide vertical sheet that illuminated the brine shrimp along its midline. A high-speed, high-resolution (1024×512 pixels) Photron APX camera (Photron USA, San Diego, CA, USA) was

positioned perpendicular to the laser sheet to record brine shrimp and particle movement at 60 frames s^{-1}. Images were processed using DaVis 7.0 software (LaVision, Goettingen, Germany) using sequential cross-correlation without pre-processing. A mask was added to exclude movements of the brine shrimp itself in order to analyze only those water movements generated by the brine shrimp. An initial correlation window of 12×12 pixels was selected using multi-pass with decreasing smaller size to a final interrogation window of 8×8 pixels with 50% overlap. All vectors above the threshold of 2 mm s^{-1} were considered to represent significant flows generated by the movements of the brine shrimp.

RESULTS

Tethered brine shrimp generated a flow that was produced by the upstroke and downstroke of their feeding and swimming appendages (Fig. 3). A weaker flow was generated by the upstroke when the appendages moved in a caudal to rostral direction (range=2–4 mm s^{-1}; Fig. 3B), compared with the stronger flow generated by the downstroke when the appendages moved in a rostral to caudal direction (range=3–7 mm s^{-1}; Fig. 3D). Little flow was observed during the pre-upstroke phase (Fig. 3A), the transition between the upstroke and downstroke (Fig. 3C) or in the post-downstroke phase (Fig. 3E) of appendage movement. Vortices were visualized ~1 cm above the abdomen (upstroke) or head (downstroke), and moved along the body axis, and were no longer visible 1 cm beyond the body. It appeared that vortices were short lived, or were shed obliquely, and were thus out of the plane of the laser sheet. Vortices were only seen in the immediate vicinity of the brine shrimp, and it is unlikely that vortices generated by more than one live brine shrimp would overlap given the spacing of the tethering platforms in feeding trials. Flow velocities appeared to vary somewhat among individual brine shrimp, which was likely a reflection of brine shrimp size (TL=7–12 mm), where larger brine shrimp generated higher flow rates.

The frequency at which tethered brine shrimp move (~2–4 beats s^{-1}) did not differ under light and dark conditions (Student's *t*-test, $P>0.05$), although it appears that in the dark the beat frequencies tended to be somewhat lower than those in the light. Overall, the frequency did not vary significantly over time under either light or dark conditions (ANOVA, $P>0.05$). However, there was no difference in beat frequency at the beginning and end of a 30 min period in the light (paired *t*-test, $P>0.05$), whereas under dark conditions the beat frequency was significantly higher at 30 min (2.4±0.5 beats s^{-1}) than at 0 min (2.1±0.5 beats s^{-1}; paired *t*-test, $P<0.05$).

Behavioral experiments

Aulonocara stuartgranti successfully fed on tethered brine shrimp in both light and dark trials. They demonstrated differences in number of prey strikes, detection distance, detection angle, prey preference and time to first detection depending on light conditions. Treatment with cobalt chloride resulted in a change in prey detection behavior, especially in the dark.

Experiment I: normal light and dark trials

In light trials, the fish swam throughout the water column, appearing to explore the tank. Upon first prey detection, the fish would swim a few millimeters above the substrate until it detected other prey, and then it would return to a more general exploration of the tank. This sequence was repeated until the end of the trial. In dark trials, the fish appeared to spend more time in the bottom half of the tank prior to first prey detection, but then similar behavior near the bottom of the tank was observed. When swimming immediately above the substrate, all fish searched for prey with a series of quick thrusts (mediated by several beats of the caudal fin) followed by glides [with decreasing swimming velocity (D.K.B. and J.F.W., unpublished data)], at the end of which they appeared to pause. Prey detection was indicated by the initiation of either a turn towards the prey or a forward glide over the prey followed by a reversal in

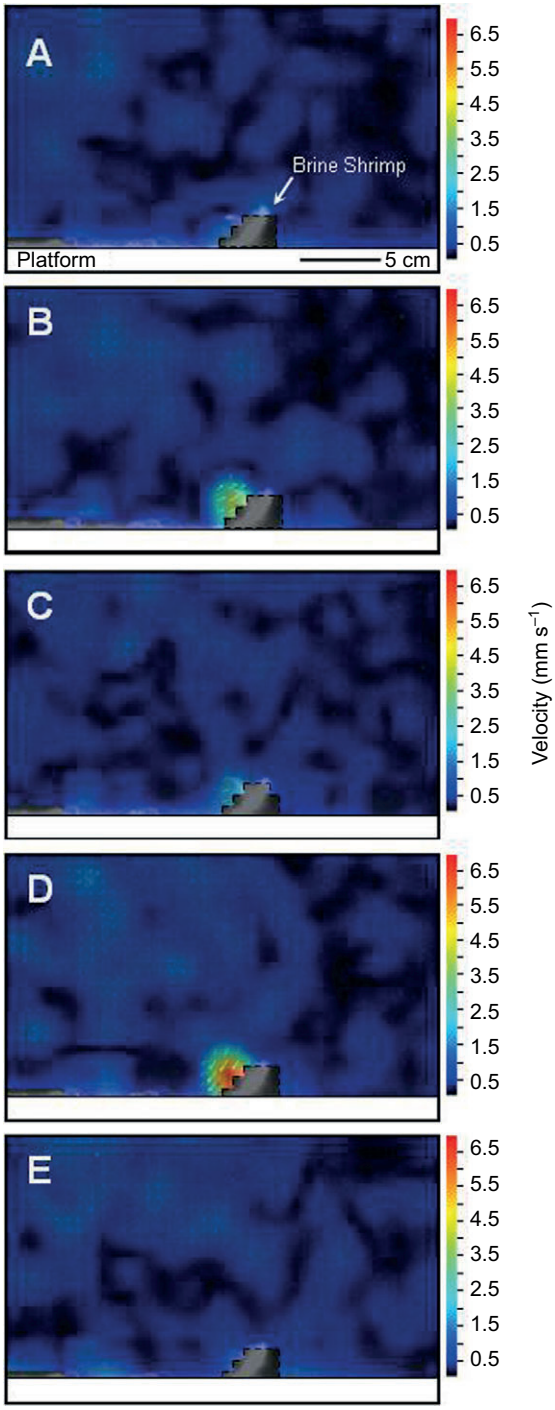

Fig. 3. Velocity vector field (arrows) and the color map of flow magnitudes (red=maximum, ~7 mm s^{-1}; dark blue=minimum) during one beat cycle (time elapsed=167 ms) above a single adult brine shrimp tethered ventral-side-up to a platform. Each box represents a phase of movement identified during a beat cycle: (A) pre-upstroke, (B) upstroke, (C) transition between upstroke and downstroke, (D) downstroke and (E) post-downstroke. The dashed box represents the masked area around the brine shrimp.

Table 1. Number and frequency (%) of prey detections leading to strikes in Experiments I (*N*=6 fish) and II (*N*=7 fish) that occur during the glide or the pause phase of swimming in *Aulonocara stuartgranti*

			Live prey		Dead prey		All prey	
			Pause	Glide	Pause	Glide	Pause	Glide
Experiment I		Light	67 (73.6%)	24 (26.4%)	52 (66.7%)	26 (33.3%)	119 (70.4%)	50 (29.6%)
		Dark	3 (7.7%)	36 (92.3%)	1 (6.7%)	14 (93.3%)	3 (7.4%)	50 (92.6%)
Experiment II	Pre-cobalt	Light	32 (76.2%)	10 (23.8%)	21 (55.3%)	17 (44.7%)	53 (66.3%)	27 (33.7%)
		Dark	2 (12.5%)	14 (87.5%)	1 (33.3%)	2 (66.7%)	3 (15.8%)	16 (84.2%)
	Cobalt	Light	15 (78.9%)	4 (21.1%)	13 (72.2%)	5 (27.8%)	28 (75.7%)	9 (24.3%)
		Dark	0	0	0	0	0	0
	Post-cobalt	Light	25 (73.5%)	9 (26.5%)	18 (51.4%)	17 (48.6%)	43 (62.3%)	26 (37.1%)
		Dark	1 (12.5%)	7 (87.5%)	0 (0%)	4 (100%)	1 (8.3%)	11 (91.7%)

Percentages were calculated for live prey (*N*=6), dead prey (*N*=6) or all prey (live + dead, *N*=12) for each type of trial (light, *N*=3; dark, *N*=3).

swimming direction, which was facilitated by pectoral fin movements (observed in dark trials only). In light trials, *A. stuartgranti* tended to detect prey during a pause (70.4% of the time; Table 1), whereas in dark trials more prey were detected during a glide (92.6% of the time).

Prey detection was defined by an approach ending in a strike. In the event of a miss (as visualized in video), only the first strike on that prey was included in data analysis. No differences were detected in number of prey strikes, detection distance or detection angle among the three replicate light or dark trials for an individual (GLMM, $P>0.05$). Furthermore, the two tethering methods used did not influence number of prey strikes (GLMM, $P>0.05$) or detection distance (GLMM, $P>0.05$), but mean detection angle differed in light trials only (Watson's U^2-test, $U^2=0.24$, $P=0.02$), suggesting a difference in visual cues associated with tethering method. Interestingly, fish detected more prey tethered to Petri dishes with an approach to their right side (mean angle=20.0±6.3 deg) and detected more prey tethered to mesh platforms with an approach to their left side (mean angle=351.6±3.7 deg, i.e. a mean of 9.4 deg to the left).

All individuals successfully struck at and consumed prey in both light and dark trials. There were more prey strikes in light trials than in dark trials (GLMM, $F=129.98$, $P<0.001$; Fig. 4A), but in the dark trials there were more strikes on live prey than dead prey (GLMM, $F=7.36$, $P<0.01$). Furthermore, strikes on live prey preceded strikes on dead prey in both light and dark trials (paired *t*-test; light, $t=5.55$, $P<0.01$; dark, $t=5.23$, $P<0.01$; Table 2). Mean detection distance for strikes on both types of prey in light trials was twice as long as that in dark trials (GLMM, $F=71.10$, $P<0.001$; Table 3). Mean prey detection angle was significantly different in light and dark trials (Watson's U^2-test, $U^2=0.33$, $P<0.005$) and at detection, prey were not distributed uniformly around the fish (Rayleigh test; light, $Z=119.96$, $P<0.001$; dark, $Z=11.75$, $P<0.001$; Fig. 5A). No differences were found in prey detection angle for live

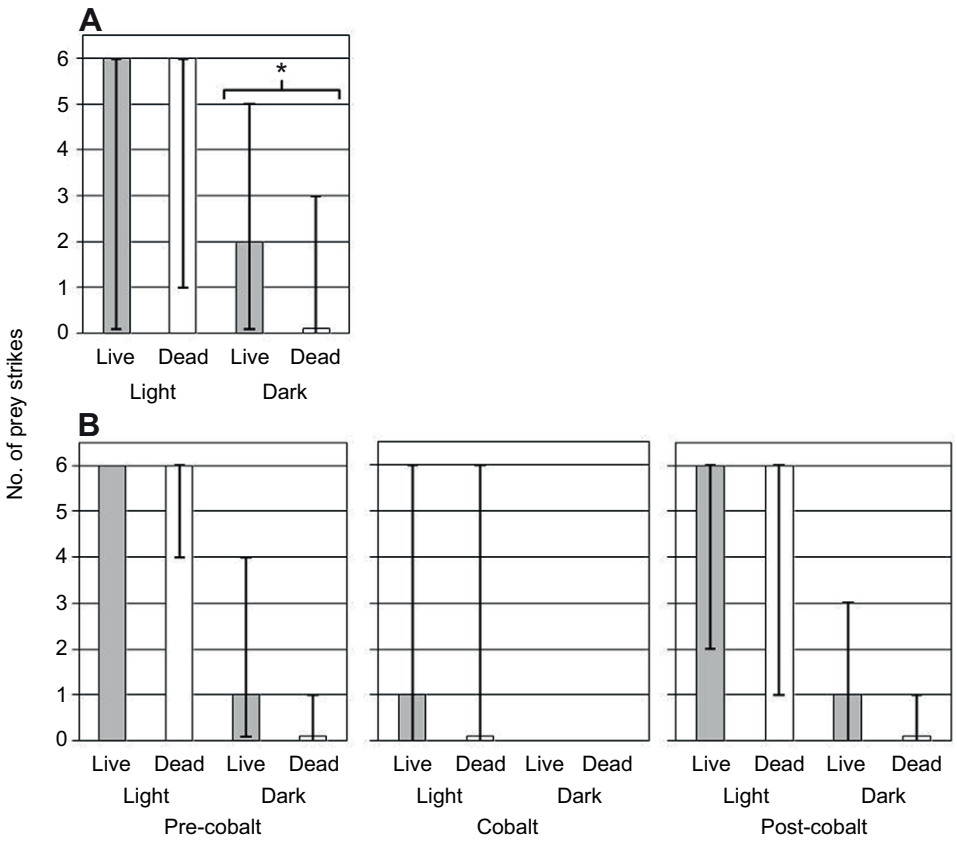

Fig. 4. Number of strikes (median ± min./max.) on live (*N*=6) and dead (*N*=6) brine shrimp in (A) Experiment I and (B) Experiment II. The asterisk indicates a significant difference between number of strikes on live and dead prey ($P<0.05$).

Table 2. Mean prey preference scores for live (*N*=6) or dead (*N*=6) prey in light (*N*=3) and dark (*N*=3) trials in Experiments I (*N*=6 fish) and II (*N*=7 fish) following Taplin (Taplin, 2007)

		Light trials		Dark trials	
		Live	Dead	Live	Dead
Experiment I		5.49*	7.51	5.74*	7.26
Experiment II	Pre-cobalt	5.12*	7.88	5.93	7.07
	Cobalt	6.45	6.55	No strikes	No strikes
	Post-cobalt	6.29	6.71	6.19*	6.82

If the fish demonstrated a preference for a type of prey (indicated by a significantly lower preference score), it was always for live prey (*P<0.05).

vs dead prey in light or dark trials (Watson's U^2-test, P>0.05). In light trials, most prey (e.g. live and dead) were detected in front of the fish (anterior 180 deg) rather than being detected around and directly behind the head in dark trials. Although the average time to first detection in dark trials was twice that in light trials, there was no statistically significant difference (univariate ANOVA, P>0.05; Table 4) because of variability among trials.

Experiment II: lateral line ablation trials

Significant differences were found among the variables measured (number of prey strikes, detection distance and detection angle) during the different treatments. Specific comparisons are described below.

Results for light and dark trials prior to cobalt treatment (pre-cobalt trials) were similar to those in Experiment I. The fish detected more prey during a pause (66.3% of the time) in light trials than during a glide (84.2% of the time) in dark trials (Table 1). The number of strikes on live *vs* dead prey did not differ in either light or dark trials (GLMM, P>0.05; Fig. 4B). Although fish tended to strike first at live prey in light trials (paired t-test, t=3.77, P<0.01), preference for prey type was not evident in dark trials (paired t-test, t=1.62, P=0.16; Table 2). Mean detection distance was greater for both live and dead prey in light trials than in dark trials (GLMM, F=20.07, P<0.001; Table 3). In light trials, live prey were detected at a greater distance than dead prey (GLMM, F=10.37, P=0.002), but this was not the case in dark trials (GLMM, P>0.05, Table 3). In light trials, detection angles were not uniformly distributed around the fish (Rayleigh test, Z=32.17, P<0.001) and most prey were detected in front of the fish (anterior 180 deg), whereas in dark trials detection angles were statistically uniform (Rayleigh test, P>0.05; Fig. 5B) and prey were detected in all directions around the fish.

In light trials with cobalt treatment, only four of the seven fish demonstrated feeding behavior, even though all of them actively swam around the experimental tank. The four fish that did feed generally struck at all 12 live and dead prey (Fig. 4B), which occurred during a pause 75.7% of the time (Table 1). Both detection distance (GLMM, P>0.05) and detection angle (Watson's U^2-test, P>0.05) were similar to those in other light trials in Experiment II (Table 3, Fig. 5B). However, fish did not show a preference for live prey (paired t-test, P>0.05; Table 2), suggesting that lateral line inactivation influenced prey detection behavior. In dark trials, no strike behavior was observed among the seven fish despite the fact that they swam frequently over the tethered brine shrimp.

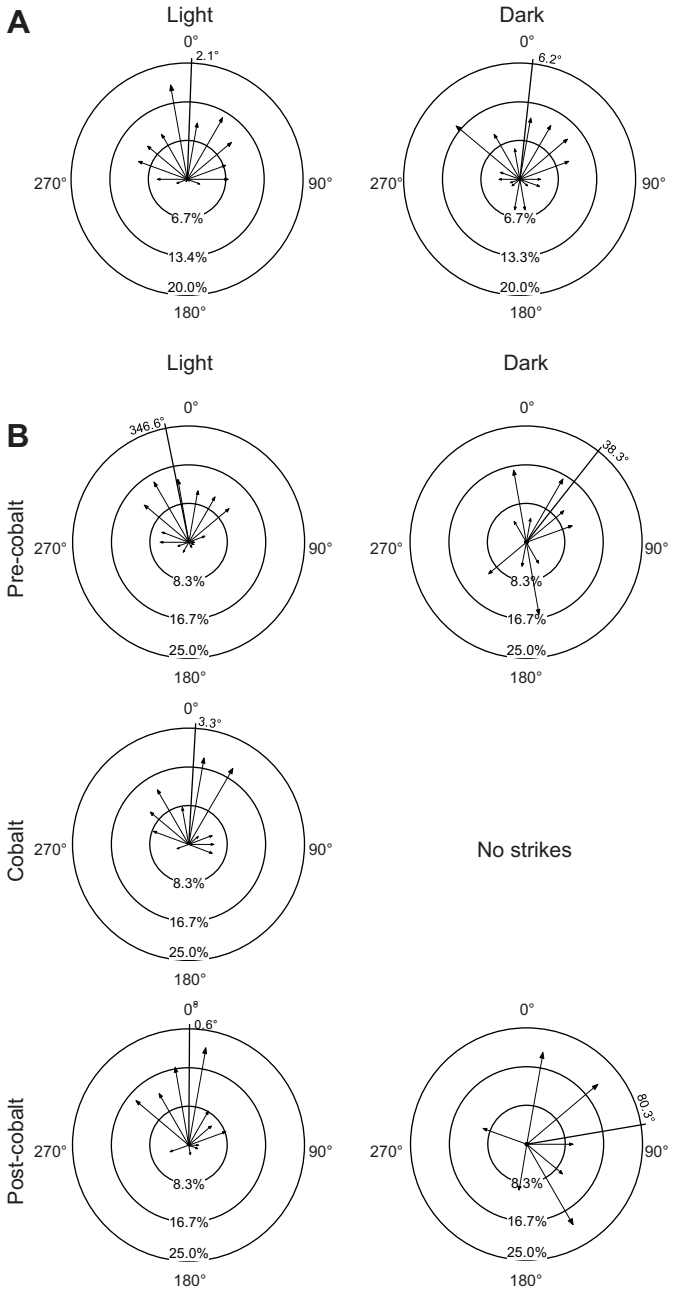

Fig. 5. Orientation to prey at time of detection in the light and dark trials of (A) Experiment I (*N*=6 fish) and (B) Experiment II (*N*=7 fish). Arrows represent the proportion of the total number of combined live and dead detection events grouped into 20 deg intervals; the line represents the mean angle. The midpoint between the eyes is the center of the polar plot, facing 0 deg.

Table 3. Detection distance (mean ± s.e.m.; cm) for live (*N*=6) and dead (*N*=6) brine shrimp prey in Experiments I (*N*=6 fish) and II (*N*=7 fish)

		Light trials		Dark trials	
		Live	Dead	Live	Dead
Experiment I		8.47±0.65	7.17±0.66	4.42±0.64	3.93±0.83
Experiment II	Pre-cobalt	9.72±0.57	7.13±0.42	3.27±0.47	2.24±0.60
	Cobalt	9.95±1.50	8.46±0.90	No strikes	No strikes
	Post-cobalt	8.41±0.74	7.63±0.38	2.91±0.60	2.05±0.52

Detection distance in light trials (*N*=3) is significantly longer than in dark trials (*N*=3; GLMM, P<0.05). The only significant difference between detection distance for live and dead prey was in pre-cobalt light trials (P<0.01).

Table 4. Mean (min.–max.) time to first detection (min) of prey (live, *N*=6; dead, *N*=6) in light (*N*=3) and dark (*N*=3) trials in Experiments I (*N*=6 fish) and II (*N*=7 fish)

		Light trials	Dark trials
Experiment I		4.2 (0.1–23.5)	8.3 (0.6–21.9)
Experiment II	Pre-cobalt	5.6 (1.0–15.9)	10.5 (0.6–29.6)
	Cobalt	9.2 (0.3–28.1)	No strikes
	Post-cobalt	6.6 (0.1–19.7)	7.8 (0.6–20.2)

Differences in time to first detection were not significantly different between light and dark trials in Experiments I and II or among treatments in Experiment II (univariate ANOVA, $P>0.05$).

In post-cobalt recovery trials, carried out 21–28 days after cobalt treatment, more strikes occurred in the light trials than in the dark trials (GLMM, $F=56.80$, $P<0.001$; Fig. 4B) and there was no difference in the number of strikes on live and dead prey in these trials (GLMM, $P>0.05$). As in pre-cobalt trials, prey tended to be detected during a pause in light trials (62.3% of the time) compared with a glide in dark trials (91.7% of the time; Table 1). Fish demonstrated longer detection distances in light *vs* dark trials (GLMM, $F=18.58$, $P<0.001$) and detection distance in light trials was similar to that in pre-cobalt and cobalt trials (GLMM, $P>0.05$; Table 3). The range of detection angles in light and dark post-cobalt trials was consistent with that in the pre-cobalt trials: most prey were detected in front of the fish (anterior 180 deg; Rayleigh test, $Z=21.84$, $P<0.001$), whereas in dark trials prey were detected in all directions around the fish (Rayleigh test, $P>0.05$; Fig. 5B). The same total strikes occurred in post-cobalt trials as in pre-cobalt trials (GLMM, $P>0.05$).

Thus, prey detection behavior appeared to be restored in post-cobalt trials, but certain aspects of behavior did not return to pre-cobalt levels. For instance, unlike pre-cobalt trials, fish struck at live and dead prey equally in post-cobalt light trials (paired *t*-test, $P>0.05$), but live prey were struck at first in dark trials (paired *t*-test, $t=2.81$, $P=0.031$). Interestingly, the same individuals used in pre-cobalt trials showed a preference for live prey in the light, but a statistically insignificant tendency to prefer live prey in dark trials (paired *t*-test, $t=1.62$, $P=0.16$; Table 2). These two results suggest that the lateral line system may not have completely recovered from cobalt chloride treatment.

Time to first prey detection was similar in all light trials in Experiment II (pre-cobalt, cobalt and post-cobalt; univariate ANOVA, $P>0.05$) and although not significant, the first prey strike appeared to occur sooner in pre- and post-cobalt light trials (within the first 1–5 min) than in dark trials (within the first 5–10 min; Table 4). In light trials with cobalt treatment, of the four fish that did strike at prey, they did so either within the first 10 min or near the end of the 30 min trial.

When two fish were immersed in conditioned tap water in the same type of container used for cobalt treatment (=cobalt sham) and run through a light and dark trial, their feeding behavior (e.g. detection distance, number of prey captured) was comparable to that in the light and dark pre-cobalt trials in Experiment II. Most importantly, both fish consumed prey during these dark cobalt sham trials. These results show that handling had no effect on feeding behavior.

DISCUSSION

This study has demonstrated that *A. stuartgranti*: (1) uses the same feeding strategy (swimming and hovering over the sandy substrate) as observed by others in the field; (2) detects and consumes live invertebrate prey in the dark; (3) alters aspects of prey detection behavior when feeding on the same prey under light and dark conditions, suggesting the importance of multimodal input in prey detection; and (4) appears to use its lateral line system to detect hydrodynamic stimuli produced by prey, especially in the dark, as demonstrated by treatment with cobalt chloride.

The ability of fishes to feed under low light conditions and nocturnally has indeed been established in many taxa [e.g. yellow perch (Richmond et al., 2004) and bluegill (Vinyard and O'Brien, 1976) (reviewed in Webb et al., 2008)]. With the results of this study, *Aulonocara* joins a relatively short list of teleost fishes in which the ability to detect prey [live or simulated (vibrating sphere)] using the lateral line system has been experimentally demonstrated [e.g. mottled sculpin (Coombs and Janssen, 1990; Coombs and Patton, 2009); yellow perch and Eurasian ruffe (Janssen, 1997); goldfish (Coombs, 1994; Engelmann et al., 2002); rainbow trout (Engelmann et al., 2002); common bully (Bassett et al., 2006); scorpionfish (Bassett et al., 2007); oscar (Mogdans and Nauroth, 2011); and bastard cod (Bassett and Montgomery, 2011; Yoshizawa et al., 2010; reviewed in Webb et al., 2008)].

The results of this study provide the first experimental evidence to support prior assertions based on field observations (Fryer, 1959; Konings, 1990; Konings, 2007) that *Aulonocara* uses its lateral line system to detect benthic prey. *Aulonocara* use a thrust, glide and pause swimming strategy, and detect prey during either a glide or subsequent pause at short detection distances (<1 body length). This is additional evidence of the need to detect hydrodynamic stimuli generated by prey against self-generated flows, especially in species with increased sensitivity provided by widened lateral line canals (Denton and Gray, 1988; Denton and Gray, 1989). The results of these experiments have illustrated the importance of the lateral line system for detection of live prey in the dark, but suggest that multimodal input appears to be necessary for robust responses to prey under light conditions. Our results shed light on the role of the non-visual senses in feeding behavior in cichlids more generally (see also Mogdans and Nauroth, 2011), and argue for the adaptive significance of the convergent evolution of widened lateral line canals among teleost fishes.

Swimming and prey detection behavior

All fish exhibited a saltatory search strategy [defined in O'Brien et al. and Bassett et al. (O'Brien et al., 1989; Bassett et al., 2007)] in light and dark trials in both Experiments I and II. Swimming behavior was defined as consisting of three phases (thrust, glide and pause), which is similar to that described for the ruffe (Janssen, 1997). The phase during which *A. stuartgranti* detected prey (pause *vs* glide) differed depending on light condition; prey tended to be detected during a pause in light trials, whereas prey tended to be detected during a glide in dark trials. The detection of prey during a pause is consistent with the use of vision because movement of the background across the visual field can make it increasingly difficult to discern prey from the background, especially when prey are cryptic. Prey detection capabilities of the lateral line system may be compromised because of self-generated hydrodynamic noise (Bassett, 2008), especially when detection sensitivity is enhanced [e.g. with widened canals (Denton and Gray, 1988; Denton and Gray, 1989)]. Thus, background noise (e.g. environmental water flow or flow generated by swimming movements) becomes even more of a challenge for prey detection. Nevertheless, prey detection in dark trials, which depends on the lateral line system, occurred more often during glides [as in ruffe (Janssen, 1997)]. During a glide in the light, a fish may move to within a distance appropriate for detection

by the lateral line system (e.g. one to two body lengths) and at a decreasing velocity, such that self-generated noise does not overwhelm lateral line input. In the dark, detection during a pause would not be effective unless the fish had already detected the prey and moved within strike range. Interestingly, *Aulonocara* swimming velocity at prey detection in the dark was approximately half that of the swimming velocity in light trials (D.K.B. and J.F.W., unpublished data).

Some benthic fishes are known to re-orient to prey after initial detection using their lateral line system [sculpins (Coombs and Conley, 1997) and gobies (Bassett et al., 2006; Bergstrom and Mensinger, 2009)]. In contrast, *Aulonocara* maintains the same trajectory towards a prey once it is detected under both light and dark conditions. However, it is interesting to note that in the dark, *Aulonocara* frequently glides over prey and then performs a 180 deg swimming reversal, which serves to position the prey under the lower jaw before striking (present study; D.K.B. and J.F.W., unpublished data). *Aulonocara* most likely uses its ventrally directed mandibular, lower preopercular and perhaps infraorbital canals (see Fig. 1B,C) to detect its benthic prey during slow glides just millimeters above the substrate.

Generally, fishes use their lateral line system to detect water flows within one to two body lengths (Kalmijn, 1988; Coombs, 1999), but benthic predators have been shown to detect and successfully capture free-swimming prey within half a body length [oyster toadfish *Opsanus tau* (Price and Mensinger, 1999; Palmer et al., 2005); mottled sculpin *Cottus bairdi* (Hoekstra and Janssen, 1986); and freshwater sculpins *Cottus* spp. and the round goby *Apollonia melanostoma* (Bergstrom and Mensinger, 2009)]. These benthic species all have narrow canals [except for the round goby, which has reduced canals (Webb, 1989)] and are either ambush predators [oyster toadfish (Phillips and Swears, 1979)] or use a saltatory search strategy [sculpins (Hoekstra and Janssen, 1985) and gobies (Bassett et al., 2007) (M.A.B.S. and A. Mensinger, unpublished observations)]. These benthic fishes detect prey in the water column while generating little if any hydrodynamic noise, which is associated with swimming. The results of the present study demonstrate that, like the ruffe (Janssen, 1997), *A. stuartgranti* can also detect prey within half a body length using its lateral line system, and thus its lateral-line-mediated detection capabilities are comparable to those of the benthic predators mentioned above.

Evidence for multimodal sensory interaction in prey detection

The results of this study are consistent with the use of lateral-line-mediated prey detection. Differences in parameters that define prey detection behavior, including prey preference (tendency to strike first at live *vs* dead prey) in light and dark trials, suggests that *A. stuartgranti* uses different combinations of sensory input depending on light conditions.

It could be argued that live brine shrimp present a stronger stimulus than dead brine shrimp because of the generation of a combination of visual, hydrodynamic, olfactory and perhaps tactile cues generated by the movement of their appendages. Thus, significant differences in the order of prey strikes (preference for live *vs* dead prey) under conditions in which different subsets of sensory modalities are available can be used to reveal the nature of sensory input that is necessary and sufficient for the initiation of prey detection behavior. For instance, results for Experiment I show a preference for live prey in both light and dark trials (Table 2). Multiple sensory modalities likely contribute to prey detection behavior during light trials, but lateral line (and olfactory and tactile) cues are sufficient to generate a preference for live prey in dark trials. In this study, it was assumed that similar olfactory cues were presented by offering live and freshly dead brine shrimp, but the movements of live brine shrimp may generate a stronger olfactory cue (in addition to a more obvious visual cue) that reinforces the preference for live prey. Tactile stimulation may have also contributed to some prey strikes (especially in the dark), as the fish swim just millimeters above the substrate and their bodies and/or pelvic fins could come in contact with the brine shrimp, resulting in the initiation of strike behavior. In pre-cobalt trials in Experiment II (same methodology as Experiment I), in which all sensory systems were available, fish showed a preference for live prey only in light trials. The statistically insignificant prey preference in the dark (P=0.16) suggests that fish may not be able to discern the difference between live and dead prey using a tactile sense.

The results of Experiment I suggest that visual cues are used for prey detection in light trials. This is based on the fact that detections occurred while the fish were stationary (e.g. in a pause), at longer detection distances and with a smaller range of detection angles. Fish showed an equally high number of strikes on both live and dead prey, a shorter time to first prey strike and a predominance of prey detections (live and dead prey) during a pause, regardless of whether the fish had been treated with cobalt chloride. In light trials with cobalt treatment, only some of the fish struck at prey even when visual cues were available. In dark trials (Experiment I, and in pre- and post-cobalt trials in Experiment II), more prey were detected during a glide, at shorter detection distances and with a broader range of detection angles (including 180 deg swimming reversals). The fish also struck at more live (*vs* dead) prey and generally during glides in the absence of cobalt treatment, indicating the role of the lateral line system when vision was not available. In dark trials with cobalt treatment, none of the fish struck at prey in the dark, even when the same fish struck at prey in light trials just a few hours earlier. Thus, it is concluded that lateral line input is required for prey detection behavior under dark as well as light conditions.

In cobalt trials, fish did not show a preference for live prey in the light, and did not strike at any prey in the dark. Although vision and olfaction are not thought to be affected by cobalt chloride (Liao, 2006; Yoshii and Kurihara, 1983), the absence of both preference for live prey in light trials and any prey strikes in dark trials (Experiment II) shows that, under these conditions, olfactory and tactile cues were not sufficient for the localization of prey and initiation of prey strikes in the dark. During post-cobalt recovery trials, fish did not show a preference for live prey in either light or dark trials as they did in Experiment I, which suggests that the lateral line system may not have fully recovered from cobalt chloride treatment.

Cobalt chloride ablation of the lateral line system

There has been much discussion about methods used for the chemical or pharmacological ablation of the lateral line system using aminoglycoside antibiotics (e.g. Song et al., 1995; Janssen, 2000; Santos et al., 2006; Van Trump et al., 2010; Brown et al., 2011), so a consideration of ablation methods using cobalt chloride deserves a short discussion here. Karlsen and Sand (Karlsen and Sand, 1987) reported that after treatment with cobalt chloride in calcium-free water, a fish's sensitivity to water flows returned within hours to weeks when placed in calcium-enriched water. Cobalt chloride blocks the calcium channels in the membranes of the sensory hair cells that compose the neuromasts, which is reversible when the cobalt ions are replaced with calcium ions (that normally occur in freshwater and seawater). Subsequent studies used a wide

range of concentrations and exposure times [e.g. 0.003–1.0 mmol l^{-1} for 1 h to 1–2 weeks (Karlsen and Sand, 1987); 2 mmol l^{-1} for 3 h (Montgomery et al., 1997); 0.15 mmol l^{-1} for 3–4 h (Liao, 2006); and 0.05 mmol l^{-1} for 24 h (Patton et al., 2010)]. In the present study, the concentration and duration of cobalt chloride treatment (0.1 mmol l^{-1} for 3 h) appeared to inactivate the lateral line system of *A. stuartgranti* as demonstrated by the behavioral results. Interestingly, behavioral changes were observed despite the fact that calcium was present in water during cobalt chloride treatment (60 mg l^{-1}; Hach hardness test kit, Loveland, CO, USA) and in the experimental tank (140–160 mg l^{-1}). It was also demonstrated that handling (cobalt sham trials) did not affect feeding behavior. Results of this study show that treatment with cobalt chloride significantly affected prey detection behavior, especially in the dark. This is interpreted as being the result of successful lateral line inactivation by cobalt chloride.

Interestingly, prey detection behavior in post-cobalt (recovery) trials was not as robust as that prior to cobalt treatment (e.g. pre-cobalt trials), providing evidence that 3–4 weeks was not long enough for the fish to fully recover. Although most of the behavioral parameters measured (e.g. detection distance, detection angles, median number of strikes and time to first detection) were not significantly different in post-cobalt trials than in pre-cobalt trials, the number of strikes on live prey was lower and the preference for live prey seen in pre-cobalt trials was not evident in light post-cobalt trials. This result suggests that *A. stuartgranti* can regain sensitivity to hydrodynamic stimuli within several hours after exposure to cobalt chloride, but that this sensitivity may not be strong enough to elicit a robust response, including preference for live prey in the dark, when visual cues are not available. Furthermore, lateral line morphology must be taken into account when interpreting the present results concerning recovery time. Prior studies were carried out in a species with narrow canals that contain small canal neuromasts [the roach *Rutilus rutilus* (Karlsen and Sand, 1987)]. Larger neuromasts (a characteristic of widened canals, including those of *Aulonocara*) have more hair cells, so collectively, these neuromasts likely have more calcium channels and may require a longer period to recover. Thus, the effective recovery period may indeed be longer than previously reported, especially for species with larger neuromasts and widened canals. Here recovery occurs at least 3 to 4 weeks post treatment compared with 2 to 3 weeks reported by Karlsen and Sand (Karlsen and Sand, 1987). It is recommended that in future studies, the morphology of the lateral line system should be carefully considered and the course of post-cobalt recovery should be monitored by assaying several parameters of prey detection behavior (e.g. prey preferences) to determine when complete recovery has indeed been achieved.

Finally, to provide some morphological verification of the effects of cobalt chloride, juvenile fishes were treated and then stained with a fluorescent mitochondrial stain, DASPEI, to visualize the neuromasts. When juvenile *A. stuartgranti* [standard length (SL)=40–44 mm] were stained (0.01% DASPEI for 30 min) immediately after cobalt treatment (0.1 mmol l^{-1}, for 3 h in tank water, as in Experiment II), many neuromasts showed an unstained central region that is interpreted as negative staining of hair cells (M.A.B.S. and E. A. Becker, unpublished data), suggesting a physiological effect of cobalt chloride treatment. When juvenile *A. stuartgranti* (SL=23–36 mm) were treated at a lower concentration (0.05 mmol l^{-1} for 3 h in tank water; paired with control fish not treated with cobalt chloride) and then different individuals were stained with DASPEI (as above) at different time points over the course of 3 weeks, a decrease in staining intensity was observed for several days. More than 1 week after cobalt treatment, the intensity of DASPEI fluorescence had increased so that it was comparable to that in control fish (E. A. Becker and M.A.B.S., unpublished data). Thus, it is concluded that cobalt chloride does indeed have an effect on neuromasts, which is apparent over the course of 1 week, but that it may have more subtle effects that could not be visualized with DASPEI over the 3–4 week recovery period. Although providing verification that differences in feeding behavior were likely not due to non-specific effects of treatment with cobalt chloride, the impact of cobalt chloride (a calcium channel blocker) on neuromast staining with DASPEI (a mitochondrial stain) deserves more study.

Sensory biology and trophic niches of cichlids

The demonstration that *A. stuartgranti* can detect, successfully strike at and consume live benthic prey in the dark reveals the importance of non-visual senses in the feeding biology of cichlids, which have traditionally been considered to be diurnal visual feeders. This finding, coupled with what is known about the ecology of *Aulonocara* (Grant et al., 1987), can now provide hints concerning the role of non-visual senses in this genus. Some *Aulonocara* species are reported to occupy relatively low-light environments (e.g. >40 m depth, up to 70 m) and live and/or maintain territories in caves adjacent to the sandy substrates in which they feed (Meyer et al., 1987; Konings, 1990). Furthermore, evidence of nocturnal courtship behavior (D.K.B., unpublished observations) suggests that non-visual sensory modalities may be important for other aspects of their behavior. Although the present study has shown that *A. stuartgranti* can feed in the dark in the laboratory, these fishes are not known to be active at night in Lake Malawi (A. Konings, personal communication). However, it remains possible that these fishes are indeed nocturnally active, perhaps at depths greater than those at which they have been observed, and/or in the caves that some *Aulonocara* species occupy.

The ability to feed in low-light environments, or to feed nocturnally on inconspicuous and especially infaunal invertebrate prey, may reduce competition with visual feeders (Schleuter and Eckmann, 2006) by allowing access to novel spatial and temporal niches and providing refuge from a fish's own diurnally active predators (Helfman, 1993; Bassett, 2008). Modulation of prey detection behavior depending on light conditions, as we have demonstrated in *A. stuartgranti*, may be beneficial for fishes that are normally active in a range of light conditions, in habitats where food resources are diverse (e.g. cryptic, non-cryptic) and/or in habitats where prey are unpredictably distributed. Non-visual detection of prey may confer an advantage for species in habitats with increased turbidity levels (Bergman, 1991; Schleuter and Eckmann, 2006) in which the ability to visually detect prey is compromised.

Some ambush predators are able to detect prey in the water column using neuromasts in narrow canals [e.g. mottled sculpin (Coombs et al., 2001; Kanter and Coombs, 2003) and trout and goldfish (Sand and Bleckmann, 2008)] or superficial neuromasts [common bully *Gobiomorphus cotidianus* (Bassett et al., 2006)]. However, neuromasts in canals, and in widened canals in particular, present some important advantages for detection of benthic (as opposed to free-swimming) prey. First, canal neuromasts are protected from abrasion and fouling by sediment and thus appear to be advantageous for close-range detection of hydrodynamic flows generated by prey living on or in gravel, sand or mud. Second, canal neuromasts are able to better detect prey in background flows – those generated by currents moving past a fish or generated by the

swimming motion of a fish (Kanter and Coombs, 2003; Engelmann et al., 2000; Engelmann et al., 2002; Bassett et al., 2006). Third, neuromasts in widened canals (which are generally larger with more variable morphology) are more sensitive than neuromasts in narrow, well-ossified canals (Denton and Gray, 1988; Denton and Gray, 1989; Janssen, 1997). The evolution of widened canals in *Aulonocara* coupled with their particular prey search and prey detection behaviors makes a strong case for the evolution of widened canals in this genus as an adaptation for the detection of hydrodynamic stimuli generated by benthic invertebrate prey.

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