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The effect of light intensity on prey detection behavior in two Lake Malawi cichlids, *Aulonocara stuartgranti* and *Tramitichromis* sp.

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Abstract

Two Lake Malawi sand-dwelling cichlids (*Aulonocara stuartgranti*, *Tramitichromis* sp.) that have different lateral line phenotypes, but feed on benthic invertebrates, have been shown to use lateral line and/or visual cues to detect prey under light versus dark conditions. The current study examined how ecologically relevant variation in light intensity (0-800 lux) influences detection of prey (mobile, immobile) in each species by analyzing six well-defined behavioral parameters. Both species fed at light intensities ≥1 lux; prey type and/or time of day (but not light intensity) predicted all four parameters analyzed with generalized linear mixed models in *A. stuartgranti*, whereas the interaction of light intensity and time of day predicted three of these parameters in *Tramitichromis* sp. Data for all six parameters suggest that the critical light intensity is 1-12 lux for both species, the integration of visual and lateral line input explains differences in the detection of mobile and immobile prey and the behavioral changes that occur at the transition from 1 to 0 lux in *A. stuartgranti*, and that *Tramitichromis* sp. likely uses binocular vision to locate prey. The sensory biology of species that exploit similar food resources will have important implications for the trophic ecology of African cichlid fishes.

Keywords

Vision, lateral line, detection distance, prey detection, sensory ecology

Abbreviations

AICC	Akaike information criterion
GLMM	Generalized linear mixed model

Lx	Lux
PAR	Photosynthetically active radiation
SL	Standard length
TL	Total length

Introduction

Light in aquatic habitats varies in quality and quantity over time and space (Kirk 2011) and influences the ability of visual predators to detect and capture mobile prey (Vinyard and O'Brien 1976; Confer et al. 1978; Lythgoe 1979; Ryer and Olla 1999; Vogel and Beauchamp 1999; Rickel and Genin 2005). Fishes occupying similar habitats may demonstrate variation in visually-mediated prey detection abilities, such as visual thresholds and absorption spectra of visual pigments, which may provide a competitive advantage under particular light conditions (Vogel and Beauchamp 1999; Hofmann et al. 2009). Many fishes are also able to detect prey at low light intensities (e.g., dawn, dusk, at depth, or with increased turbidity), but with reduced capabilities compared to that at higher light intensities. The distance at which free swimming prey are detected dramatically decreases below a certain light intensity ("critical light intensity," Confer et al. 1978) in salmonids (Dunbrack and Dill 1984; Henderson and Northcote 1985) and some freshwater percomorphs (bluegill, *Lepomis macrochirus*, Vinyard and O'Brien 1976; largemouth bass, *Micropterus salmoides*, Howick and O'Brien 1983; yellow perch, *Perca flavescans*, Richmond et al. 2004).

Given the importance of multimodal sensory integration in the formulation of behavior, the contributions of the non-visual sensory systems to prey detection (e.g., mechanosensory

lateral line, auditory, olfactory, gustatory, somatosensory/tactile, and in some cases, the electrosensory system; reviewed in Montgomery et al. 2014) must also be considered. Morphological and/or physiological specializations of non-visual sensory systems, including the olfactory system (Parzefall 1993; Montgomery et al. 1999), gustatory system (Atema 1971) and the lateral line system (Janssen 1997; Schwalbe et al. 2012, reviewed in Webb 2014), have been used to predict how these senses provide alternatives to vision for prey detection in light-limited environments. Futhermore, the integration of different combinations of sensory inputs may explain variation in behavior under different environmental conditions (Partridge and Pitcher 1980; Moller 2002; Montgomery et al. 2003; Gardiner and Motta 2012). Several species of fishes have been shown to modulate feeding strategies using a combination of visual and non-visual cues that allow them to feed under a range of light conditions, including darkness (Townsend and Risebrow 1982; Batty et al. 1986; Diehl 1988; Schwalbe et al. 2012).

The mechanosensory lateral line system is known to play important roles in prey detection, as well as in predator avoidance, communication, and navigation around obstacles (Webb et al. 2008; Montgomery et al. 2014). The system demonstrates a great deal of variation, which is defined by the morphology of the cranial and trunk lateral line canals and neuromast receptor organs within them, and the distribution of superficial neuromasts on the skin of the head, trunk and tail (reviewed in Webb 2014). Widened lateral line canals, one of five cranial lateral line canal phenotypes found among bony fishes, has evolved convergently in ~12 teleost families (including deep sea taxa) and appears to be an adaptation for enhanced sensitivity to water flows and prey detection (Denton and Gray 1988, 1989; Montgomery and Coombs 1992; discussed in Schwalbe et al. 2012; reviewed in Webb 2014).

92 The speciose cichlid fishes of the African Rift Lakes are typically described as visual
93 feeders (Fryer and Iles 1972) and most genera have narrow cranial lateral line canals, but all
94 members of a few genera (e.g., *Alticorpus, Aulonocara*, *Aulonocranus*, *Trematocara,*
95 *Trematocranus*, Konings 2007) have widened lateral line canals suggesting the capacity for
96 lateral line mediated prey detection (Konings 1990). Two genera of non-mbuna, haplochromine
97 cichlids in Lake Malawi, *Aulonocara* (widened canals) and *Tramitichromis* (narrow canals; Fig.
98 1), provide an interesting taxon pair for comparison of prey detection strategies since both feed
99 on benthic invertebrates in the sand, and thus appear to be ecologically similar. Schwalbe et al.
100 (2012) and Schwalbe and Webb (2014) analyzed the behavioral responses of *Aulonocara*
101 *stuartgranti* and *Tramitichromis* sp. to tethered live and dead prey (=adult brine shrimp, *Artemia*
102 sp.), in experiments carried out under light and dark conditions in which the lateral line system
103 was experimentally inactivated. These studies demonstrated that *A. stuartgranti* uses a
104 combination of inputs to its visual and lateral line systems to detect prey in the light, but depends
105 on its lateral line system to detect prey in the dark. Furthermore, these studies showed that
106 deactivation of the lateral line system of *A. stuartgranti* significantly affected prey detection
107 behavior and revealed that other senses (olfaction, gustation, and somatosensory/tactile) were
108 insufficient to initiate prey detection behavior in the dark. In contrast, *Tramitichromis* sp. did not
109 feed in the dark, and the inactivation of the lateral line system had little effect on prey detection
110 behavior in the presence of light, demonstrating that it is a visual predator.
111 *Aulonocara* and *Tramitichromis* species appear to share a food resource (benthic
112 invertebrates in sandy substrates), but occupy different depth ranges (*Aulonocara* species at
113 depths of 5-120 m and *Tramitichromis* species at depths of < 15 m; Fryer and Iles 1972; Konings
114 1990, 2007) and use different strategies to detect and capture benthic invertebrate prey in the

field. Species of *Aulonocara* swim just above the substrate to sense hydrodynamic flows generated by benthic invertebrates in or on the substrate in the field (Konings 2007). In contrast, species of *Tramitichromis* typically capture invertebrate prey by plunging into the substrate, filling their mouth with sand, and sifting out prey with their gill rakers in the field (= "sand sifting," Fryer, 1959). This sand sifting behavior appears to be synonymous with the "winnowing" behaviors observed in some surfperches (Laur and Ebeling 1983) and vision likely contributes to the ability to locate patches of high quality food resources where "winnowing" takes place (Holbrook and Schmitt 1984; Schmitt and Holbrook 1984).

This study uses the same methods used in prior studies (Schwalbe et al. 2012; Schwalbe and Webb 2014) to test the hypothesis that variation in light intensity (0-800 lx) will have different effects on the detection of live (mobile) and dead (immobile) benthic invertebrate prey in *A. stuartgranti* and *Tramitichromis* sp.

Materials and methods

Study species

Adult *Aulonocara stuartgranti* and *Tramitichromis* sp. (unidentifiable to species level, J. Stauffer, pers. commun.), is referred to as *Tramitichromis* throughout. These fish were reared in the laboratory from breeding stock originally acquired from commercial suppliers (*A. stuartgranti*: Bluegrass Aquatics, Louisville, KY, USA; *Tramitichromis*: Old World Exotic Fish, Inc., Homestead, FL, USA and Life Fish Direct, Draper, UT, USA). They were housed in small groups by species in 190 L 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 standard white fluorescent light on a 12h:12h diurnal cycle and, equipped with appropriate mechanical and biological filtration. Fish were fed daily with cichlid pellets (New Life Spectrum Cichlid Formula; New Life International, Inc., Homestead, FL) and supplemented with live adult brine shrimp. Individual fish were not used in feeding experiments if breeding behavior was observed. Animal care and all experimental procedures followed an approved IACUC protocol.

Light environment in the experimental tank

Light in the experimental tank was provided by two fluorescent light fixtures (Lithonia Lighting, Model GRW 2 14 CSW CO M4, Conyers, GA, USA) fitted with full spectrum bulbs (BlueMax lamps, Full Spectrum Solutions, Jackson, MI, USA) positioned above the tank and within an opaque curtain enclosure. The curtain (black canvas) was suspended from a rectangular plywood frame placed 2 m above the top of the tank in order to exclude ambient light from entering the set-up during all behavioral trials (Fig. 2a). Light intensity was varied by changing the height of the lights above the water surface and using combinations of different neutral density filters covering the lights (Lee Filters, Burbank, CA, USA). Light intensity (in lux [lx], lumen/m^2, and photosynthetically active radiation [PAR], μmol photons/m^2/s) and color spectrum were measured using a spectrometer (range: 340-1028 nm, Jaz spectrometer, Ocean Optics, Dunedin, FL, USA) connected to a 2 m optical fiber (QP400-2-UV/VIS, Ocean Optics) fitted with a cosine corrector (CC-3, Ocean Optics). Water temperature was monitored during experiments and the fluorescent bulbs did not raise the temperature of the experimental tank.

160 Light intensities used in this study were based on the following data and calculations.
161 First, light levels present during sunrise/sunset to darkness are known for other freshwater
162 habitats (Harden Jones 1956; Ali 1959) and can range from 1000 lx (early twilight) to 0 lx (new
163 moon, Table 1). Second, few direct measurements of light intensities at different depths in Lake
164 Malawi are available, so the light intensity at specific depths were estimated with the following
165 equation:

$$I_t = I_S \times e^{-\varepsilon \times T}$$

166 where I_s and I_t are the light intensities at the surface (S) and at depth (T); and ε is the light
167 extinction coefficient. The average light intensity at the surface of Lake Malawi at midday on a
168 clear sunny day is approximately 2000 μmol photons/m^2/s (~108,000 lx). This photon flux was
169 derived from cloudless surface irradiance for Lake Malawi (Guildford et al. 2000). Using light
170 extinction coefficient of either 0.10 m^{-1} (Patterson et al. 2000), 0.13 m^{-1} (Guildford et al. 2007),
171 or 0.43 m^{-1} (Guildford et al. 2007) depending on location and season, the light intensity at many
172 depths can be estimated under these conditions (Table 1).
173 Full spectrum bulbs were used because they provide the range of wavelengths that
174 correspond to the range of known absorption peaks of retinal photopigments in species of
175 *Aulonocara* and *Tramitichromis*. For instance, absorption peaks for *A. hueseri* are at 415 nm
176 (violet), 484 nm (blue-green) and 526 nm (green; Jordan et al. 2006) and absorption peaks for *T.*
177 *intermedius* are at 455 nm (blue), 532 nm (green) and 569 nm (red; Parry et al. 2005). In the
178 experimental tank, full spectrum bulbs generated major and minor light peaks at 404, 435, 487,
179 545, 587, and 611 nm, and neutral density filters were used to change light intensity did not
180 appreciably change the light spectrum in the experimental tank (Fig. 2b)
181

182 Experiments

183

184 Behavioral trials and video analysis of six well-defined behavioral parameters (number of prey
185 strikes, detection distance, detection angle, detection-to-strike velocity, swimming phase [glide,
186 pause] at detection and, prey type preference [order of prey strikes]) were carried out as in
187 Schwalbe et al. (2012) and Schwalbe and Webb (2014) with slight modifications. A total of sixty
188 trials were conducted using *A. stuartgranti* (30 trials, n = 6 fish, 75-85 mm total length [TL], 4
189 females, 2 male) and *Tramitichromis* (30 trials, n = 6 fish, 75-98 mm TL, 1 female, 5 males) in
190 order to quantify variation in behavioral responses to live (mobile) and dead (immobile) prey (=
191 tethered adult brine shrimp) at five light intensities between 0 and 800 lx.

192 Trials were conducted in an experimental tank (120 x 75 x 60 cm; 560 L) with 5 cm of
193 sand covering the bottom of the tank. Light intensity and spectral measurements (with ±0.01
194 accuracy, measured in lx and PAR) were taken directly above the center of each mesh platform
195 (to which live and dead prey were tethered, see below) before and after each trial, and light
196 intensity and spectrum were found to be consistent at all six platforms and trials (Figs. 2b, c).
197 Each fish was acclimated to a particular light intensity in the experimental tank for at least 30
198 minutes prior to a trial. The transition between photopic (cone-mediated) and scotopic (rod-
199 mediated) vision occurs at approximately 1 lx, and light-adapted fish may take 30 minutes (and
200 up to 3 hours) to become dark-adapted (Ali 1959). Thus, the 30+ minute light adaptation period
201 was judged to be sufficient to allow the fish's visual system to adjust to the light level for a given
202 trial.

203 Before each trial, 12 adult brine shrimp (*Artemia* sp.) were tethered in pairs (1 live and 1
204 dead, freshly frozen) on each of six mesh platforms (10 x 10 cm), which were positioned in a 2 x

205 3 matrix so that the top of each platform flush with the sand surface. The water filtration system
206 for the experimental tank was then turned off to eliminate acoustic and hydrodynamic noise. At
207 the start of a trial, a fish was released into the experimental arena from behind an opaque divider
208 and feeding behavior was recorded for 30 min using an HD digital video camera (Sony © HDR-
209 CX550V, 30 frames per second) mounted directly above the tank, which provided a dorsal view
210 of the experimental arena. Trials at 1-800 lx were carried out with standard fluorescent room
211 lights on for all but the lowest light levels (1-12 lx). Dark trials (0 lx) were conducted with room
212 lights off, but with infrared illumination (peak = 840 nm, range 800-880 nm; Speco Provideo,
213 IR-200/24, Amityville, NY) to allow video recording of behavior.
214 Each fish was run through five trials, one trial per day each at a single light intensity,
215 progressing from highest to lowest intensity on subsequent days (e.g., 800, 112, 12, 1, and then 0
216 lx). Trials were carried out in this order to increase the likelihood that a fish would respond to
217 prey at lower light intensities (especially in the dark, 0 lx), as was suggested by preliminary
218 results. Trials were conducted over four months and the mean time between the first (800 lx) and
219 last (0 lx) trial for a given fish was 11 days (range = 6-19 days).
220 "Light" trials (1-800 lx) started midday to late afternoon (11:00-17:00) and "dark" trials
221 (0 lx) took place shortly after sunset (19:00-21:00; soon after room lights had automatically shut
222 off; as in Schwalbe et al. 2012; Schwalbe and Webb 2014). Dark trials (0 lx) were not carried out
223 during the day (during the light phase of the lab's light:dark cycle) in order to avoid the
224 introduction of extraneous light. In addition, it was known that placing fish in low light or
225 darkness during normal daylight hours would disrupt feeding behavior (M.A.B. Schwalbe and A.
226 Mensinger, pers. obs.), and that species that normally feed both in full light during the day and at

night (e.g., during the dark phase of a lab's light:dark cycle) were unresponsive in dark (0 lx) trials carried out during the day.

To assess the number of prey detections that lead to prey strikes, unconsumed prey were counted at the end of each 30-minute trial and strike success was also confirmed in video recordings. Video sequences leading to each prey strike were exported to Premier Pro (Adobe, CS5) for further analysis. Analysis of sequential video frames was used to identify the phase of swimming behavior (thrust, glide, or pause) during which prey detections occurred. Detection distance and detection angle were measured in these images using ImageJ (NIH, v. 1.41o). Detection distance was defined as the distance from the tip of a fish's mouth to the prey, measured in the frame immediately before the fish oriented towards it (e.g. before a turn defining detection). For each prey strike, detection-to-strike velocity was calculated by dividing detection distance by the time interval between detection and initiation of a strike. Detection angle was measured in the same video frame in which detection distance was measured, and was defined as the angle between a line extending anteriorly along midline of the fish (body axis) and a line drawn from the prey to the tip of the fish's mouth.

Statistical analysis

Four of the six behavioral parameters were analyzed using generalized linear mixed models (GLMMs; number of prey strikes, detection distance, detection-to-strike velocity, phase of search behavior during which detections occurred). In addition, a ranking method (Taplin 2007) was used to analyze prey preferences (live versus dead prey) and circular statistics were used to analyze detection angles. All continuous data (e.g. detection distance and detection-to-strike

250 velocity) were tested for normality (Kolmogorov-Smirnov test) and were $\log_{10}$ transformed to
251 achieve normality (detection distance and detection-to-strike velocity). All statistical tests were
252 considered significant at $P < 0.05$ and values are given as means ± s.e.m.
253 Start time (= time of day, 0-24 hr) for trials conducted at the five different light intensities
254 was analyzed with nonparametric tests (e.g. Kruskal-Wallis test and Mann-Whitney U test) to
255 determine whether time of day affected feeding behavior. This analysis showed that the times at
256 which light trials (1-800 lx) and dark trials (0 lx) started did not differ between species (Mann-
257 Whitney U test, $P > 0.05$), but trial start time varied among light intensities in each species
258 (Kruskal-Wallis test, *A. stuartgranti*: $K = 22.804$, $P < 0.001$; *Tramitichromis*: $K = 20.141$, $P <$
259 0.001). Thus, time of day (=trial start time) was included in all GLMM analyses.
260 Four-way GLMM analyses (SPSS, IBM, v. 22) were used to test whether species (*A.*
261 *stuartgranti, Tramitichromis)*, light intensity (0-800 lx), prey type (live, dead), and/or trial start
262 time (0-24 hr) predict differences in each of four behavioral parameters (number of prey strikes,
263 detection distance, detection-to-strike velocity, and phase of search behavior during which
264 detections occurred). Three-way GLMM analyses were used to further examine whether light
265 intensity, prey type, and/or trial start time predict differences in the four behavioral parameters in
266 each species separately. The selection of random (individual) and fixed effects (species, light
267 intensity, prey type, and trial start time), including repeated measures for the same individual,
268 was addressed in all analyses. Different types of GLMMs were used to account for the different
269 types of data collected in this study (summarized in Table 2) and the most parsimonious model
270 was selected for each behavioral parameter based on the corrected Akaike information criterion
271 (AICC).

The order in which live (mobile) and dead (immobile) prey were struck was analyzed in each species following Taplin (2007). This method assumes that when presented with equal numbers of two or more types of prey, the order in which prey are consumed provides information about prey preference – that prey consumed first are more highly preferred than prey consumed second, third, etc. and the last prey consumed is the least preferred. While differences in handling time, encounter rates, and relative mobility of prey can potentially complicate the results of this sort of analysis (Durham et al. 2012, McWilliam et al. 2013), such variation was minimized in the current study by offering equal numbers of live and dead brine shrimp tethered in the same arrangement to platforms placed in a 2 x 3 matrix in all trials. The null hypothesis for this analysis was that live and dead prey would be consumed randomly during a trial. Videos were analyzed so that each prey consumed was assigned a rank number (first prey consumed=1, second prey consumed=2, etc.), and any remaining prey were assigned an average of the remaining preference scores, and considered "tied for last." A pair of preference scores for live and dead prey at each light intensity was calculated for each fish. The pairs of scores from all of the fish were considered independent samples and thus grouped by light intensity and species for analysis. A score of 6.5 (based on presentation of six live and six dead prey, 12 total prey in a trial) indicated no preference, a score of <6.5 revealed a preference for that prey type, and a score of >6.5 indicated no preference or that prey type was ignored or avoided. Scores for live and dead prey at each light intensity and for each species, were compared separately using paired *t*-tests (SPSS, IBM, v. 22).

Detection angles were analyzed with circular statistics (Oriana v. 3, Kovach Computing Services, Anglesey, UK). Rayleigh tests were performed for each species to test whether detections of live and dead prey at each of the five light intensities (0-800 lx) occurred at

uniformly or non-uniformly distributed positions around the fishes' body relative to the body axis (e.g. to define the receptive field). Watson's U^2 tests were used to determine if detection angles differed with prey type and with light intensity within and between species.

Results

Aulonocara stuartgranti and *Tramitichromis* sp. actively swam around the experimental tank during trials at all light intensities, including darkness (0 lx), and used a saltatory search strategy (a cyclic sequence of a caudal fin thrust, glide, and pause) while exploring the tank. Of the 360 total prey presented to fish during all 60 trials, *A. stuartgranti* struck at 299 prey (=83%) and *Tramitichromis* struck at 231 prey (=64%; see Figs. 3, 4). Prey were detected by both species during a glide or a pause, but never during a thrust (see Fig. 5).

Four-way GLMM analyses (Table 3) indicated that species alone did not predict differences in any of the four behavioral parameters (number of prey detections, detection distance, detection-to-strike velocity, or swimming phase at prey detection), and that only the interaction of species, light intensity, and prey type had a significant effect only on number of prey detections. The interaction of light intensity and time of day predicted differences in all four behaviors. Light intensity alone predicted differences in all four behaviors, and time of day predicted differences in three behavioral parameters (number of prey detections, detection distance, detection-to-strike velocity), but not in swimming phase at prey detection. Separate three-way GLMM analyses for each species (Table 4, see below) revealed interesting trends that are indicative of species differences in prey detection behavior. Analyses of prey preference (live

317 vs. dead prey) and prey detection angle, carried out using other statistical methods, also indicated
318 differences in behavior between species, but did not consider time of day.
319
320 Feeding behavior of *Aulonocara stuartgranti*
321
322 GLMM analyses (Table 4) showed that light intensity did not significantly predict any of the
323 four behavioral parameters in *A. stuartgranti* (number of prey detections, detection distance,
324 detection-to-strike velocity, or swimming phase at prey detection; GLMMs, $P > 0.05$; Table 4).
325 However, time of day predicted the number of prey detections, and the interaction of time of day
326 and prey type predicted both detection distance and detection-to-strike velocity (GLMMs, $P <$
327 0.05; Table 4). Neither light intensity, time of day, prey type, nor their interactions, predicted
328 swimming phase at prey detection.
329 An examination of data for each of the behavioral parameters revealed informative
330 trends. *A. stuartgranti* struck at high numbers of both live (mobile) and dead (immobile) prey at
331 light intensities of 1-800 lx (Figs. 3a, 4a). They detected prey during a pause about half of the
332 time, but detected 61% of prey in a pause at the highest light intensity (800 lx; Fig. 5a).
333 Detection distance and detection-to-strike velocity appeared to not vary among light intensities
334 of 12-800 lx, but fish tended to detect live prey from greater distances (mean = 8.7-9.6 cm) than
335 dead prey (6.0-6.9 cm) and to detect live prey at higher detection-to-strike velocities (9.7-10.4
336 cm/s) than dead prey (6.9-7.4 cm/s; Figs. 4c, e). At 1 lx, fish tended to detect live and dead prey
337 from similar distances (mean = 6.5 and 6.3 cm, respectively; Fig. 4c) and similar detection-to-
338 strike velocities (6.8 cm/s and 7.8 cm/s, respectively; Fig. 4e). Live and dead prey (combined)
339 were detected at non-uniformly distributed positions around the fishes' bodies at light intensities

≥1 lx (Rayleigh test, $P < 0.001$; ±90° from body axis) with no differences in the distribution of angles among pairs of light intensities with the exception of the two highest light intensities (112 lx versus 800 lx; Watson's U^2 test, $U^2 = 0.19$, $P < 0.05$; Fig. 6a). Finally, fish tended to prefer live prey at all light intensities, but only demonstrated a statistically significant preference for live prey at 112 lx, but not at 800 lx (Table 5; Fig. 7a), which is not easily explained.

In the dark (0 lx), prey detection behavior of *A. stuartgranti* was different than at light intensities ≥ 1 lx. Fish struck at only 22 prey (=30.6% of total prey presented; Fig. 3a), and tended to detect prey at even shorter distances (Fig. 4c) and at slower detection-to-strike velocities (Fig. 4e) than when at least some light was present. Fish tended to detect more live prey than dead prey (mean of 2.7 and 1.0, respectively; Figs. 3a, 4a), showed a statistically significant preference for live prey (Table 5, Fig. 7a), and detected live prey from more than twice the distance than dead prey (3.2 and 1.4 cm, respectively; Fig. 4c). In addition, detection-to-strike velocity at 0 lx was about one half of that at higher light intensities (~3.5-5 cm/s at 0 lx versus ~7-10 cm/s at ≥ 1 lx), but fish tended to detect live prey at somewhat higher detection-to-strike velocities than dead prey (Fig. 4e). In the dark, 95% of prey were detected during a glide and only a few prey (5%) were detected during a pause (Fig. 5a). Prey (live and dead combined) were detected at positions uniformly distributed around fishes' bodies (Rayleigh test, $P > 0.05$) at a wide range of angles (± 180° from body axis, Fig. 6a), but the distribution of detection angles did not differ for live versus dead prey (Watson's U^2 test, $P > 0.05$).

Feeding behavior of *Tramitichromis*

GLMM analyses (Table 4) showed that, in contrast to *A. stuartgranti*, the interaction of light intensity and time of day predicted three of four behavioral parameters (number of prey detections, detection distance, and detection-to-strike velocity). As in *A. stuartgranti*, neither light intensity, time of day, nor prey type, or their interactions, predicted swimming phase at prey detection. Prey type did not predict any of the four behavioral parameters in *Tramitichromis*, and the interaction of light intensity and prey type predicted only detection distance (Table 4).

An examination of trends for each of the behavioral parameters revealed that *Tramitichromis* tended to strike at high numbers of prey (Fig. 3b, 4b), and >60% of prey (live and dead, combined) were detected during a pause at light intensities of 1-800 lx (Fig. 5b). At light intensities of 12-800 lx, fish struck at live and dead prey from similar, long detection distances (means = 9.8-10.1 and 8.5-10.0 cm, respectively) and at high detection-to-strike velocities (9.6-10.5 and 8.7-9.3 cm/s, respectively). In contrast, at 1 lx, fish tended to strike at both live and dead prey at similar, but shorter detection distances (6.9 and 6.3, respectively) and lower detection-to-strike velocities (7.0 and 6.1, respectively; Figs. 4d, f) than at higher light intensities. Both live and dead prey were detected at non-uniform positions around the body (Rayleigh test, $P < 0.001$), which defined a very narrow range of detection angles from the body axis (±40°); distributions were the same for live prey and dead prey at light intensities of 1-800 lx (Watson's U^2, $P > 0.05$; Fig. 6b). Fish tended to prefer live prey at different light intensities, but only showed a statistically significant preference for live prey at the highest light intensity (800 lx; Table 5; Fig. 7b).

Despite being active in the dark (0 lx), *Tramitichromis* only struck at only 3 prey (=4.2% of the 72 prey presented). These strikes are likely to have been the result of random encounters with prey as opposed to being the result of active search and directed strikes.

Discussion

The multiple statistical analyses presented here, and the detailed examination of trends in the detection of live and dead prey at different light intensities in each species indicate that light intensity affects prey detection behavior in different ways in *Aulonocara stuartgranti* and *Tramitichromis*.

Feeding behavior of *Aulonocara stuartgranti* and *Tramitichromis*

Prey type and/or time of day, but not light intensity, were predictors of three of the four behavioral parameters (number of prey detections, detection distance, and detection-to-strike velocity) analyzed using GLMMs in *A. stuartgranti*. The lack of significance for light intensity is consistent with the use of lateral line cues (see also Schwalbe et al. 2012), but also suggests that *A. stuartgranti* may use a light-independent circadian rhythm to interpret time of day. This is consistent with the occurrence of *Aulonocara* species at depths up to120 m in Lake Malawi where light is limited or absent (Konings 1990, 2007) and in caves where spawning has been reported (Grant et al. 1987), and thus where normal diurnal variation in light intensity may not be a consistent or reliable cue for the regulation of behavior. The significance of prey type as a predictor of detection distance and detection-to-strike velocity is illustrated by apparent differences in numbers of live and dead prey detected at the same light intensities (Fig. 4; see also Schwalbe et al. 2012; Schwalbe and Webb 2014), the tendency to prefer live prey at all light intensities, and the statistically significant preference for live prey in the dark.

In contrast, in *Tramitichromis,* it is the interaction of light intensity and time of day that predict these same three behavioral parameters. The importance of light intensity not surprising because *Tramitichromis* uses visual, but not lateral line cues, for prey detection and does not feed in the dark (Schwalbe and Webb 2014). Furthermore, these two factors are correlated both in the lab where the fish were reared (on a 12:12 hr light/dark cycle) as well as in the relatively shallow waters in their natural habitat in Lake Malawi, which is just 9-17° south of the equator where fish experience 11-13 hours of daylight per day on an annual basis (http://astro.unl.edu). Thus, these fish have evolved and are reared in environments where light intensity and time of day are tightly correlated. The independent roles of these two factors in predicting behavior would need to be addressed in additional experiments, which were out of the scope of this study.

Swimming phase (glide, pause) during which prey were detected was predicted neither by light intensity nor by time of day in either species. The ability to detect prey during a glide or pause will affect both the stabilization of the visual field (for vision-mediated detection) and/or the magnitude of environmental and self-generated hydrodynamic noise (for lateral line-mediated detection). *A. stuartgranti* and *Tramitichromis* both detected between 40% and 70% of prey during a pause at light intensities of ≥1 lx, suggesting the importance of stabilizing the visual field for prey detection at these light intensities. Prey type (which defines the presence or absence of an additional visual motion stimulus) did not predict swimming phase at detection for *Tramitichromis* ($P < 0.053$), but a larger sample size may have yielded a different statistical outcome. Prey type also did not predict swimming phase at prey detection for *A. stuartgranti*, but the shift to 95% of prey detections during a glide in the dark (where stabilization of visual field is irrelevant), and their preference for live prey (that generate hydrodynamic flows detected in the dark; Schwalbe and Webb 2014), are important indicators of the overall importance of prey type.

A. stuartgranti detected live prey at distances of less than half of a body length and at lower detection-to-strike velocities at a low light intensity (1 lx) and in the dark (0 lx). In the presence of at least some light, lower detection-to-strike velocities would also reduce self-generated hydrodynamic noise (Montgomery et al. 2009), enhancing lateral line-mediated prey detection, which would suggest that fish would tend to detect prey during a pause. However, the high proportion of detections (95%) at relatively low detection-to-strike velocities, while not eliminating self-generated noise, would bring a fish into the vicinity of potential prey that are generating detectable hydrodynamic flows (Schwalbe et al. 2012).

Role of vision and critical light intensities

The importance of vision in *A. stuartgranti* and *Tramitichromis* is further supported by a consideration of critical light intensities and the potential differences in the use of binocular vision. Prey detection at relatively long distances is consistent with vision-mediated prey detection in fishes (Vinyard and O'Brien 1976, Confer et al. 1978, Henderson and Northcote 1985, Mazur and Beauchamp 2003), and at higher light intensities detection of free swimming prey generally occurs at longer distances (Vinyard and O'Brien 1976; Richmond et al. 2004; Bergstrom and Mensinger 2009). In this study, both *A. stuartgranti* and *Tramitichromis* tended to demonstrate the longest detection distances at the highest light intensities, which is thus consistent with vision-mediated prey detection. Detection distances may not increase as light intensity increases further in a given species (Schmidt and O'Brien 1982), but may decrease sharply below a "critical light intensity" (Confer et al. 1978). Trends in behavioral parameters in the current study reveals that the critical light intensity for fish feeding on tethered adult brine

shrimp is between 12 and 1 lx for both *A. stuartgranti* and *Tramitichromis*. This is comparable to the critical light intensities for other freshwater teleosts in studies feeding on free-swimming *Daphnia* (11-50 lx, in bluegill, Vinyard and O'Brien 1976; in lake trout, brook trout, and bluegill, Confer et al. 1978), amphipods (5-25 lx, in round goby, logperch, slimy sculpin, and spoonhead sculpin, Bergstrom and Mensinger 2009), or on small fish (~6-18 lx, in largemouth bass, Howick and O'Brien 1983; lake trout, Vogel and Beauchamp 1999). At low light intensities (below the critical intensity, at 1 lx), the ability of *A. stuartgranti* to detect more prey than *Tramitichromis*, but at comparable distances suggests that *A. stuartgranti* may have superior visual abilities for prey capture at these lower light intensities. This is consistent with their distribution at a wider depth range than *Tramitichromis* and the observation of reproductive behaviors in caves (Grant et al. 1987), but whether *Aulonocara* species possess adaptations for increased sensitivity and/or visual acuity as found in known crepuscular or nocturnal teleosts (reviewed in Warrant 2004; Schmitz and Wainwright 2011) requires further study.

The potential for binocular vision can be revealed by looking at behavioral evidence for differences in the size of visual fields under different light conditions and between species. While visual predators may respond differently to stimuli in different portions of their visual fields (Collin 1989; McComb and Kajiura 2007; Miyazaki et al. 2011), it is detection angle that is reflects the overall size of the visual field, which is defined by the size, shape, and position of the eyes (Collin and Shand 2003). *A. stuartgranti* demonstrates a wide range of detection angles at light intensities $\geq$ 1 lx (±90° from body axis) and an even wider range of angles in darkness (0 lx, ±180° from body axis). This shift is correlated with differences in behavioral parameters at 1 lx versus 0 lx, which are interpreted as a shift between primarily vision-mediated prey detection to lateral line-mediated prey detection. Lateral line-mediated detection of prey around the body is

477 enabled by the more sensitive widened cranial lateral line canals that characterize *Aulonocara*
478 species, and by the broad distribution of canal and superficial neuromasts on the skin of the head,
479 trunk and tail, which is typical of cichlids and of most teleosts (reviewed in Webb 2014). In
480 contrast, *Tramitichromis* detected prey at a range of angles (±40° from body axis) that was less
481 than half of that for *A. stuartgranti* (±90° from body axis) at light intensities of 1-800 lx, with
482 one exception (Watson's U^2 test, $P < 0.05$). This suggests that *Tramitichromis,* but likely not *A.*
483 *stuartgranti,* uses binocular vision and depth perception to detect prey at a distance (as
484 demonstrated in other teleosts, Sivak 1978; Blanco-Vives et al. 2011; Miyazaki et al. 2011).
485 Furthermore, *Tramitichromis* tends to swim higher above the substrate than *A. stuartgranti* when
486 searching for prey in the laboratory (Schwalbe and Webb 2014). Coupled with the use of
487 binocular vision, this search strategy could explain the tendency for *Tramitichromis* to detect
488 benthic prey at somewhat longer distances than *A. stuartgranti* (Figs. 4c, d).
489 The movements of the appendages of the live prey used in this study presumably generate
490 a visual motion stimulus, and an enhanced dispersal of an odor plume (not evaluated here), in
491 addition to a hydrodynamic stimulus, which addresses the importance of multimodal integration
492 in the formulation of prey detection behavior. However, prey type predicted detection distance
493 and detection-to-strike velocity only in *A. stuartgranti*, which tended to strike at live prey at
494 longer detection distances and at higher velocities than for dead prey at the same light intensities
495 (12-800 lx). At 1 lx, detection distances were about one body length or less, which is within the
496 effective range of the lateral line system (Coombs 1999). Behavior is consistent with the use of
497 the lateral line system in addition to vision for detection of live prey by *A. stuartgranti* in full
498 light.

In contrast, in *Tramitichromis*, prey type did not predict any of the four behavioral parameters analyzed using GLMMs, although the interaction of prey type and light intensity did predict detection distance. However, the examination of data trends showed that *Tramitichromis* demonstrates comparable values and trends for live and dead prey with reference to number of prey detections, detection distance (despite the significance of its interaction with prey type) and detection-to-strike velocity at light intensities of 1-800 lx. These results also substantiate results of a prior laboratory study (Schwalbe and Webb 2014) that showed that *Tramitichromis* is a visual predator, which is not dependent on the detection of hydrodynamic stimuli generated by live prey. However, the lack of significance of prey type indicates that *Tramitichromis* does not respond to a visual motion stimulus that are likely to have been generated by live (but not dead) prey, which is surprising given the feeding strategies that these fish employ in nature. In the relatively shallow, well-lit waters of Lake Malawi, *Tramitichromis* species typically capture prey by plunging into the substrate, filling their mouth with sand, and sifting out prey with their gill rakers (= sand sifting, Fryer 1959). The sensory basis for the plunge and sift feeding behavior needs to be determined experimentally, but the results of this study suggest that it is a visual stimulus and not an associated motion stimulus generated by live prey that influences where *Tramitichromis* initiates feeding behavior in the field.

The connection between experimental light conditions and light levels in Lake Malawi

As in other lakes, the photic conditions in Lake Malawi are dynamic and many factors influence the light environment, including habitat type, water depth, and proximity to the lake bottom (Sabbah et al. 2011), as well as meteorological events, eutrophication, turbidity, and both diurnal

522 and seasonal changes in light quality and quantity. In shallow water, full spectrum light is
523 typically present and middle wavelengths transmit best, but shorter and longer wavelengths
524 attenuate rapidly (Dalton et al. 2010). Further, the irradiance spectrum differs between waters
525 overlying sandy and rocky substrates, where light transmission in water above sand is shifted to
526 longer wavelengths compared to that above rocky habitats (Sabbah et al. 2011).

527 The extent to which species of *Aulonocara* and *Tramitichromis* forage using vision at
528 different depths can be approximated by comparing behavioral data from the current study to
529 estimates of depths at which particular light intensities are predicted to occur in Lake Malawi.
530 The light extinction coefficients in Table 1 are representative of pelagic ($\varepsilon = 0.10\ m^{-1}$, Patterson
531 et al. 2000; $\varepsilon = 0.13\ m^{-1}$, Guildford et al. 2007) and nearshore ($\varepsilon = 0.43\ m^{-1}$, Guildford et al.
532 2007) habitats in Lake Malawi, but disparities in water clarity between these areas are likely
533 influenced by nutrient loading and sedimentation from deforestation, intense agricultural
534 practices, and erosion in nearshore areas (Bootsma and Jorgensen 2006). Estimations based on
535 low light extinction coefficients (e.g. $\varepsilon = 0.10\ m^{-1}$ or $0.13\ m^{-1}$) suggest that *Aulonocara* species
536 could visually detect prey at 71 to 92 m (≥12 lx) and with some visual limitations at ~89 to 115
537 m where light levels are at ~1 lx. Some *Aulonocara* species are found to depths of 120 m
538 (Konings 2007), so they may be able to visually detect prey in these depths at midday when light
539 intensities are highest. Alternatively, when light extinction coefficients are used ($\varepsilon = 0.43\ m^{-1}$),
540 the maximum depths at which *Aulonocara* species could reliably detect prey are greatly reduced
541 (to 21 m and 27 m, respectively). In the lab, *Tramitichromis* was able detect prey at a light
542 intensity of 1 lx, which translates to depths of 89 to 115 m if the light extinction coefficient is
543 low. However, these fish are typically found in shallower waters (<15 m, Konings 1990, 2007),
544 so the ability of *Tramitichromis* to find prey at 1 lx is more relevant for the potential for feeding

early or late during the day. Given its dependence on vision for prey detection (Schwalbe and Webb 2014), *Tramitichromis* species may be limited to shallow habitats so that the visual detection of prey is not compromised. In contrast, *Aulonocara* species can feed at low light intensities and in the dark, which can explain the wider range of depths at which they occur in Lake Malawi. They may also be crepuscular or nocturnal in habit, which may also facilitate other behaviors (e.g., social interactions) at low light intensities.

Conclusions

A. stuartgranti fed on prey at a range of ecologically relevant light intensities, including darkness, and *Tramitichromis* was also able to feed at low light intensities, but not in darkness. In *A. stuartgranti*, the influence of time of day on several aspects of its behavior suggests that it may use circadian rhythms to regulate behavior in nature where diurnal light cues may not be available (e.g. at greater depth, in caves). The integration of visual and non-visual (e.g., lateral line) sensory modalities can explain the statistically non-significant trends in behavior. Similarly, the dramatic change in behavior from 1 lx to 0 lx is consistent with a transition from primarily vision-mediated to exclusively lateral line-mediated prey detection behavior. In contrast to *A. stuartgranti*, *Tramitichromis* depends on vision-mediated prey detection (Schwalbe and Webb 2014); in this study its behavior was significantly affected by the interaction of light intensity with time of day, but these two factors could not be teased apart. Finally, in an ecological context, the tendency of *Tramitichromis* species to live in shallower, well-lit habitats, in contrast to *Aulonocara* species, which live at a wide range of depths and light environments, suggests that sensory capabilities may allow *Aulonocara* species to escape competition with *Tramitichromis*

species for prey resources, thus facilitating niche differentiation between these taxa. Field observations in Lake Malawi are needed to test this hypothesis, which would provide an important link between the morphology, feeding behavior, and ecology of cichlid fishes.

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Figure Legends

Fig. 1 Lateral line canals and canal neuromasts in *Aulonocara stuartgranti* (widened canals) and *Tramitichromis* (narrow canals) visualized using fluorescent vital staining (4-di-2-ASP, 63 μM, 5 min; **a**, **c**), μCT imaging (reconstructed from 14 μm slices; **b**, **d**, **e**, **g**, **h**), and scanning electron microscopy (**f**). **a** Lateral view of *A. stuartgranti* revealing series of larger infraorbital (IO), mandibular (MD), and preopercular (PO) canal neuromasts and very small superficial neuromasts on the skin (juvenile, 25 mm standard length [SL]). Neuromast number and distribution is the same in *Tramitichromis* **b** μCT reconstruction of *A. stuartgranti* (adult, 78 mm SL) indicating the location of the supraorbital (SO), IO, MD, and PO canals in dermatocranial bones. **c** Ventral view of the head of *A. stuartgranti* (juvenile, 28 mm SL), revealing canal neuromasts in the MD and PO canals. **d** *A. stuartgranti* (adult, 78 mm SL) and **e** *Tramitichromis* (adult, 79 mm SL) in ventral view. Asterisks (*) denote the locations of the MD and PO canal neuromasts, as visualized in **c**; canal neuromasts are found in floor of the canal, between canal pore positions in the canal roof. Note the much larger pores in *A. stuartgranti* (**d**) than in *Tramitichromis* (**e**). **f** MD canal neuromast in a juvenile *A. stuartgranti*. Ciliary bundles of the sensory hair cells are evident in an elongate sensory strip in the middle of the diamond-shaped neuromast. Double-headed arrow below the sensory strip indicates the axis of physiological sensitivity of the hair cells, as well as the long axis of the canal in which the neuromast is found. Scale bar = 10 μm. **g** *A. stuartgranti* and **h** *Tramitichromis* in frontal-ventral view with the pores of the SO, IO, MD, and PO canals that are directed ventrally, toward the source of stimuli generated by benthic prey. The pores on the right side of each fish in **g** and **h** have been enhanced to increase their visibility.

Fig. 2 Experimental setup and light conditions used to record feeding behavior of *A. stuartgranti* and *Tramitichromis*. **a** Diagram of experimental tank with front panel of light curtain removed. Light sources originate from two fluorescent light fixtures (full spectrum light) and two infrared (IR) lights. **b** Sample light spectra generated by fluorescent lights in behavioral trials. The y-axis was compressed to illustrate peaks in the visual spectrum (400-700 nm) that occurred in 1-800 lx trials. These peaks were consistent when light intensity was decrease with the addition of neutral density filters among trials (see Table 1). The peak at 840 nm is from two IR lights in 0 and 1 lx trials only. **c** Mean (± s.e.m.) light intensities measured before and after trials indicating that light intensity did not differ before and after trials at any of the light intensities used (students *t*-test, $P > 0.05$)

Fig. 3 Total number of prey detections by prey type (white bars = live tethered brine shrimp, grey bars = dead tethered brine shrimp) for **a** *A. stuartgranti* ($n = 6$ fish) and **b** *Tramitichromis* ($n = 6$ fish) at five different light intensities. Maximum number of possible prey detections = 72 for each light intensity

Fig. 4 Three behavioral parameters defining prey detection in *A. stuartgranti* ($n = 6$ fish) and *Tramitichromis* ($n = 6$ fish) at five different light intensities. (**a**, **c**, **e**) Mean (± s.e.m.) number of prey detections (maximum 6 live, 6 dead tethered brine shrimp), detection distance, and detection-to-strike velocity, for *A. stuartgranti* feeding on live (—■—) and dead (---□---) prey, and (**b**, **d**, **f**) *Tramitichromis* feeding on live (—●—) and dead (---○---) prey

Fig. 5 Frequency of prey strikes (live and dead prey combined, 12 total prey/trial) during glide or pause phases of swimming at five different light intensities in **a** *A. stuartgranti* (n = 6 fish) and **b** *Tramitichromis* (n = 6 fish)

Fig. 6 Detection angle for live and dead prey combined (=12 total prey/trial) at five different light intensities for **a** *A. stuartgranti* (n = 6 fish) and **b** *Tramitichromis* (n = 6 fish). Black lines represent the proportion of prey detections grouped into 20° intervals. Fish snout is at the center and fish is facing 0° (indicated by the grey arrow in the top plot in **a**). The thin line represents the mean angle for all trials. Results for *Tramitichromis* at 0 lx were not included here due to the small number of strikes (n = 3 strikes)

Fig. 7 Mean (± s.e.m.) prey preference scores (following Taplin 2007) for **a** *A. stuartgranti* (n = 6 fish) and **b** *Tramitichromis* (n = 6 fish) feeding on six live (white bars) and six dead (gray bars) tethered adult brine shrimp in trials at five different light intensities. Preferences scores were calculated by taking the mean of the rank order in which prey were captured. The dotted line (= 6.5) indicates the mean preference score with no preference for either prey type. Scores <6.5 (below dotted line) indicate a preference. Significantly different preference scores between live and dead prey indicated by an asterisk (*, paired t-test, $P < 0.05$, Table 5)

Table 1 The relationship of measured light intensity (mean lux and PAR, ± s.e.m. measured immediately after behavioral trials) and predicted depths at which these intensities occur in Lake Malawi. Calculations were based on midday sunlight levels, three light extinction coefficients (0.10 m^{-1}, Patterson et al. 2000; 0.13 m^{-1}, 0.43 m^{-1}, Guildford et al. 2007), and light intensities under natural conditions (Harden Jones 1955, Ali 1959). Light intensities were achieved by varying the height of two fluorescent fixtures (ballasts) and/or covering these fixtures with several neutral density filters

Light Intensity		Light Extinction Coefficient			
Lux	PAR	$\varepsilon = 0.10\ m^{-1}$	$\varepsilon = 0.13\ m^{-1}$	$\varepsilon = 0.43\ m^{-1}$	
Lumen/m^2	µmol photons/m^2/s	Depth (m)	Depth (m)	Depth (m)	Light intensities under natural conditions
800 (800.8 ± 5.4)	11.0 (11.0 ± 0.10)	52	40	12	Very cloudy day
112 (112.4 ± 1.9)	1.5 (1.51 ± 0.03)	72	55	17	Twilight
12 (12.0 ± 0.3)	0.2 (0.16 ± 0.01)	92	71	21	Twilight
1 (1.4 ± 0.1)	0.03 (0.03 ± 0.01)	115	89	27	Full moon/deep twilight
0 (0 ± 0.1)	0 (0.000 ± 0.003)	NA	NA	NA	New moon

Table 2 Determination of GLMM types used to analyze four parameters of feeding behavior at five different light intensities (0-800 lx) in interspecific and intraspecific comparisons

Source	Distribution	Link	Covariance Structure	AICC
4-way GLMMs				
Number of prey strikes	Multinomial	Negative log-log	AR(1)	1,713.0
Detection distance	Normal*	Identity	AR(1)	177.9
Detection-to-strike velocity	Normal*	Identity	AR(1)	-32.4
Swimming phase at prey detection	Binomial	Probit	AR(1)	1932.8
3-way GLMMs				
Aulonocara stuartgranti				
Number of prey strikes	Multinomial	Probit	AR(1)	698.1
Detection distance	Normal*	Identity	AR(1)	44.9
Detection-to-strike velocity	Normal*	Identity	AR(1)	-24.6
Swimming phase at prey detection	Binomial	Probit	AR(1)	1065.9
Tramitichromis				
Number of prey strikes	Multinomial	Negative log-log	AR(1)	955.4
Detection distance	Normal*	Identity	AR(1)	130.6
Detection-to-strike velocity	Normal*	Identity	AR(1)	-7.2
Swimming phase at prey detection	Binomial	Probit	AR(1)	869.1

Note: the table includes information on the error distribution and link function. The first-order auto-regressive process [AR(1)] was used for the covariance structure in all models. The most parsimonious model was selected based on the corrected Akaike information criterion (AICC).

*Data was log10 transformed to achieve normality (normality assessed with Kolmogorov-Smirnov test).

Table 3 Summary of 4-way GLMM statistics for prey detection behavior for two species (*A. stuartgranti*, *n* = 6 fish; *Tramitichromis,* *n* = 6 fish) feeding on two prey types (live, dead) at five light intensities (0-800 lx). Only those factors that are significant for at least one behavioral parameter are listed. See Table 2 for details of GLMMs used

	Number of prey detections		**Detection distance**		**Detection-to-strike velocity**		**Swimming phase at prey detection**	
Source	*F* (*df*)	*P* value	*F* (*df*)	*P* value	*F* (*df*)	*P* value	*F* (*df*)	*P* value
Species (S)		n.s.		n.s.		n.s.		n.s.
Light intensity (L)	14.390 (1,99)	**<0.001**	9.480 (1,481)	**0.002**	8.919 (1,511)	**0.003**	8.276 (1,18)	**0.010**
Time of day (T)	22.203 (1,99)	**<0.001**	17.342 (1,512)	**<0.001**	16.838 (1,513)	**<0.001**	0.038 (1,129)	0.847
Prey type (P)	4.549 (1,99)	**0.035**	1.145 (1,503)	0.285	3.876 (1,504)	**0.050**	3.037 (1,514)	0.082
S × L	8.950 (1,99)	**0.004**		n.s.		n.s.		n.s.
S × P		n.s.		n.s.		n.s.	4.288 (1,514)	**0.039**
L × T	14.101 (1,99)	**<0.001**	10.479 (1,482)	**0.001**	9.957 (1,512)	**0.002**	6.044 (1,62)	**0.017**
S × L × T	10.464 (1,99)	**0.002**		n.s.		n.s.		n.s.

n.s. = not significant ($P > 0.05$)

P values < 0.05 are shown in bold

Table 4 Summary of 3-way GLMM statistics for prey detection behavior for *A. stuartgranti* (*n* = 6 fish) and *Tramitichromis* (*n* = 6 fish) feeding on two prey types (live, dead) at five different light intensities (0-800 lx). See Table 2 for details of GLMMs used

	Aulonocara stuartgranti		*Tramitichromis*	
Model term	*F* (*df*)	*P* value	*F* (*df*)	*P* value
Number of prey detections				
Light intensity (L)	0.282 (1,47)	0.598	11.867 (1,47)	**0.001**
Time of day (T)	11.212 (1,47)	**0.002**	23.887 (1,47)	**<0.001**
Prey type (P)	1.649 (1,47)	0.205	0.109 (1,47)	0.743
L × T	0.293 (1,47)	0.591	12.162 (1,47)	**0.001**
L × P	0.845 (1,47)	0.363	0.078 (1,47)	0.780
T × P	0.968 (1,47)	0.330	0.003 (1,47)	0.956
L × T × P	0.675 (1,47)	0.416	0.037 (1,47)	0.847
Detection distance				
Light intensity (L)	2.772 (1,242)	0.097	6.185 (1,217)	**0.014**
Time of day (T)	26.812 (1,291)	**<0.001**	5.655 (1,223)	**0.018**
Prey type (P)	8.220 (1,286)	**0.004**	0.000 (1,217)	0.986
L × T	3.408 (1,240)	0.066	6.677 (1,217)	**0.010**
L × P	0.002 (1,286)	0.965	4.019 (1,220)	**0.046**
T × P	4.604 (1,286)	**0.033**	0.026 (1,217)	0.872
L × T × P	0.004 (1,286)	0.949	4.211 (1,220)	**0.041**
Detection-to-strike velocity				
Light intensity (L)	3.158 (1,200)	0.077	4.695 (1,223)	**0.031**
Time of day (T)	16.895 (1,290)	**<0.001**	8.058 (1,221)	**0.005**
Prey type (P)	20.107 (1,286)	**<0.001**	0.012 (1,218)	0.912
L × T	3.465 (1,198)	0.064	5.495 (1,223)	**0.020**

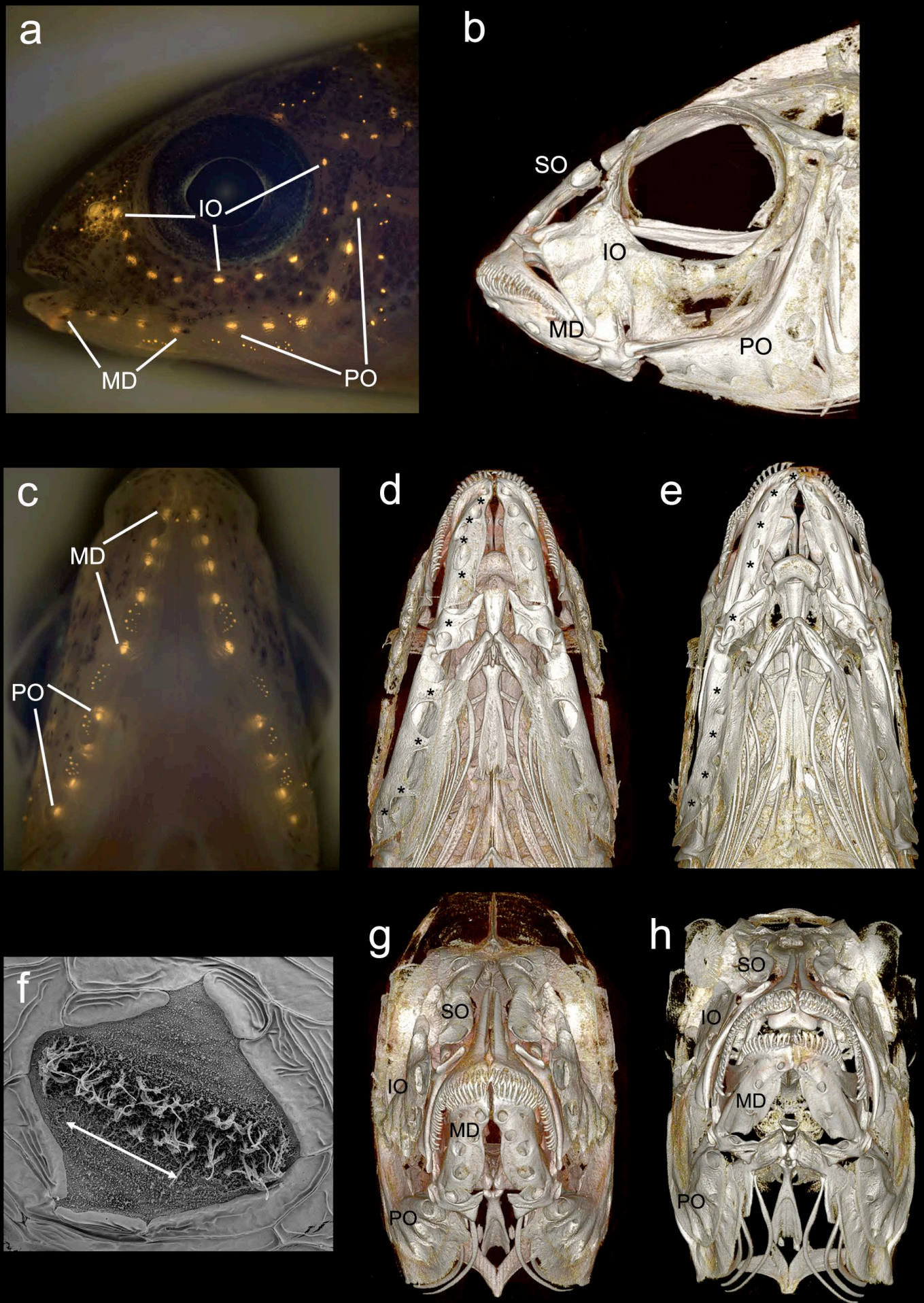
L × P	0.385 (1,286)	0.535	2.220 (1,219)	0.138
T × P	14.330 (1,286)	**<0.007**	0.014 (1,218)	0.907
L × T × P	0.393 (1,286)	0.531	2.300 (1,219)	0.131
Swimming phase at prey detection				
Light intensity (L)	0.289 (1,291)	0.592	3.208 (1,223)	0.075
Time of day (T)	1.593 (1,291)	0.208	0.068 (1,223)	0.794
Prey type (P)	0.147 (1,291)	0.701	3.794 (1,223)	0.053
L × T	0.128 (1,291)	0.720	2.873 (1,223)	0.091
L × P	0.220 (1,291)	0.639	0.433 (1,223)	0.511
T × P	0.202 (1,291)	0.653	2.588 (1,223)	0.109
L × T × P	0.211 (1,291)	0.646	0.438 (1,223)	0.509

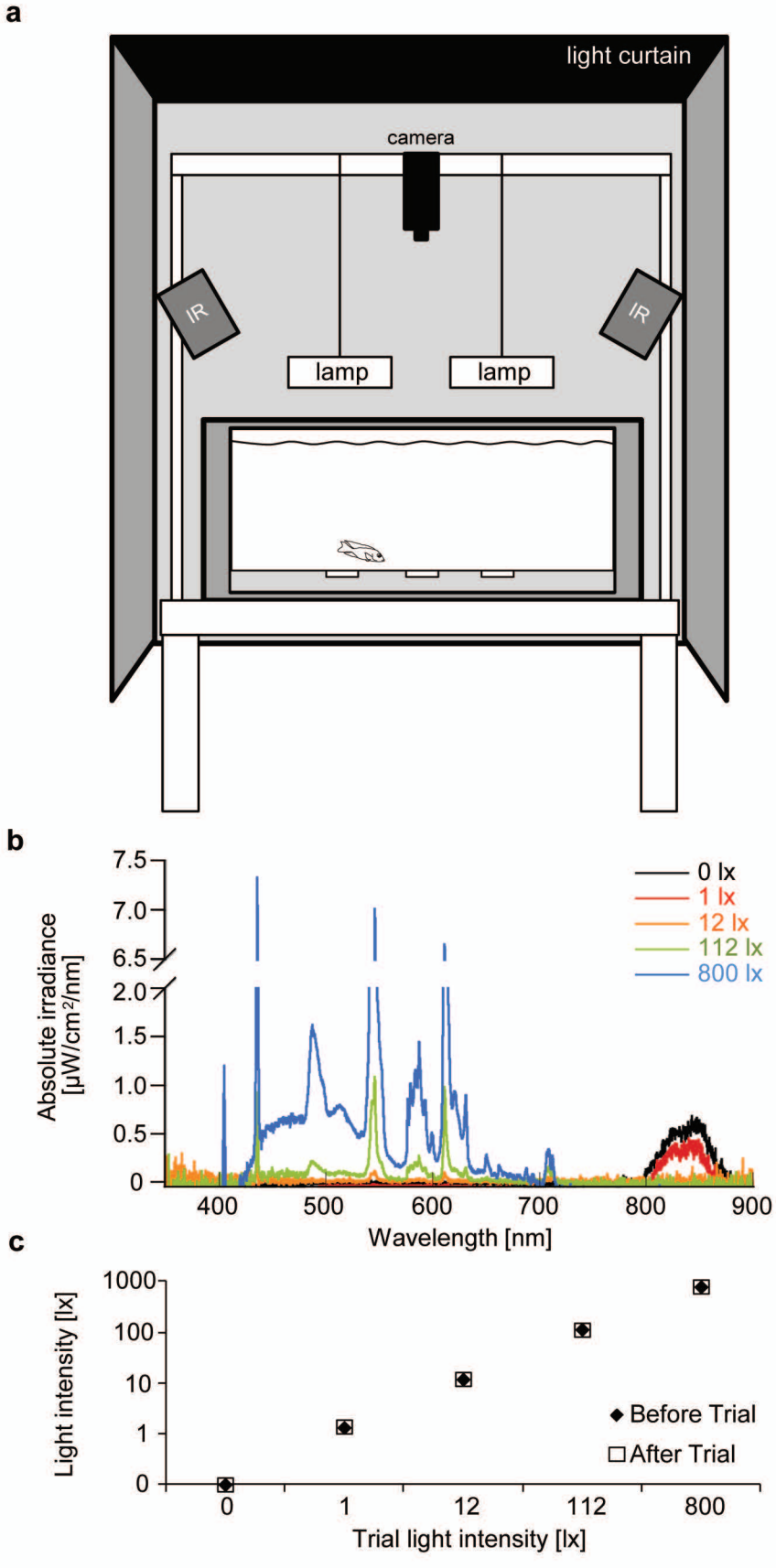
P values < 0.05 are shown in bold

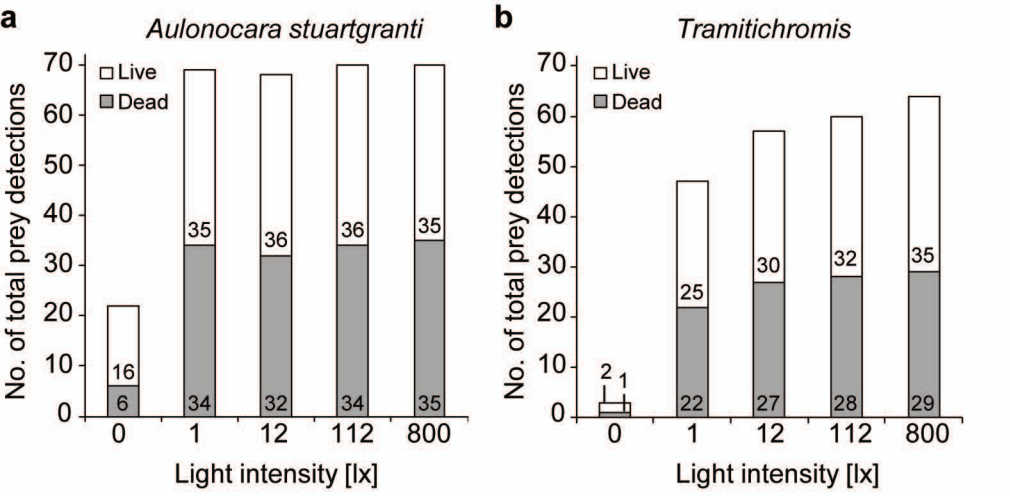
Table 5 Summary of the paired *t*-tests comparing prey preference scores of live versus dead prey of *A. stuartgranti* and *Tramitichromis* (following Taplin 2007) by light intensity

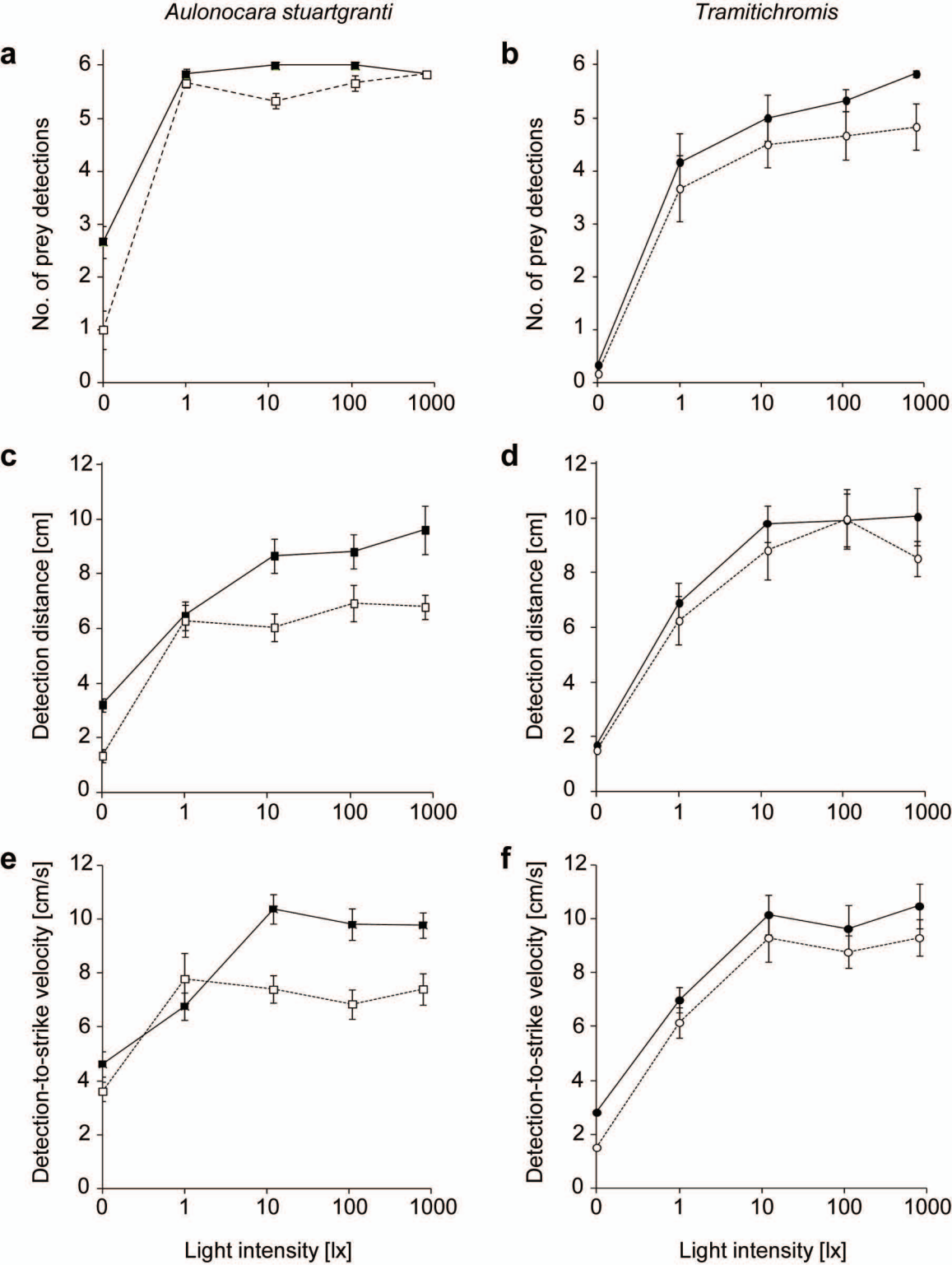
Light intensity (lx)	*T (df)*	*P* value
Aulonocara stuartgranti		
0	-2.853 (5)	**0.036**
1	-0.618 (5)	0.564
12	-1.395 (5)	0.222
112	-6.102 (5)	**0.002**
800	-1.892 (5)	0.117
Tramitichromis		
0	-0.797 (5)	0.461
1	-2.396 (5)	0.062
12	-1.379 (5)	0.226
112	-2.441 (5)	0.059
800	-13.647 (5)	**<0.001**

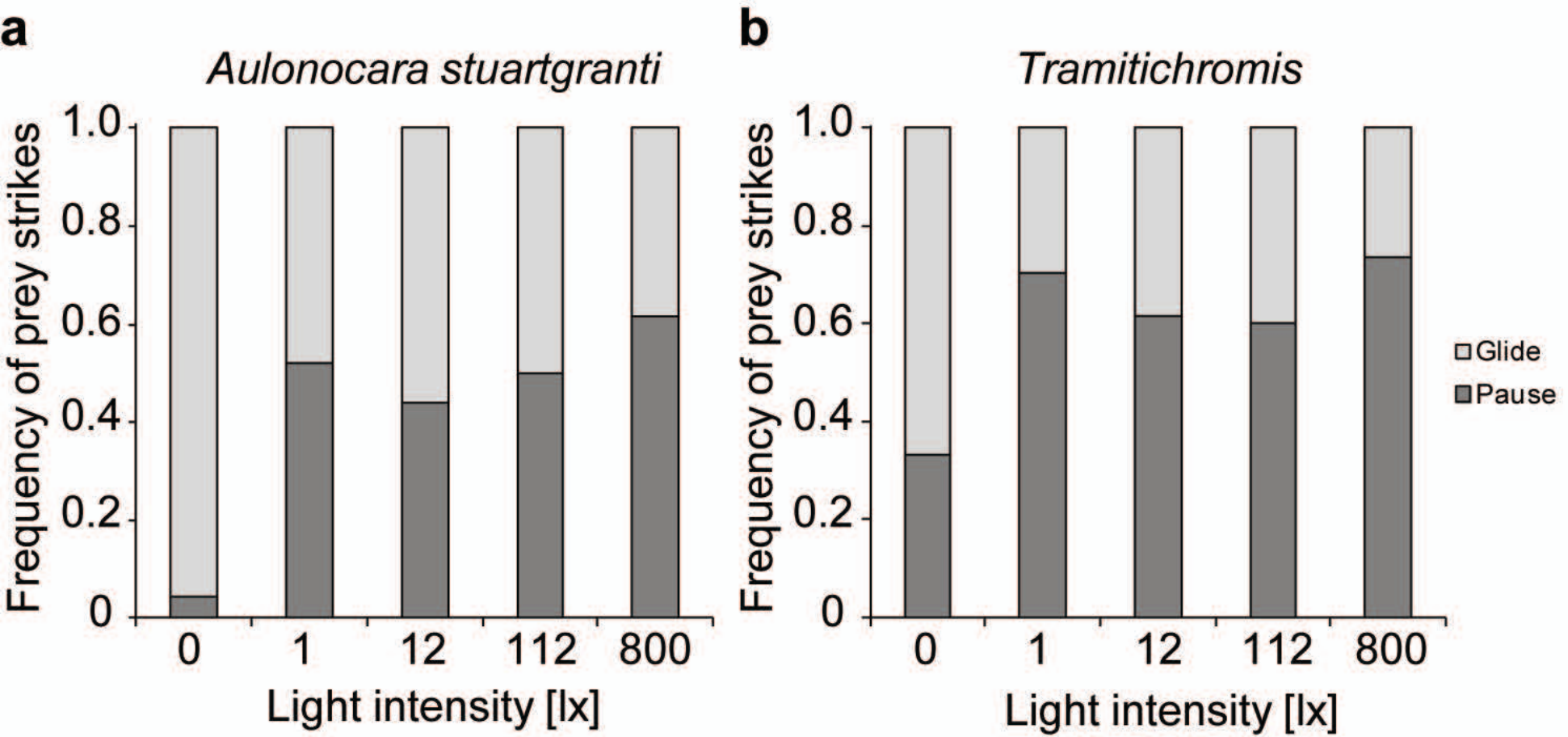
P values < 0.05 are shown in bold

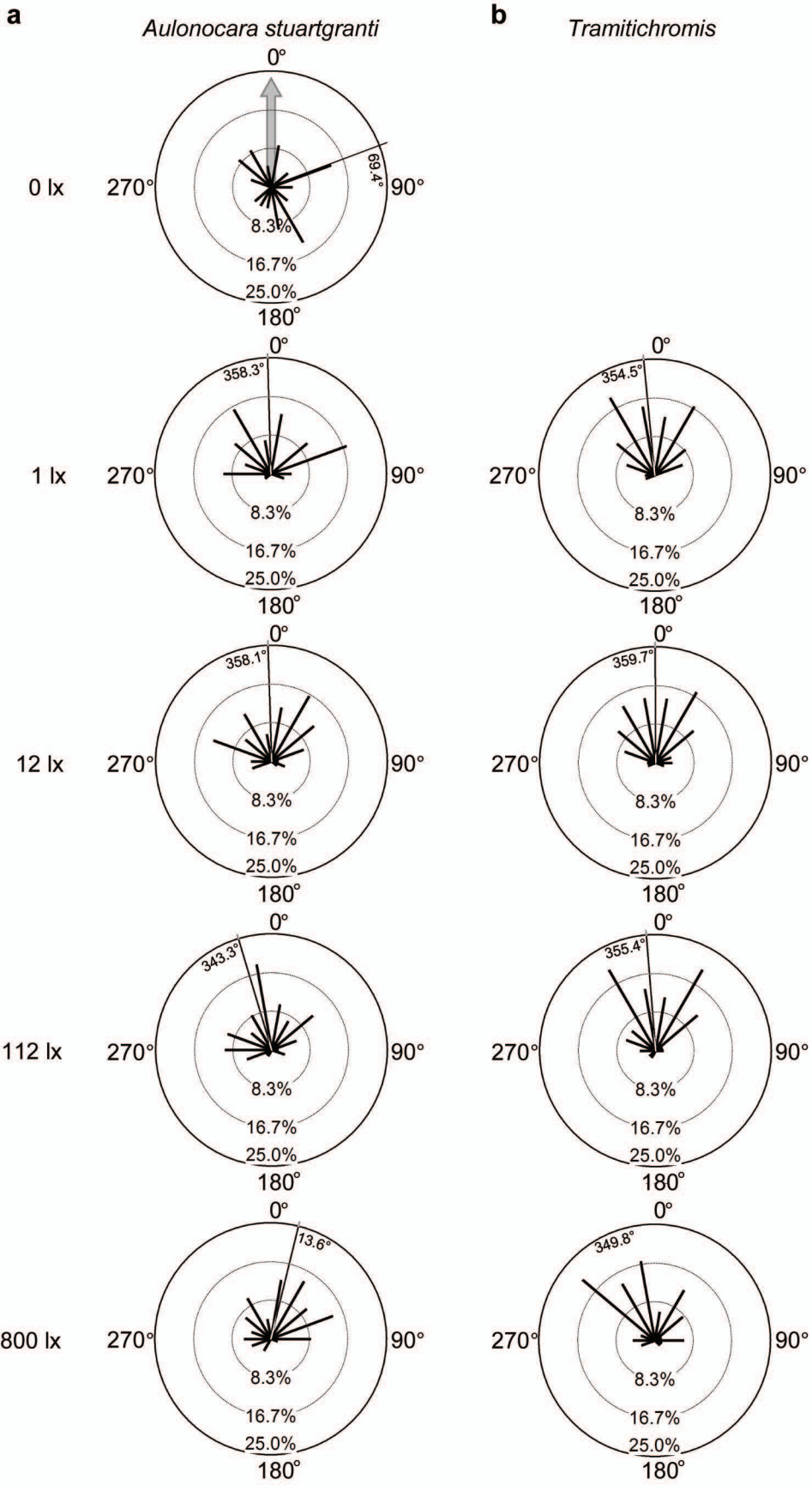

a
IO
MD
PO
b
SO
IO
MD
PO
c
MD
PO
d
e
f
g
SO
IO
MD
PO
h
SO
IO
MD
PO


a
light curtain
camera
IR
IR
lamp
lamp
b
Absolute irradiance [μW/cm²/nm]
7.5
7.0
6.5
2.0
1.5
1.0
0.5
0
400
500
600
700
800
900
Wavelength [nm]
0 lx
1 lx
12 lx
112 lx
800 lx
c
Light intensity [lx]
1000
100
10
1
0
0
1
12
112
800
Trial light intensity [lx]
Before Trial
After Trial


a
Aulonocara stuartgranti
Live
Dead
No. of total prey detections
0
10
20
30
40
50
60
70
16
6
35
34
36
32
36
34
35
35
0
1
12
112
800
Light intensity [lx]
b
Tramitichromis
Live
Dead
No. of total prey detections
0
10
20
30
40
50
60
70
2
1
25
22
30
27
32
28
35
29
0
1
12
112
800
Light intensity [lx]


Aulonocara stuartgranti
Tramitichromis
a
b
c
d
e
f
No. of prey detections
Detection distance [cm]
Detection-to-strike velocity [cm/s]
Light intensity [lx]
0
1
10
100
1000
0
2
4
6
8
10
12


a
Aulonocara stuartgranti
Frequency of prey strikes
1.0
0.8
0.6
0.4
0.2
0
0
1
12
112
800
Light intensity [lx]
b
Tramitichromis
Frequency of prey strikes
1.0
0.8
0.6
0.4
0.2
0
0
1
12
112
800
Light intensity [lx]
Glide
Pause


a
Aulonocara stuartgranti
b
Tramitichromis
0 lx
1 lx
12 lx
112 lx
800 lx
0°
90°
180°
270°
8.3%
16.7%
25.0%
69.4°
358.3°
354.5°
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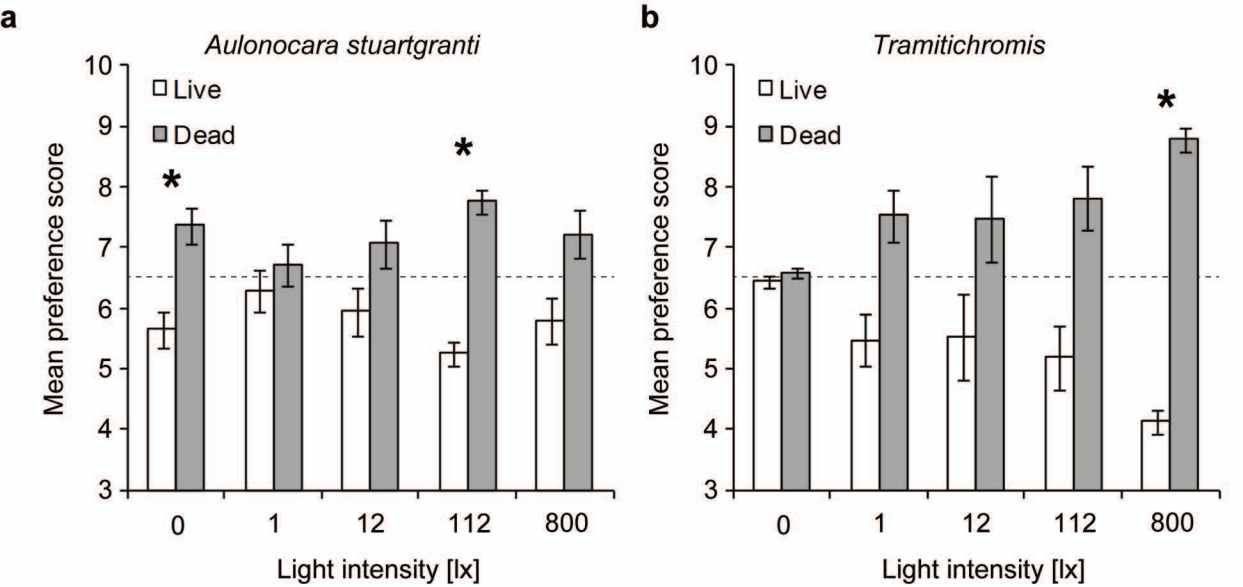
359.7°
343.3°
355.4°
13.6°
349.8°


a
Aulonocara stuartgranti
Live
Dead
Mean preference score
10
9
8
7
6
5
4
3
*
*
0
1
12
112
800
Light intensity [lx]
b
Tramitichromis
Live
Dead
Mean preference score
10
9
8
7
6
5
4
3
*
0
1
12
112
800
Light intensity [lx]