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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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Aulonocara stuartgranti **and** *Tramitichromis* **sp.**

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Tables: 5

Figures: 7 (2 color)

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23

24 **Abstract**

25

26 Two Lake Malawi sand-dwelling cichlids (*Aulonocara stuartgranti*, *Tramitichromis* sp.) that

27 have different lateral line phenotypes, but feed on benthic invertebrates, have been shown to use

28 lateral line and/or visual cues to detect prey under light versus dark conditions. The current study

29 examined how ecologically relevant variation in light intensity (0-800 lux) influences detection

30 of prey (mobile, immobile) in each species by analyzing six well-defined behavioral parameters.

31 Both species fed at light intensities ≥1 lux; prey type and/or time of day (but not light intensity)

32 predicted all four parameters analyzed with generalized linear mixed models in *A. stuartgranti*,

33 whereas the interaction of light intensity and time of day predicted three of these parameters in

34 *Tramitichromis* sp. Data for all six parameters suggest that the critical light intensity is 1-12 lux

35 for both species, the integration of visual and lateral line input explains differences in the

36 detection of mobile and immobile prey and the behavioral changes that occur at the transition

37 from 1 to 0 lux in *A. stuartgranti*, and that *Tramitichromis* sp. likely uses binocular vision to

38 locate prey. The sensory biology of species that exploit similar food resources will have

39 important implications for the trophic ecology of African cichlid fishes.

40

44 **Abbreviations**

45 AICC   Akaike information criterion

46 GLMM   Generalized linear mixed model

47	Lx	Lux
48	PAR	Photosynthetically active radiation
49	SL	Standard length
50	TL	Total length

51

52 **Introduction**

53

54 Light in aquatic habitats varies in quality and quantity over time and space (Kirk 2011) and

55 influences the ability of visual predators to detect and capture mobile prey (Vinyard and O'Brien

56 1976; Confer et al. 1978; Lythgoe 1979; Ryer and Olla 1999; Vogel and Beauchamp 1999;

57 Rickel and Genin 2005). Fishes occupying similar habitats may demonstrate variation in

58 visually-mediated prey detection abilities, such as visual thresholds and absorption spectra of

59 visual pigments, which may provide a competitive advantage under particular light conditions

60 (Vogel and Beauchamp 1999; Hofmann et al. 2009). Many fishes are also able to detect prey at

61 low light intensities (e.g., dawn, dusk, at depth, or with increased turbidity), but with reduced

62 capabilities compared to that at higher light intensities. The distance at which free swimming

63 prey are detected dramatically decreases below a certain light intensity ("critical light intensity,"

64 Confer et al. 1978) in salmonids (Dunbrack and Dill 1984; Henderson and Northcote 1985) and

65 some freshwater percomorphs (bluegill, *Lepomis macrochirus*, Vinyard and O'Brien 1976;

66 largemouth bass, *Micropterus salmoides*, Howick and O'Brien 1983; yellow perch, *Perca*

67 *flavescans*, Richmond et al. 2004).

68 Given the importance of multimodal sensory integration in the formulation of behavior,

69 the contributions of the non-visual sensory systems to prey detection (e.g., mechanosensory

70   lateral line, auditory, olfactory, gustatory, somatosensory/tactile, and in some cases, the

71   electrosensory system; reviewed in Montgomery et al. 2014) must also be considered.

72   Morphological and/or physiological specializations of non-visual sensory systems, including the

73   olfactory system (Parzefall 1993; Montgomery et al. 1999), gustatory system (Atema 1971) and

74   the lateral line system (Janssen 1997; Schwalbe et al. 2012, reviewed in Webb 2014), have been

75   used to predict how these senses provide alternatives to vision for prey detection in light-limited

76   environments. Futhermore, the integration of different combinations of sensory inputs may

77   explain variation in behavior under different environmental conditions (Partridge and Pitcher

78   1980; Moller 2002; Montgomery et al. 2003; Gardiner and Motta 2012). Several species of fishes

79   have been shown to modulate feeding strategies using a combination of visual and non-visual

80   cues that allow them to feed under a range of light conditions, including darkness (Townsend and

81   Risebrow 1982; Batty et al. 1986; Diehl 1988; Schwalbe et al. 2012).

82   The mechanosensory lateral line system is known to play important roles in prey

83   detection, as well as in predator avoidance, communication, and navigation around obstacles

84   (Webb et al. 2008; Montgomery et al. 2014). The system demonstrates a great deal of variation,

85   which is defined by the morphology of the cranial and trunk lateral line canals and neuromast

86   receptor organs within them, and the distribution of superficial neuromasts on the skin of the

87   head, trunk and tail (reviewed in Webb 2014). Widened lateral line canals, one of five cranial

88   lateral line canal phenotypes found among bony fishes, has evolved convergently in ~12 teleost

89   families (including deep sea taxa) and appears to be an adaptation for enhanced sensitivity to

90   water flows and prey detection (Denton and Gray 1988, 1989; Montgomery and Coombs 1992;

91   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

115   field. Species of *Aulonocara* swim just above the substrate to sense hydrodynamic flows

116   generated by benthic invertebrates in or on the substrate in the field (Konings 2007). In contrast,

117   species of *Tramitichromis* typically capture invertebrate prey by plunging into the substrate,

118   filling their mouth with sand, and sifting out prey with their gill rakers in the field (= "sand

119   sifting," Fryer, 1959). This sand sifting behavior appears to be synonymous with the

120   "winnowing" behaviors observed in some surfperches (Laur and Ebeling 1983) and vision likely

121   contributes to the ability to locate patches of high quality food resources where "winnowing"

122   takes place (Holbrook and Schmitt 1984; Schmitt and Holbrook 1984).

123   This study uses the same methods used in prior studies (Schwalbe et al. 2012; Schwalbe

124   and Webb 2014) to test the hypothesis that variation in light intensity (0-800 lx) will have

125   different effects on the detection of live (mobile) and dead (immobile) benthic invertebrate prey

126   in *A. stuartgranti* and *Tramitichromis* sp.

127

128   **Materials and methods**

129

130   Study species

131

132   Adult *Aulonocara stuartgranti* and *Tramitichromis* sp. (unidentifiable to species level, J.

133   Stauffer, pers. commun.), is referred to as *Tramitichromis* throughout. These fish were reared in

134   the laboratory from breeding stock originally acquired from commercial suppliers (*A.*

135   *stuartgranti*: Bluegrass Aquatics, Louisville, KY, USA; *Tramitichromis*: Old World Exotic Fish,

136   Inc., Homestead, FL, USA and Life Fish Direct, Draper, UT, USA). They were housed in small

137   groups by species in 190 L aquaria at 26±1°C and 1.0±0.2 p.p.t. salinity (using Cichlid Lake Salt,

138   Seachem Laboratories, Inc., Madison, GA, USA) with standard white fluorescent light on a

139   12h:12h diurnal cycle and, equipped with appropriate mechanical and biological filtration. Fish

140   were fed daily with cichlid pellets (New Life Spectrum Cichlid Formula; New Life International,

141   Inc., Homestead, FL) and supplemented with live adult brine shrimp. Individual fish were not

142   used in feeding experiments if breeding behavior was observed. Animal care and all

143   experimental procedures followed an approved IACUC protocol.

144

145   Light environment in the experimental tank

146

147   Light in the experimental tank was provided by two fluorescent light fixtures (Lithonia Lighting,

148   Model GRW 2 14 CSW CO M4, Conyers, GA, USA) fitted with full spectrum bulbs (BlueMax

149   lamps, Full Spectrum Solutions, Jackson, MI, USA) positioned above the tank and within an

150   opaque curtain enclosure. The curtain (black canvas) was suspended from a rectangular plywood

151   frame placed 2 m above the top of the tank in order to exclude ambient light from entering the

152   set-up during all behavioral trials (Fig. 2a). Light intensity was varied by changing the height of

153   the lights above the water surface and using combinations of different neutral density filters

154   covering the lights (Lee Filters, Burbank, CA, USA). Light intensity (in lux [lx], lumen/m^2, and

155   photosynthetically active radiation [PAR], μmol photons/m^2/s) and color spectrum were

156   measured using a spectrometer (range: 340-1028 nm, Jaz spectrometer, Ocean Optics, Dunedin,

157   FL, USA) connected to a 2 m optical fiber (QP400-2-UV/VIS, Ocean Optics) fitted with a cosine

158   corrector (CC-3, Ocean Optics). Water temperature was monitored during experiments and the

159   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

10

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

228 trials carried out during the day.

229   To assess the number of prey detections that lead to prey strikes, unconsumed prey were

230 counted at the end of each 30-minute trial and strike success was also confirmed in video

231 recordings. Video sequences leading to each prey strike were exported to Premier Pro (Adobe,

232 CS5) for further analysis. Analysis of sequential video frames was used to identify the phase of

233 swimming behavior (thrust, glide, or pause) during which prey detections occurred. Detection

234 distance and detection angle were measured in these images using ImageJ (NIH, v. 1.41o).

235 Detection distance was defined as the distance from the tip of a fish's mouth to the prey,

236 measured in the frame immediately before the fish oriented towards it (e.g. before a turn defining

237 detection). For each prey strike, detection-to-strike velocity was calculated by dividing detection

238 distance by the time interval between detection and initiation of a strike. Detection angle was

239 measured in the same video frame in which detection distance was measured, and was defined as

240 the angle between a line extending anteriorly along midline of the fish (body axis) and a line

241 drawn from the prey to the tip of the fish's mouth.

242

243 Statistical analysis

244

245 Four of the six behavioral parameters were analyzed using generalized linear mixed models

246 (GLMMs; number of prey strikes, detection distance, detection-to-strike velocity, phase of

247 search behavior during which detections occurred). In addition, a ranking method (Taplin 2007)

248 was used to analyze prey preferences (live versus dead prey) and circular statistics were used to

249 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).

272   The order in which live (mobile) and dead (immobile) prey were struck was analyzed in

273   each species following Taplin (2007). This method assumes that when presented with equal

274   numbers of two or more types of prey, the order in which prey are consumed provides

275   information about prey preference – that prey consumed first are more highly preferred than prey

276   consumed second, third, etc. and the last prey consumed is the least preferred. While differences

277   in handling time, encounter rates, and relative mobility of prey can potentially complicate the

278   results of this sort of analysis (Durham et al. 2012, McWilliam et al. 2013), such variation was

279   minimized in the current study by offering equal numbers of live and dead brine shrimp tethered

280   in the same arrangement to platforms placed in a 2 x 3 matrix in all trials. The null hypothesis for

281   this analysis was that live and dead prey would be consumed randomly during a trial. Videos

282   were analyzed so that each prey consumed was assigned a rank number (first prey consumed=1,

283   second prey consumed=2, etc.), and any remaining prey were assigned an average of the

284   remaining preference scores, and considered "tied for last." A pair of preference scores for live

285   and dead prey at each light intensity was calculated for each fish. The pairs of scores from all of

286   the fish were considered independent samples and thus grouped by light intensity and species for

287   analysis. A score of 6.5 (based on presentation of six live and six dead prey, 12 total prey in a

288   trial) indicated no preference, a score of <6.5 revealed a preference for that prey type, and a score

289   of >6.5 indicated no preference or that prey type was ignored or avoided. Scores for live and

290   dead prey at each light intensity and for each species, were compared separately using paired *t*-

291   tests (SPSS, IBM, v. 22).

292   Detection angles were analyzed with circular statistics (Oriana v. 3, Kovach Computing

293   Services, Anglesey, UK). Rayleigh tests were performed for each species to test whether

294   detections of live and dead prey at each of the five light intensities (0-800 lx) occurred at

295   uniformly or non-uniformly distributed positions around the fishes' body relative to the body

296   axis (e.g. to define the receptive field). Watson's U^2 tests were used to determine if detection

297   angles differed with prey type and with light intensity within and between species.

298

299   **Results**

300

301   *Aulonocara stuartgranti* and *Tramitichromis* sp. actively swam around the experimental tank

302   during trials at all light intensities, including darkness (0 lx), and used a saltatory search strategy

303   (a cyclic sequence of a caudal fin thrust, glide, and pause) while exploring the tank. Of the 360

304   total prey presented to fish during all 60 trials, *A. stuartgranti* struck at 299 prey (=83%) and

305   *Tramitichromis* struck at 231 prey (=64%; see Figs. 3, 4). Prey were detected by both species

306   during a glide or a pause, but never during a thrust (see Fig. 5).

307   Four-way GLMM analyses (Table 3) indicated that species alone did not predict

308   differences in any of the four behavioral parameters (number of prey detections, detection

309   distance, detection-to-strike velocity, or swimming phase at prey detection), and that only the

310   interaction of species, light intensity, and prey type had a significant effect only on number of

311   prey detections. The interaction of light intensity and time of day predicted differences in all four

312   behaviors. Light intensity alone predicted differences in all four behaviors, and time of day

313   predicted differences in three behavioral parameters (number of prey detections, detection

314   distance, detection-to-strike velocity), but not in swimming phase at prey detection. Separate

315   three-way GLMM analyses for each species (Table 4, see below) revealed interesting trends that

316   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

340 ≥1 lx (Rayleigh test, $P < 0.001$; ±90° from body axis) with no differences in the distribution of

341 angles among pairs of light intensities with the exception of the two highest light intensities (112

342 lx versus 800 lx; Watson's U^2 test, $U^2 = 0.19$, $P < 0.05$; Fig. 6a). Finally, fish tended to prefer

343 live prey at all light intensities, but only demonstrated a statistically significant preference for

344 live prey at 112 lx, but not at 800 lx (Table 5; Fig. 7a), which is not easily explained.

345   In the dark (0 lx), prey detection behavior of *A. stuartgranti* was different than at light

346 intensities ≥ 1 lx. Fish struck at only 22 prey (=30.6% of total prey presented; Fig. 3a), and

347 tended to detect prey at even shorter distances (Fig. 4c) and at slower detection-to-strike

348 velocities (Fig. 4e) than when at least some light was present. Fish tended to detect more live

349 prey than dead prey (mean of 2.7 and 1.0, respectively; Figs. 3a, 4a), showed a statistically

350 significant preference for live prey (Table 5, Fig. 7a), and detected live prey from more than

351 twice the distance than dead prey (3.2 and 1.4 cm, respectively; Fig. 4c). In addition, detection-

352 to-strike velocity at 0 lx was about one half of that at higher light intensities (~3.5-5 cm/s at 0 lx

353 versus ~7-10 cm/s at ≥ 1 lx), but fish tended to detect live prey at somewhat higher detection-to-

354 strike velocities than dead prey (Fig. 4e). In the dark, 95% of prey were detected during a glide

355 and only a few prey (5%) were detected during a pause (Fig. 5a). Prey (live and dead combined)

356 were detected at positions uniformly distributed around fishes' bodies (Rayleigh test, $P > 0.05$)

357 at a wide range of angles (± 180° from body axis, Fig. 6a), but the distribution of detection

358 angles did not differ for live versus dead prey (Watson's U^2 test, $P > 0.05$).

359

360 Feeding behavior of *Tramitichromis*

361

362   GLMM analyses (Table 4) showed that, in contrast to *A. stuartgranti*, the interaction of light

363   intensity and time of day predicted three of four behavioral parameters (number of prey

364   detections, detection distance, and detection-to-strike velocity). As in *A. stuartgranti*, neither

365   light intensity, time of day, nor prey type, or their interactions, predicted swimming phase at prey

366   detection. Prey type did not predict any of the four behavioral parameters in *Tramitichromis*, and

367   the interaction of light intensity and prey type predicted only detection distance (Table 4).

368   An examination of trends for each of the behavioral parameters revealed that

369   *Tramitichromis* tended to strike at high numbers of prey (Fig. 3b, 4b), and >60% of prey (live

370   and dead, combined) were detected during a pause at light intensities of 1-800 lx (Fig. 5b). At

371   light intensities of 12-800 lx, fish struck at live and dead prey from similar, long detection

372   distances (means = 9.8-10.1 and 8.5-10.0 cm, respectively) and at high detection-to-strike

373   velocities (9.6-10.5 and 8.7-9.3 cm/s, respectively). In contrast, at 1 lx, fish tended to strike at

374   both live and dead prey at similar, but shorter detection distances (6.9 and 6.3, respectively) and

375   lower detection-to-strike velocities (7.0 and 6.1, respectively; Figs. 4d, f) than at higher light

376   intensities. Both live and dead prey were detected at non-uniform positions around the body

377   (Rayleigh test, $P < 0.001$), which defined a very narrow range of detection angles from the body

378   axis ($\pm 40°$); distributions were the same for live prey and dead prey at light intensities of 1-800

379   lx (Watson's U^2, $P > 0.05$; Fig. 6b). Fish tended to prefer live prey at different light intensities,

380   but only showed a statistically significant preference for live prey at the highest light intensity

381   (800 lx; Table 5; Fig. 7b).

382   Despite being active in the dark (0 lx), *Tramitichromis* only struck at only 3 prey (=4.2%

383   of the 72 prey presented). These strikes are likely to have been the result of random encounters

384   with prey as opposed to being the result of active search and directed strikes.

17

385

Discussion

386

387

388 The multiple statistical analyses presented here, and the detailed examination of trends in the

389 detection of live and dead prey at different light intensities in each species indicate that light

390 intensity affects prey detection behavior in different ways in *Aulonocara stuartgranti* and

391 *Tramitichromis*.

392

393 Feeding behavior of *Aulonocara stuartgranti* and *Tramitichromis*

394

395   Prey type and/or time of day, but not light intensity, were predictors of three of the four

396 behavioral parameters (number of prey detections, detection distance, and detection-to-strike

397 velocity) analyzed using GLMMs in *A. stuartgranti*. The lack of significance for light intensity is

398 consistent with the use of lateral line cues (see also Schwalbe et al. 2012), but also suggests that

399 *A. stuartgranti* may use a light-independent circadian rhythm to interpret time of day. This is

400 consistent with the occurrence of *Aulonocara* species at depths up to120 m in Lake Malawi

401 where light is limited or absent (Konings 1990, 2007) and in caves where spawning has been

402 reported (Grant et al. 1987), and thus where normal diurnal variation in light intensity may not be

403 a consistent or reliable cue for the regulation of behavior. The significance of prey type as a

404 predictor of detection distance and detection-to-strike velocity is illustrated by apparent

405 differences in numbers of live and dead prey detected at the same light intensities (Fig. 4; see

406 also Schwalbe et al. 2012; Schwalbe and Webb 2014), the tendency to prefer live prey at all light

407 intensities, and the statistically significant preference for live prey in the dark.

18

408   In contrast, in *Tramitichromis,* it is the interaction of light intensity and time of day that

409   predict these same three behavioral parameters. The importance of light intensity not surprising

410   because *Tramitichromis* uses visual, but not lateral line cues, for prey detection and does not feed

411   in the dark (Schwalbe and Webb 2014). Furthermore, these two factors are correlated both in the

412   lab where the fish were reared (on a 12:12 hr light/dark cycle) as well as in the relatively shallow

413   waters in their natural habitat in Lake Malawi, which is just 9-17° south of the equator where

414   fish experience 11-13 hours of daylight per day on an annual basis (http://astro.unl.edu). Thus,

415   these fish have evolved and are reared in environments where light intensity and time of day are

416   tightly correlated. The independent roles of these two factors in predicting behavior would need

417   to be addressed in additional experiments, which were out of the scope of this study.

418   Swimming phase (glide, pause) during which prey were detected was predicted neither

419   by light intensity nor by time of day in either species. The ability to detect prey during a glide or

420   pause will affect both the stabilization of the visual field (for vision-mediated detection) and/or

421   the magnitude of environmental and self-generated hydrodynamic noise (for lateral line-

422   mediated detection). *A. stuartgranti* and *Tramitichromis* both detected between 40% and 70% of

423   prey during a pause at light intensities of ≥1 lx, suggesting the importance of stabilizing the

424   visual field for prey detection at these light intensities. Prey type (which defines the presence or

425   absence of an additional visual motion stimulus) did not predict swimming phase at detection for

426   *Tramitichromis* ($P < 0.053$), but a larger sample size may have yielded a different statistical

427   outcome. Prey type also did not predict swimming phase at prey detection for *A. stuartgranti,* but

428   the shift to 95% of prey detections during a glide in the dark (where stabilization of visual field is

429   irrelevant), and their preference for live prey (that generate hydrodynamic flows detected in the

430   dark; Schwalbe and Webb 2014), are important indicators of the overall importance of prey type.

19

431 *A. stuartgranti* detected live prey at distances of less than half of a body length and at lower

432 detection-to-strike velocities at a low light intensity (1 lx) and in the dark (0 lx). In the presence

433 of at least some light, lower detection-to-strike velocities would also reduce self-generated

434 hydrodynamic noise (Montgomery et al. 2009), enhancing lateral line-mediated prey detection,

435 which would suggest that fish would tend to detect prey during a pause. However, the high

436 proportion of detections (95%) at relatively low detection-to-strike velocities, while not

437 eliminating self-generated noise, would bring a fish into the vicinity of potential prey that are

438 generating detectable hydrodynamic flows (Schwalbe et al. 2012).

439

440 Role of vision and critical light intensities

441

442   The importance of vision in *A. stuartgranti* and *Tramitichromis* is further supported by a

443 consideration of critical light intensities and the potential differences in the use of binocular

444 vision. Prey detection at relatively long distances is consistent with vision-mediated prey

445 detection in fishes (Vinyard and O'Brien 1976, Confer et al. 1978, Henderson and Northcote

446 1985, Mazur and Beauchamp 2003), and at higher light intensities detection of free swimming

447 prey generally occurs at longer distances (Vinyard and O'Brien 1976; Richmond et al. 2004;

448 Bergstrom and Mensinger 2009). In this study, both *A. stuartgranti* and *Tramitichromis* tended to

449 demonstrate the longest detection distances at the highest light intensities, which is thus

450 consistent with vision-mediated prey detection. Detection distances may not increase as light

451 intensity increases further in a given species (Schmidt and O'Brien 1982), but may decrease

452 sharply below a "critical light intensity" (Confer et al. 1978). Trends in behavioral parameters in

453 the current study reveals that the critical light intensity for fish feeding on tethered adult brine

454 shrimp is between 12 and 1 lx for both *A. stuartgranti* and *Tramitichromis*. This is comparable to

455 the critical light intensities for other freshwater teleosts in studies feeding on free-swimming

456 *Daphnia* (11-50 lx, in bluegill, Vinyard and O'Brien 1976; in lake trout, brook trout, and

457 bluegill, Confer et al. 1978), amphipods (5-25 lx, in round goby, logperch, slimy sculpin, and

458 spoonhead sculpin, Bergstrom and Mensinger 2009), or on small fish (~6-18 lx, in largemouth

459 bass, Howick and O'Brien 1983; lake trout, Vogel and Beauchamp 1999). At low light

460 intensities (below the critical intensity, at 1 lx), the ability of *A. stuartgranti* to detect more prey

461 than *Tramitichromis*, but at comparable distances suggests that *A. stuartgranti* may have superior

462 visual abilities for prey capture at these lower light intensities. This is consistent with their

463 distribution at a wider depth range than *Tramitichromis* and the observation of reproductive

464 behaviors in caves (Grant et al. 1987), but whether *Aulonocara* species possess adaptations for

465 increased sensitivity and/or visual acuity as found in known crepuscular or nocturnal teleosts

466 (reviewed in Warrant 2004; Schmitz and Wainwright 2011) requires further study.

467   The potential for binocular vision can be revealed by looking at behavioral evidence for

468 differences in the size of visual fields under different light conditions and between species.

469 While visual predators may respond differently to stimuli in different portions of their visual

470 fields (Collin 1989; McComb and Kajiura 2007; Miyazaki et al. 2011), it is detection angle that

471 is reflects the overall size of the visual field, which is defined by the size, shape, and position of

472 the eyes (Collin and Shand 2003). *A. stuartgranti* demonstrates a wide range of detection angles

473 at light intensities ≥ 1 lx (±90° from body axis) and an even wider range of angles in darkness (0

474 lx, ±180° from body axis). This shift is correlated with differences in behavioral parameters at 1

475 lx versus 0 lx, which are interpreted as a shift between primarily vision-mediated prey detection

476 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.

499   In contrast, in *Tramitichromis*, prey type did not predict any of the four behavioral

500   parameters analyzed using GLMMs, although the interaction of prey type and light intensity did

501   predict detection distance. However, the examination of data trends showed that *Tramitichromis*

502   demonstrates comparable values and trends for live and dead prey with reference to number of

503   prey detections, detection distance (despite the significance of its interaction with prey type) and

504   detection-to-strike velocity at light intensities of 1-800 lx. These results also substantiate results

505   of a prior laboratory study (Schwalbe and Webb 2014) that showed that *Tramitichromis* is a

506   visual predator, which is not dependent on the detection of hydrodynamic stimuli generated by

507   live prey. However, the lack of significance of prey type indicates that *Tramitichromis* does not

508   respond to a visual motion stimulus that are likely to have been generated by live (but not dead)

509   prey, which is surprising given the feeding strategies that these fish employ in nature. In the

510   relatively shallow, well-lit waters of Lake Malawi, *Tramitichromis* species typically capture prey

511   by plunging into the substrate, filling their mouth with sand, and sifting out prey with their gill

512   rakers (= sand sifting, Fryer 1959). The sensory basis for the plunge and sift feeding behavior

513   needs to be determined experimentally, but the results of this study suggest that it is a visual

514   stimulus and not an associated motion stimulus generated by live prey that influences where

515   *Tramitichromis* initiates feeding behavior in the field.

516

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

518

519   As in other lakes, the photic conditions in Lake Malawi are dynamic and many factors influence

520   the light environment, including habitat type, water depth, and proximity to the lake bottom

521   (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

545   early or late during the day. Given its dependence on vision for prey detection (Schwalbe and

546   Webb 2014), *Tramitichromis* species may be limited to shallow habitats so that the visual

547   detection of prey is not compromised. In contrast, *Aulonocara* species can feed at low light

548   intensities and in the dark, which can explain the wider range of depths at which they occur in

549   Lake Malawi. They may also be crepuscular or nocturnal in habit, which may also facilitate other

550   behaviors (e.g., social interactions) at low light intensities.

551

552   **Conclusions**

553

554   *A. stuartgranti* fed on prey at a range of ecologically relevant light intensities, including

555   darkness, and *Tramitichromis* was also able to feed at low light intensities, but not in darkness.

556   In *A. stuartgranti*, the influence of time of day on several aspects of its behavior suggests that it

557   may use circadian rhythms to regulate behavior in nature where diurnal light cues may not be

558   available (e.g. at greater depth, in caves). The integration of visual and non-visual (e.g., lateral

559   line) sensory modalities can explain the statistically non-significant trends in behavior. Similarly,

560   the dramatic change in behavior from 1 lx to 0 lx is consistent with a transition from primarily

561   vision-mediated to exclusively lateral line-mediated prey detection behavior. In contrast to *A.*

562   *stuartgranti*, *Tramitichromis* depends on vision-mediated prey detection (Schwalbe and Webb

563   2014); in this study its behavior was significantly affected by the interaction of light intensity

564   with time of day, but these two factors could not be teased apart. Finally, in an ecological

565   context, the tendency of *Tramitichromis* species to live in shallower, well-lit habitats, in contrast

566   to *Aulonocara* species, which live at a wide range of depths and light environments, suggests that

567   sensory capabilities may allow *Aulonocara* species to escape competition with *Tramitichromis*

25

568 species for prey resources, thus facilitating niche differentiation between these taxa. Field

569 observations in Lake Malawi are needed to test this hypothesis, which would provide an

570 important link between the morphology, feeding behavior, and ecology of cichlid fishes.

571

585

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736

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.

761 **Fig. 2** Experimental setup and light conditions used to record feeding behavior of *A. stuartgranti*

762 and *Tramitichromis*. **a** Diagram of experimental tank with front panel of light curtain removed.

763 Light sources originate from two fluorescent light fixtures (full spectrum light) and two infrared

764 (IR) lights. **b** Sample light spectra generated by fluorescent lights in behavioral trials. The y-axis

765 was compressed to illustrate peaks in the visual spectrum (400-700 nm) that occurred in 1-800 lx

766 trials. These peaks were consistent when light intensity was decrease with the addition of neutral

767 density filters among trials (see Table 1). The peak at 840 nm is from two IR lights in 0 and 1 lx

768 trials only. **c** Mean (± s.e.m.) light intensities measured before and after trials indicating that light

769 intensity did not differ before and after trials at any of the light intensities used (students *t*-test, *P*

770 > 0.05)

771

772 **Fig. 3** Total number of prey detections by prey type (white bars = live tethered brine shrimp,

773 grey bars = dead tethered brine shrimp) for **a** *A. stuartgranti* (*n* = 6 fish) and **b** *Tramitichromis* (*n*

774 = 6 fish) at five different light intensities. Maximum number of possible prey detections = 72 for

775 each light intensity

776

777 **Fig. 4** Three behavioral parameters defining prey detection in *A. stuartgranti* (*n* = 6 fish) and

778 *Tramitichromis* (*n* = 6 fish) at five different light intensities. (**a**, **c**, **e**) Mean (± s.e.m.) number of

779 prey detections (maximum 6 live, 6 dead tethered brine shrimp), detection distance, and

780 detection-to-strike velocity, for *A. stuartgranti* feeding on live (—■—) and dead (---□--) prey, and

781 (**b**, **d**, **f**) *Tramitichromis* feeding on live (—●—) and dead (---○---) prey

782

35

783   **Fig. 5** Frequency of prey strikes (live and dead prey combined, 12 total prey/trial) during glide or

784   pause phases of swimming at five different light intensities in **a** *A. stuartgranti* (n = 6 fish) and **b**

785   *Tramitichromis* (n = 6 fish)

786

787   **Fig. 6** Detection angle for live and dead prey combined (=12 total prey/trial) at five different

788   light intensities for **a** *A. stuartgranti* (n = 6 fish) and **b** *Tramitichromis* (n = 6 fish). Black lines

789   represent the proportion of prey detections grouped into 20° intervals. Fish snout is at the center

790   and fish is facing 0° (indicated by the grey arrow in the top plot in **a**). The thin line represents the

791   mean angle for all trials. Results for *Tramitichromis* at 0 lx were not included here due to the

792   small number of strikes (n = 3 strikes)

793

794   **Fig. 7** Mean (± s.e.m.) prey preference scores (following Taplin 2007) for **a** *A. stuartgranti* (n =

795   6 fish) and **b** *Tramitichromis* (n = 6 fish) feeding on six live (white bars) and six dead (gray bars)

796   tethered adult brine shrimp in trials at five different light intensities. Preferences scores were

797   calculated by taking the mean of the rank order in which prey were captured. The dotted line (=

798   6.5) indicates the mean preference score with no preference for either prey type. Scores <6.5

799   (below dotted line) indicate a preference. Significantly different preference scores between live

800   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

Source	Number of prey detections F (df)	P value	Detection distance F (df)	P value	Detection-to-strike velocity F (df)	P value	Swimming phase at prey detection 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

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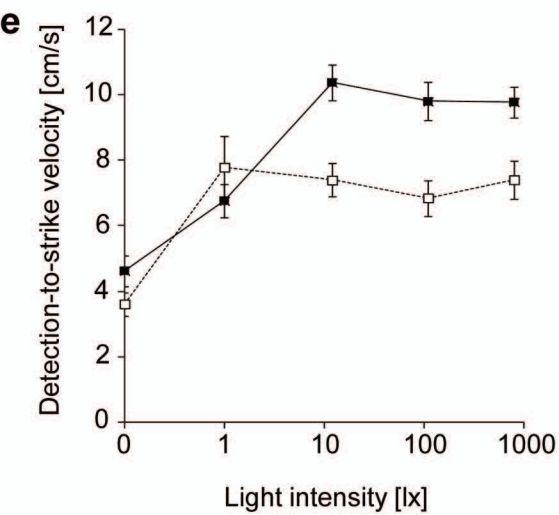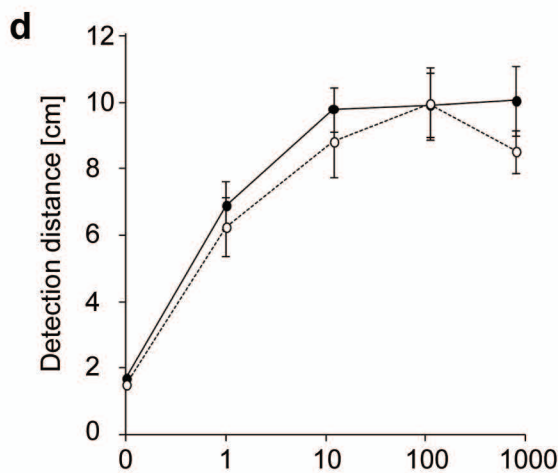
Light intensity (lx)	*T (df)*	*P* value
Aulonocara stuartgranti		
0	-2.853 (5)	**0.036**
1	-0.618 (5)	0.564
12	-1.395 (5)	0.222
112	-6.102 (5)	**0.002**
800	-1.892 (5)	0.117
Tramitichromis		
0	-0.797 (5)	0.461
1	-2.396 (5)	0.062
12	-1.379 (5)	0.226
112	-2.441 (5)	0.059
800	-13.647 (5)	**<0.001**

P values < 0.05 are shown in bold

a

light curtain

camera

IR   IR

lamp   lamp

b

Absolute irradiance [μW/cm²/nm]

0 lx	(black)
1 lx	(red)
12 lx	(orange)
112 lx	(green)
800 lx	(blue)

Wavelength [nm]

c

Light intensity [lx]

◆ Before Trial
☐ After Trial

Trial light intensity [lx]

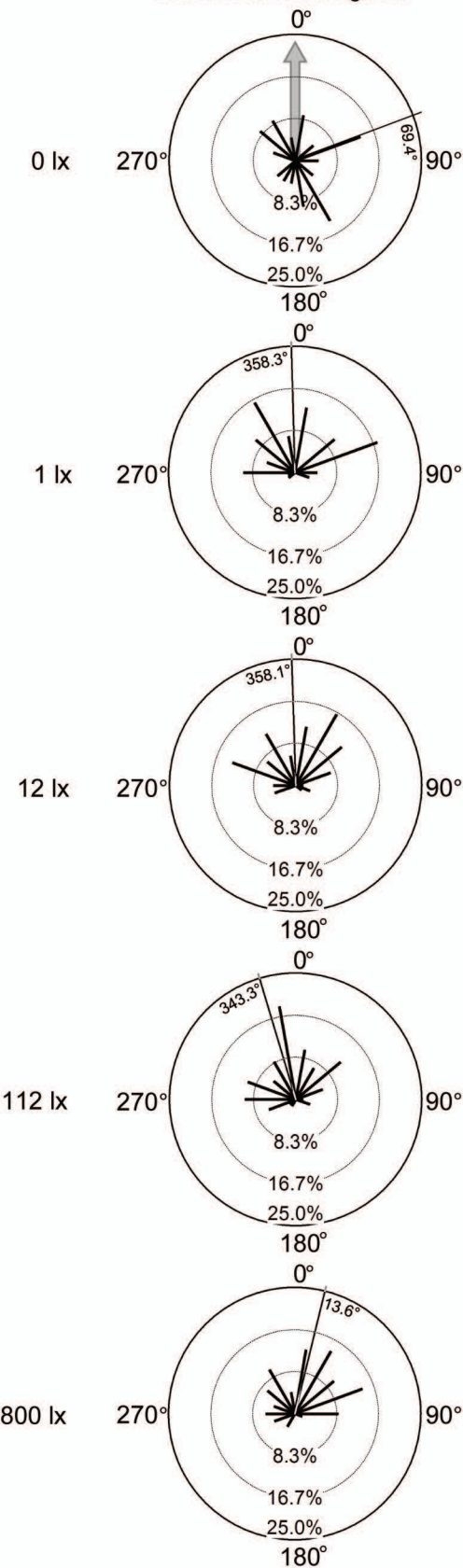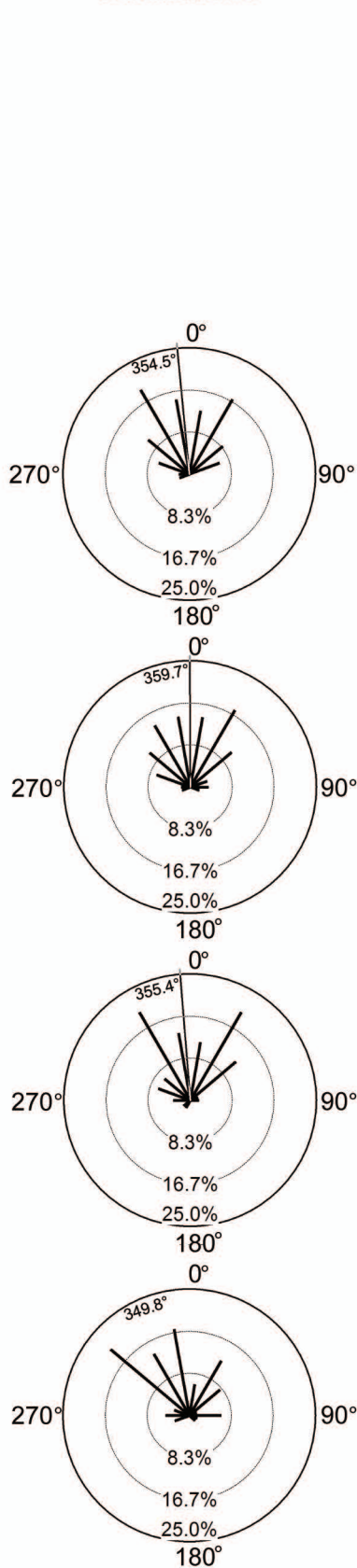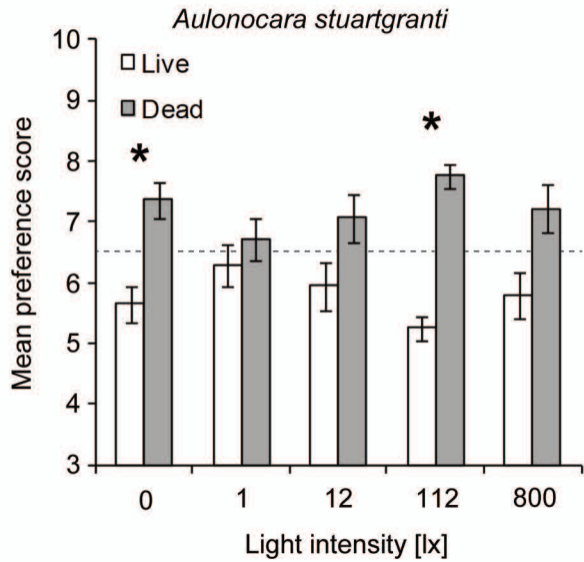**a** *Aulonocara stuartgranti*

b *Tramitichromis*

Aulonocara stuartgranti   *Tramitichromis*

a *Aulonocara stuartgranti*   **b** *Tramitichromis*

a *Aulonocara stuartgranti*   **b** *Tramitichromis*

a *Aulonocara stuartgranti*

b *Tramitichromis*

Light intensity [lx]

Mean preference score