12-9-2013

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Citation/Publisher Attribution

Schwalbe, M. A.B., & Webb, J. F. (2013). Sensory basis for detection of benthic prey in two Lake Malawi cichlids. *Zoology, 117*(2), 112-121.
Available at: http://dx.doi.org/10.1016/j.zool.2013.09.003

Sensory basis for detection of benthic prey in two Lake Malawi cichlids

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Text pages: 32

Figures: 4 Table, 5 Figures (1 in color)

Key words: *Aulonocara*, *Tramitichromis*, lateral line, vision, multimodal, cobalt chloride

Abstract

The adaptive radiations of African cichlids resulted in a diversity of feeding morphologies and strategies, but the role of sensory biology in prey detection and feeding ecology remains largely unexplored. Two endemic Lake Malawi cichlid genera, *Tramitichromis* and *Aulonocara*, feed on benthic invertebrates, but differ in lateral line morphology (narrow and widened lateral line canals, respectively) and foraging strategy. The hypothesis that they use their lateral line systems differently was tested by looking at the relative contribution of the lateral line system and vision in prey detection by *Tramitichromis* sp. and comparing results to those from a complementary study using *A. stuartgranti* (Schwalbe et al., 2012). First, behavioral trials were used to assess the ability of *Tramitichromis* sp. to detect live (mobile) and dead (immobile) benthic prey under light and dark conditions. Second, trials were run before, immediately after, and several weeks after chemical ablation of the lateral line system to determine its role in feeding behavior. Results show that *Tramitichromis* is a visual predator that neither locates prey in the dark, nor depends on lateral line input for prey detection and is thus distinct from *A. stuartgranti*, which uses its lateral line or a combination of vision and lateral line to detect prey depending on light condition. Investigating how functionally distinctive differences in sensory morphology are correlated with feeding behavior in the laboratory and determining the role of sensory systems in feeding ecology will provide insights into how sensory capabilities may contribute to trophic niche segregation.

1. Introduction

The mechanosensory lateral line system of fishes plays critical roles in prey detection, predator avoidance, communication, rheotaxis, and navigation around obstacles (reviewed in Webb et al., 2008; Bleckmann and Zelick, 2009). The system demonstrates a considerable degree of morphological variation among bony fishes (Webb, 1989b), but understanding the relationship between structure and function in the lateral line system and lateral-line mediated behavior continues to be a particularly challenging task because of the multiple levels at which both structure and function may vary.

The physiological response of the lateral line system (and ultimately behavior) depends on the properties of the different morphological components that define the system. Variation in morphology of the neuromasts (hair cell morphology, density, and orientation, neuromast shape, shape and length of the cupula into which the apical ciliary bundles of the hair cells are embedded, and patterns of neuromast innervation and central projections), and that of the lateral line canals in which canal neuromasts are found (canal diameter, pore size, presence of canal constrictions), and the hydrodynamic context (biotic, abiotic, and self-generated flows) in which the system functions all contribute to physiological, and thus behavioral, responses. Ecological correlates of lateral line morphology have been proposed (Dijkgraaf, 1963; reviewed by Webb, 1989b), but there are notable exceptions. For instance, fishes in hydrodynamically active environments tend to have narrow canals and fewer superficial neuromasts, but this relationship does not always hold in light of different sets of selection pressures (Carton and Mongtomery, 2004). In addition, some types of morphological variation (differences in canal diameter in the vicinity of canal neuromasts) do not result in differences in physiological responses by

neuromasts (Antarctic notothenioids, Coombs and Montgomery, 1992; Montgomery et al., 1994).

Testing hypotheses concerning the functional evolution of the lateral line system requires that experiments be carried out in a well-defined comparative context using closely-related species pairs with divergent morphology and the presentation of ecologically relevant stimuli. Narrow and widened cranial lateral line canals, two of the four types of lateral line canals defined among teleosts (Webb, 1989a), are of particular interest because of their distinctive morphologies and contrasting functional properties (theoretical and experimental work of Denton and Gray, 1988, 1989). Narrow canals are well-ossified with small canal pores and widened canals are typically weakly ossified with partial ossification of the canal roof over the canal neuromasts leaving large canal pores between neuromast positions that are covered by a tympanum-like epithelium typically pierced by very small pores. Narrow canals are widespread among teleosts, while widened canals have evolved convergently in just a dozen or so teleost families suggesting that the evolution of widened canals is adaptive, and further, that it represents an adaptation for prey detection.

The ability to determine the functional distinctions between narrow and widened canals has been hampered by the inability to identify appropriate species pairs that are accessible for experimental study. The percid fishes are a useful model system for illustrating the relationship between the functional morphology of the lateral line system and feeding ecology of fishes. European perch (*Perca fluviatilis*) and yellow perch (*P. flavescens*) have narrow canals and Eurasian ruffe (*Gymnocephalus cernuus*) has widened canals. The sensitivity of the large neuromasts in the widened canals of ruffe (van Netten, 2006) generally supports behavioral and ecological findings. European perch and ruffe have some seasonal and life stage-dependent diet

84 overlap in their native habitat where they co-occur (Rezsu and Specziar, 2006; Schleuter and
85 Eckmann, 2008), but ruffe occupy a greater depth range than perch and spend more time close to
86 the substrate (Bergman, 1987, 1991). In addition, ruffe are able to feed more successfully in
87 visually compromised habitats when compared to *Perca* spp. (Disler and Smirnov, 1977;
88 Bergman, 1988; Janssen, 1997; Schleuter and Eckmann, 2006) and increase in abundance and
89 replace perch in turbid water and/or low light conditions (Bergman, 1991). Interestingly, the
90 accidental introduction of ruffe in the North American Great Lakes has generated concern over
91 potential for competition with native yellow perch (*P. flavescens*, Ogle et al., 1995).
92 The speciose cichlids of the African Rift Lakes also provide opportunities for
93 comparative studies of sensory biology, feeding behavior, and ecology. There has been intense
94 study of the functional morphology of the cichlid feeding apparatus and the diverse trophic
95 niches that they occupy (Fryer and Iles, 1972; Liem, 1973, 1980; Albertson et al., 2005; Hulsey
96 et al., 2010), but only a few studies have addressed the sensory basis for prey detection (Hofman
97 et al., 2009; O'Quin et al., 2010; Mogdans and Nauroth, 2011; Schwalbe et al., 2012). The vast
98 majority of cichlid species have narrow cranial lateral line canals (e.g., Branson, 1961; Peters,
99 1973; Webb, 1989b). However, a few genera in Lake Tanganyika (*Aulonocranus* and
100 *Trematocara*) and in Lake Malawi (*Aulonocara*, *Alticorpus,* and *Trematocranus*) and have
101 widened canals (Konings, 2007).
102 One of these genera, *Aulonocara* (16-20 spp.), and a genus with narrow canals,
103 *Tramitichromis* (~6 spp.), are found at either the rock-sand interface or over sand and feed on
104 invertebrates buried in the sand (Fryer and Iles, 1972; Konings, 2007), but differ in prey search
105 strategy. *Tramitichromis* plunges into the substrate filling their mouths with sand, and sift out
106 invertebrate prey using their gill rakers ("sand sifting," Fryer, 1959). How they choose to direct

their plunges, and thus the sensory basis for the detection of their benthic prey, is still unknown. In contrast, *A. stuartgranti* swims just above the substrate, detect water flows generated by prey with their lateral line system (as confirmed with cobalt chloride ablations), and strike at individual prey in the sand (Konings, 2007; Schwalbe et al., 2012). With respect to lateral line morphology, the narrow canals of *Tramitichromis* spp. are well-ossified with small pores while the widened canals of *Aulonocara* spp. have large canal pores covered by an epithelium pierced by small perforations. A recent analysis of neuromast morphology in juvenile *Tramitichromis* sp. and *A. stuartgranti* (Becker, 2013; Becker et al., in prep.) has shown that these fishes have the same number of canal neuromasts and canal pores, despite distinct differences in canal and pore morphology (Fig. 1). They also have the same number of linear series or clusters of very small superficial neuromasts on the head, but late stage juvenile (and presumably adult) *A. stuartgranti* tend to have more superficial neuromasts within some of these series. The canal neuromasts are diamond-shaped in both species, but those in *A. stuartgranti* are a bit larger (Fig. 1B) and tend to sit in slight constrictions in the canal, which is a characteristic of many species with widened canals.

Thus, *Tramitichromis* sp. and *A. stuartgranti* present an excellent model system in which to ask questions about the relationship of lateral line morphology to its role in prey detection. These fish differ with respect to only some aspects of the morphology of the lateral line system (narrow versus widened canals, known to be functionally distinct in other taxa, and minor differences in canal neuromast size [but not general shape], and the number of superficial neuromasts). Experimental work has already determined that the lateral line system is critical for prey detection in *A. stuartgranti* (Schwalbe et al., 2012) and it is hypothesized that the role of the lateral line system in prey detection in *Tramitichromis* sp. would be different than in *A.*

130 *stuartgranti*. In order to test this, behavioral trials (as in Schwalbe et al., 2012) were conducted in
131 the laboratory in which *Tramitichromis* sp. was presented with live (mobile) and dead
132 (immobile) prey (tethered adult brine shrimp) under light and dark conditions (Experiment I).
133 Then, the role of the lateral line system in prey detection was directly addressed by temporarily
134 inactivating the lateral line system with cobalt chloride (Experiment II). Data on number of prey
135 strikes, prey detection distance and angle and preference for live or dead prey was then compared
136 with that of *A. stuartgranti* (from Schwalbe et al., 2012) to contrast the roles of the lateral line
137 system and vision in prey detection behavior.
138
139 **2. Materials and methods**
140
141 *2.1. Study Species*
142
143 Adult *Tramitichromis* sp. (= *Tramitichromis* for remainder of manuscript, unless
144 otherwise noted) were acquired from a commercial supplier (Old World Exotic Fish, Inc.,
145 Homestead, FL, USA) and housed in small groups in 190 L aquaria with mechanical and
146 biological filtration. For housing and experimental procedures, fish were maintained at 1 ppt salt
147 (Cichlid Lake Salt, Seachem Laboratories, Inc., Madison, GA, USA) at 26 ± 1°C with a 12:12 hr
148 light:dark cycle. Fish were fed daily with cichlid pellets (New Life Spectrum Cichlid Formula;
149 New Life International, Inc., Homestead, FL, USA) and supplemented with live adult brine
150 shrimp. Animal care and all experimental procedures followed an approved University of Rhode
151 Island IACUC protocol.
152

153 *2.2. Behavioral Trials*

154

155 Two experiments were conducted to determine the ability of *Tramitichromis* to detect
156 live and dead prey in light and dark trials (Experiment I) and to determine the contribution of the
157 lateral line system to prey detection in light trials (Experiment II).

158

159 *2.2.1. Experiment I – Light and Dark Trials*

160

161 Light and dark trials were conducted using *Tramitichromis* following Schwalbe et al.
162 (2012). Briefly, trials were performed in a large experimental tank (375 L) lined with sand. Adult
163 brine shrimp (*Artemia* sp.) were tethered with elastic thread in pairs (1 live, 1 dead [freshly
164 frozen]) onto each of six mesh platforms (a total of 6 live prey + 6 dead prey = 12 total prey) to
165 serve as a proxy for naturally occurring benthic prey. Platforms were placed on the bottom of the
166 tank in a 2x3 grid so that their top surfaces were flush with that of the sand. All filters in the
167 experimental tank were turned off to eliminate hydrodynamic noise during all behavioral trials.
168 At the start of a trial, a fish was released from behind an opaque barrier into the
169 experimental arena and recorded for 30 minutes using a HD digital video camera (Sony © HDR-
170 CX550V, 30 frames per second) mounted directly above the tank. Light trials were carried out
171 under standard white fluorescent illumination and dark trials were conducted under infrared (IR)
172 illumination (peak = 840 nm; Speco Provideo, IR-200/24, Amityville, NY, USA). Each of six
173 naïve male fish (total length [TL] = 99 - 110 mm) was run sequentially through three light and
174 then three dark trials for a total of 18 light trials and 18 dark trials. Each trial was performed on a
175 different day, and trials were carried out over the course of five months with a mean time

176 between the first light trial and last dark trial of 19 days for an individual fish. Several additional
177 light and dark trials were recorded in lateral view to observe the fishes' position relative to the
178 substrate.
179
180 *2.2.2. Experiment II – Chemical Ablation of the Lateral Line System*
181
182 In order to determine the role of the lateral line system in prey detection by
183 *Tramitichromis*, fish were treated with cobalt (II) chloride heptahydrate (cobalt chloride; Sigma-
184 Aldrich, St Louis, MO, USA) to deactivate the lateral line system as in Schwalbe et al. (2012).
185 The results of Experiment I (above) demonstrated that while all fish were active during dark
186 trials, the majority of fish did not feed in the dark so Experiment II consisted only of light trials.
187 Each of three fish (all males, not used in Experiment I; TL = 92 - 98 mm) was run through a
188 sequence of three different trials. First, a 30 minute "pre-cobalt" trial (identical to the light trials
189 in Experiment I) was carried out to establish a behavioral baseline. Two to three days later, the
190 fish was treated in a large container filled with 0.1 mM cobalt chloride in conditioned tap water
191 for three hours (calcium = 60 mg/L; Hach hardness test kit, Loveland, CO, USA) and returned to
192 the experimental tank (calcium = 260 mg/L). When the fish appeared to be behaving normally
193 (e.g., normal respiration and swimming, about two hours after cobalt treatment), a "cobalt trial"
194 was conducted. All fish resumed feeding on commercial pellets and/or live brine shrimp
195 immediately following cobalt trials. After 21 days (in the experimental tank), the fish was run
196 through a "post-cobalt" trial to assess recovery from cobalt treatment and allow a comparison
197 with the "pre-cobalt" and "cobalt" trials. In a previous study (Schwalbe et al., 2012), the effect of
198 handling was assess by running fish through one light and dark trial a few days before and

199 immediately after a sham cobalt chloride treatment (= 4 trials/fish). For the sham treatment, fish
200 (n = 2) were placed in a large container of conditioned tap water for three hours instead of the
201 cobalt chloride solution. Fish consumed prey during both light and dark trials before and after
202 sham treatment, so it appeared that handling had no effect on feeding behavior.
203
204 *2.3. Data analysis*
205
206 At the end of each trial, remaining prey were counted to determine the number and type
207 of prey (live and dead) that had been consumed and strike success was also confirmed in video
208 recordings. Video was analyzed using Premier Pro (Adobe, CS5) and images from video
209 sequences of prey detections (e.g. when the fish oriented towards the prey) to prey strikes were
210 exported for further analysis. These images were used to identify when detections occurred
211 relative to the start of the trial, during which phase of saltatory search strategy each prey was
212 detected (defined by O'Brien et al., 1989; a cycle of three swimming phases – caudal fin thrust,
213 glide and pause), and the order of prey strikes (live vs. dead) as an approximation of "prey
214 preference." In addition, detection distance and detection angle for each strike was measured
215 from the images using ImageJ (NIH, v. 1.41o).
216 All data were tested for normality (Kolmogorov-Smirnov test) and only detection
217 distance data needed to be $\log_{10}$ transformed to achieve normality. Separate tests using a
218 generalized linear mixed model (GLMM, SPSS, v.19) with pairwise post-hoc comparisons (least
219 significant differences, LSD) were used to detect differences in four variables (number of prey
220 strikes, detection distance, swimming phase in which strikes occurred, and order of prey capture)
221 with reference to prey type (live vs. dead) and light condition (light vs. dark). This approach

allowed the selection of random (individual) and fixed effects (species, light condition, prey type) while addressing repeated measures for the same individual. Prey preference was calculated using a method described in Taplin (2007) in which prey preference was assessed by ranking the prey according to the order in which they were consumed, and then calculating a preference score by taking the mean of the order values for each prey type. Necessary assumptions for this analysis were satisfied: multiple types of prey were offered simultaneously (e.g. live and dead tethered brine shrimp) and prey consumed last could not be distinguished from uneaten prey. Scores closer to one indicate a strong preference, whereas scores closer to twelve (= total number of prey offered) indicate no preference or rejection. Preference scores for live or dead prey in each light condition (light, dark) were compared using paired *t*-tests. Means of prey preference scores from the three replicate trials carried out for each fish were calculated prior to performing the paired *t*-test, so that the replicate variable was the fish (individual) and not the trial. Finally, Watson's U^2-tests (Oriana, Kovach Computing Services, Anglesey, UK, v.3) were used to analyze differences in detection angles with reference to prey type and light condition. Differences were considered to be significant at the $P < 0.05$ level for all statistical tests. Values are given as mean ± SE unless otherwise specified.

3. Results

Experiments I and II show that *Tramitichromis* is a visual predator that does not seek out prey in the dark and does not depend on its lateral line system for detection of benthic invertebrate prey in light trials. *Tramitichromis* is thus quite distinct from *Aulonocara stuartgranti*, which relies on the interaction of vision and lateral line for prey detection and uses

245 the lateral line system for detection of prey in the dark (Schwalbe et al. 2012).

246

247 *3.1. Experiment I – Light and Dark Trials*

248

249 *Tramitichromis* explored the tank by moving throughout the vertical extent of the water
250 column. After the first prey detection, fish generally swam within ~10 cm of the sand and struck
251 at and removed prey from the platforms. Fish alternated between moving around the entire tank
252 (vertically and horizontally) and swimming close to the sand, even after all 12 tethered brine
253 shrimp were captured. Sand sifting was frequently observed during trials and after all prey were
254 consumed.

255 In light trials, all *Tramitichromis* successfully struck at and consumed prey (94.4% of
256 total prey presented) but fish attacked more live prey than dead prey (LSD, $P = 0.005$; Table 1,
257 Fig. 2A). Strikes on live prey preceded those on dead prey (paired *t*-test, $t_5 = 8.851$, $P < 0.001$;
258 Table 2) and live prey were detected at a greater distance than dead prey (live = 11.3 ± 0.5 cm,
259 dead = 9.0 ± 0.5 cm; LSD, $P = 0.002$; Table 1, Fig. 3A). Prey was detected non-uniformly
260 around the fishes' bodies (Rayleigh test, $Z = 107.98$, $P < 0.001$; Fig. 4A) and all fish detected
261 prey in the same relatively narrow range in front of the snout (± 40° from body axis; Watson's
262 U^2-test, $P > 0.05$). *Tramitichromis* swam close to the substrate (but higher above the substrate
263 than *A. stuartgranti*) and demonstrated a saltatory search strategy (cyclic sequence of caudal fin
264 thrust, glide, and pause). Prey was never detected during a caudal fin thrust, and more prey (live
265 and dead prey combined) was detected during a pause (77.3%) than during a glide (22.7%, Fig.
266 5A).

267 The results of dark trials were quite different. The median number of strikes was zero for

both live and dead prey, which greatly contrasts with the median number of six strikes in light trials (for live or dead prey offered; Fig. 2A). All fish actively swam around the tank in dark trials as they did in light trials and some exhibited sand sifting behavior. A few strikes did occur during dark trials, but one fish was responsible for 21 of the total 23 strikes (on 216 live and dead prey presented in 18 trials). When comparing strikes on live and dead prey, no significant differences were detected in any of the measured variables used to describe prey detection behavior (e.g. prey preference, Table 2; number of prey strikes, Fig. 2A; detection distance, Fig. 3A; detection angle Fig. 4A; and swimming phase at prey detection, Fig. 5A), indicating that live prey could not be distinguished from dead prey.

However, when comparing the few strikes that did occur in dark trials ($n = 23$) to the numerous strikes in light trials ($n = 204$; Fig. 2A), significant differences were observed in some aspects of behavior. In dark trials, prey were detected at a distance one fourth of that in light trials (live and dead combined, light = 10.3 ± 0.4 cm, dark = 2.3 ± 0.3 cm; LSD, $P < 0.001$; Table 1, Fig. 3A) and more prey were detected during a glide in dark trials (60.9% of strikes) than in light trials (22.7% of strikes; LSD, $P = 0.002$, Table 1, Fig. 5A). Even though prey were detected in a wide range around the body during dark trials, the majority of prey were detected in the same narrow range as in light trials (±40° from body axis, Watson's U^2-test, $P > 0.05$, Fig. 4A). While differences were observed in several behavioral parameters in light and dark trials, *Tramitichromis* tended not to feed in the dark and when they did, prey appeared to be found rather indiscriminately as fish explored the experimental arena.

3.2. Experiment II – Chemical Ablation of the Lateral Line System

291 Given the low number of strikes by *Tramitichromis* sp. in dark trials in Experiment I,
292 only light trials were carried out to determine the effects of lateral line ablation on their prey
293 detection behavior.
294 The results for all trials - before (pre-cobalt trials), immediately following (cobalt trials),
295 and three weeks after treatment with cobalt chloride (post-cobalt trials) - were comparable to
296 results for light trials in Experiment I. All fish actively swam around the experimental arena and
297 consumed the majority of live and dead prey presented in pre-cobalt (66.7% of total prey
298 presented), cobalt (72.2%), and post-cobalt recovery (88.9%) trials. The total number of strikes
299 on live and dead prey was the same among the three trial types (GLMM, $P > 0.05$; Table 3, Fig.
300 2B). Live and dead prey were detected from similar distances in all of these trials (Table 3; Fig.
301 3B). Prey were detected non-uniformly around the body in all trials (Rayleigh test, $P < 0.04$; Fig.
302 4B) and detection angle did not vary with prey type or among sequential trials (Watson's U^2-test,
303 $P > 0.05$), like Experiment I light trials. In pre-cobalt trials, live prey were captured before dead
304 prey (paired *t*-test, $t_2 = 8.66$, $P = 0.013$), but this preference for live prey was absent in cobalt
305 trials and post-cobalt trials ($P > 0.05$; Table 2). As in the light trials in Experiment I, most prey
306 were detected during a pause, and the frequency of prey detection during a pause or glide did not
307 differ among the pre-cobalt, cobalt, and post-cobalt trials (GLMM, $P > 0.05$; Table 3, Fig. 5B).
308
309 *3.3. Comparison of feeding behavior in* Tramitichromis *and* Aulonocara stuartgranti
310
311 Interesting similarities and contrasts were found in prey detection behavior in
312 *Tramitichromis* sp. and *Aulonocara stuartgranti*. Both species swam around the tank in light and
313 dark trials using a saltatory search strategy, but *Tramitichromis* tended to swim higher above the

sand while searching for prey and pitched forward more (e.g. ~45° versus ~30° for *A. stuartgranti*) during prey strikes. In addition, *Tramitichromis* did not demonstrate the swimming reversals (e.g. swam backwards) upon prey detection that *A. stuartgranti* did, and *A. stuartgranti* did not use the sand sifting strategy used by *Tramitichromis*.

In light trials, *Tramitichromis* and *A. stuartgranti* detected similarly high numbers of live and dead prey (GLMM, $P > 0.05$, Table 4, Fig. 2A), and demonstrated a preference for live prey (*Tramitichromis*: paired *t*-test, $t_5 = 8.851$, $P < 0.001$, *A. stuartgranti*: paired *t*-test, $t_5 = 5.551$, $P = 0.003$; Table 2). In addition, both species detected more prey during a pause rather than during a glide, and did so with frequencies that were not statistically different (GLMM, $P > 0.05$; Table 4, Fig. 5A). Interestingly, *Tramitichromis* detected live prey at longer distances than *A. stuartgranti* (LSD, $P = 0.006$; Fig. 3A), but both species detected dead prey at distances that were not statistically different ($P > 0.05$). Detection angles were significantly different for *Tramitichromis* and *A. stuartgranti* (Watson U^2-test, $U^2 = 0.468$, $P < 0.001$; Fig. 4A); *Tramitichromis* detected the majority of prey in a narrower range of angles (±40° from body axis) than did *A. stuartgranti* (±90° from body axis).

In dark trials, *Tramitichromis* also demonstrated different prey detection behaviors than *A. stuartgranti*. Only half of the *Tramitichromis* ($n = 3$ of 6 fish) struck at prey while all *A. stuartgranti* ($n = 6$ fish) struck at prey. When prey was detected, *Tramitichromis* struck at fewer live prey than did *A. stuartgranti* (LSD, $P = 0.006$), but the number of strikes on dead prey was not statistically different in the two species ($P > 0.05$; Fig. 2A). Furthermore, although both species tended to detect more prey during a glide than during a pause in dark trials, *Tramitichromis* detected fewer prey during a glide than did *A. stuartgranti* (LSD, $P = 0.020$; Fig. 5A). In addition, *Tramitichromis* detected prey at shorter distances than did *A. stuartgranti* (both

337 prey types combined, LSD, $P < 0.001$; Fig. 3A). Detection angles were not statistically different
338 in dark trials (Watson's U^2-test, $P > 0.05$) and both species found prey non-uniformly around
339 their bodies (Fig. 4A). The results suggest that *Tramitichromis* is a visual predator in contrast to
340 *A. stuartgranti*, which depends on lateral line input in prey detection, especially in the dark.
341
342 **4. Discussion**
343
344 The results of Experiments I and II showed that the combination of lateral line, olfactory,
345 and tactile cues was not sufficient to elicit a prey strike response by *Tramitichromis* in the
346 absence of visual cues, but that in light trials, a combination of sensory inputs may provide some
347 additional information when used in tandem with vision. This study has demonstrated that
348 closely related taxa that feed on the same prey in the same sensory environment, but have two
349 morphologically (and likely functionally) distinct lateral line systems, use different sensory
350 systems to detect their prey under different light conditions in the laboratory.
351
352 *4.1. Feeding behavior of* Tramitichromis
353
354 The experimental design in Experiments I and II ensured that different combinations of
355 sensory cues were available to the fish allowing multimodal sensory input to be considered in the
356 interpretation of the results. In Experiment I light trials, all stimuli generated by the movement of
357 the brine shrimp were present and all sensory systems in *Tramitichromis* were intact (e.g. vision,
358 lateral line system, olfaction). In addition, the significance of prey movements for prey detection
359 – the visual motion stimulus, hydrodynamic flow, and spread of an odor plume generated by the

motion of the brine shrimp – was addressed by providing both live and dead prey in all trials. Visual cues were absent in dark trials in Experiment I, but lateral line and olfactory systems were still intact (hydrodynamic and olfactory cues were available). In Experiment II (light trials only), the ability to detect hydrodynamic cues was eliminated by temporarily inactivating the lateral line system in cobalt trials, but visual and olfactory cues were still available. A dependence on more than one sensory modality was inferred when feeding behavior was not as robust in trials in which input to one or more sensory modalities was eliminated compared to trials in which all sensory systems were available.

Tramitichromis demonstrated the most robust feeding behavior when all sensory cues were available (Experiment I light trials). In these trials, *Tramitichromis* demonstrated a preference for live prey, which were detected from greater distances than were dead prey. The visual motion stimulus generated by live brine shrimp likely strengthened the visual stimulus necessary for prey detection and was responsible for the generation of robust prey detection behavior at longer distances. More prey detections occurred during a pause than a glide in light trials, when the prey could be localized in a more stable visual field. Even though the olfactory system was intact and olfactory cues were available during light and dark trials in Experiments I and II, behaviors characteristic of olfactory mediated prey detection (e.g. following and/or locating the source of an odor by zig-zagging through its odor plume, Hara, 1993) were not observed. These results all indicate that visual detection of prey is critical for feeding in *Tramitichromis*, and that they were relatively unsuccessful in detecting prey in dark trials likely because they could not see the prey. Finally, in Experiment II, feeding behavior was similar before, immediately following, and after the recovery from lateral line ablation using cobalt chloride, providing evidence that *Tramitichromis* does not appear to depend on its lateral line

383 system for prey detection. Morphological confirmation of lateral line ablation by cobalt chloride
384 was accomplished by fluorescently staining three juvenile *Tramitichromis* sp. with 4-Di-2-ASP
385 (63μM, 5 min; also see Fig. 1) following a three hour treatment with either cobalt chloride in
386 calcium free tank water (0.1 mM), or in calcium free tank water (E. Becker, 2013). A lack of hair
387 cell staining in the central region of the neuromasts in *Tramitichromis* sp. was similar to that
388 observed in juvenile *Aulonocara stuartgranti* treated with cobalt chloride (0.05 and 0.1 mM,
389 Schwalbe et al, 2012).

390 *Tramitichromis* feeds on benthic invertebrates in the sand at the rock-sand interface in
391 Lake Malawi (Fryer, 1959; Koning, 2007), a community that is dominated by ostracods,
392 hydracarins, and chironomid larvae and also includes hydropsychid caddisfly, heptageneid
393 mayfly, and dryopoid beetle nymphs (Abdallah and Barton, 2003). *Tramitichromis* is known for
394 plunging into the sand, engulfing a mouthful of sand, and sifting it through their gill rakers, but
395 how they determine where to initiate this behavior is not known. Given the results of the current
396 study, it is likely that the fish can see minute changes in the substrate (e.g. a slightly exposed
397 invertebrate or movements by invertebrates in the substrate), perhaps in combination with
398 olfactory cues, to find these prey. Tactile cues may also elicit prey strikes and/or sand sifting
399 behavior, but lateral video recordings of behavioral trials suggest otherwise because
400 *Tramitichromis* swam several centimeters above the substrate and tended not to contact the
401 substrate with their pelvic fins.

402 Finally, the ability of one of the six *Tramitichromis* to detect both live and dead prey in
403 dark trials cannot be easily explained. *Tramitichromis intermedius* does have spectral sensitivity
404 peaks that are somewhat higher than other Lake Malawi cichlids examined (including *A.*
405 *jacobfreibergi*, Parry et al., 2005), but among all retinal cell types, the longest wavelength of

406 maximum absorbance is only about 570 nm (for the double cones). However, two recent studies
407 have demonstrated that cichlids show positive phototactic behavior (*Oreochromis mossambicus*,
408 Shcherbakov et al., 2012) and strong foraging responses *(Pelvicachromis taeniatus*, Meuthen et
409 al., 2012) in near-IR light. Thus, it is possible that this one *Tramitichromis* sp. was able to
410 successfully detect prey in dark trials illuminated with a light source in the near IR range.
411
412 *4.2. Comparison of Prey Detection Behaviors in Two Benthic Feeding Cichlids*
413
414 This study has shown that *Tramitichromis* and *A. stuartgranti* use two distinct methods
415 for detecting the same prey, likely due to the relative roles of their sensory systems. Both species
416 exhibited a saltatory search strategy (which cycles between moving through an area and pausing
417 to locate prey or reposition before the next forward movement) and different sensory systems are
418 possibly important during a pause or glide in light and dark trials. Both *Tramitichromis* and *A.*
419 *stuartgranti* appeared to visually scan for prey during a pause in light trials, when the visual field
420 was stable. In light trials, *Tramitichromis* detected more prey in a narrow range of angles relative
421 to the body axis suggesting that they may possess adequate binocular vision to localize prey (as
422 shown in other teleosts, Sivak, 1978; Bianco et al., 2011; Miyazaki et al., 2011). In contrast, *A.*
423 *stuartgranti* detected prey in a wider range of angles suggesting that binocular vision was not
424 employed. However, they struck at a higher proportion of prey during a pause in light trials,
425 suggesting that stabilization of the visual field favored successful prey detection. In dark trials,
426 *A. stuartgranti* detected prey as swimming velocity decreased during a glide, allowing
427 localization of prey as it came within the operational range of its lateral line system.
428 The temporary ablation of the lateral line system with cobalt chloride had different

429 effects on the two species. In *Tramitichromis,* prey detection behavior did not change with the
430 elimination of lateral line input, while for *A. stuartgranti*, there was a reduction in the number of
431 prey strikes in light trials and the complete elimination of prey detections in dark trials
432 (Schwalbe et al., 2012). It is concluded that *Tramitichromis* does not depend on lateral line input
433 for successful prey detection in contrast to *A. stuartgranti*, which depends on both vision and the
434 lateral line system in light trials, and uses its lateral line system to detect prey in the dark. The
435 correlation of this behavioral data with the difference in lateral line canal morphology in
436 *Tramitichromis* and *A. stuartgranti* suggest that the widened lateral line canals are an adaptation
437 for prey detection, especially in the absence of visual cues.
438
439 *4.3. Could sensory biology contribute to the feeding ecology of African cichlids?*
440
441 There has been a long history of discussion about the role of feeding mechanisms in the
442 definition of cichlid trophic niches (Fryer and Iles, 1972; Liem, 1973, 1980; McKaye and Marsh,
443 1983; Albertson et al., 2003) and the ways in which trophic niche differentiation and ecological
444 segregation occur among African cichlids (Goldschmidt et al., 1990; Reinthal, 1990; Sturmbauer
445 et al., 1992; Hori et al., 1993; Bouton et al., 1997; Genner et al., 1999a, b; Duponchelle et al.,
446 2005; Martin and Genner, 2009; Genner and Turner, 2012). In their landmark monograph, Fryer
447 and Iles (1972) reviewed the feeding biology and evolution of cichlid fishes of the African Rift
448 Lakes, but the ecological concepts of habitat partitioning and mechanisms underlying the
449 evolution of trophic diversity among cichlids has only been examined in detail more recently
450 (reviewed in Genner and Turner, 2005; Albertson, 2008). For instance, within the rock-dwelling
451 mbuna flock, it has been hypothesized that fine-scale niche partitioning occurs among species

452 that forage on a combination of algae, *aufwuchs*, phytoplankton, and other seasonally available
453 food (Reinthal, 1990; Bouton et al., 1997; Genner et al., 1999b). However, there appears to be a
454 continuum in the degree of niche overlap among these species depending on whether or not
455 shared resources are limiting (Bouton et al., 1997; Genner et al., 1999b; Duponchelle et al.,
456 2006), but a high degree of overlap may occur regardless of the availability of shared resources
457 (Martin and Genner, 2009).

458 Recent field observations by other investigators and results from the current study permit
459 some speculation about the sorts of behavioral and ecological interactions that may be occurring
460 between species of *Tramitichromis* and *Aulonocara*. A small number of stomach content
461 analyses show potential for diet overlap in these taxa (Fryer, 1959; Konings, 2007). Species of
462 *Tramitichromis* and *Aulonocara* have lake-wide distributions (Konings, 2007), presenting the
463 opportunity for spatial overlap. Where they co-occur, *Aulonocara* might experience interference
464 competition from *Tramtichromis* given its prey search strategies. For instance, members of these
465 two genera have been observed foraging in the same areas where *Tramitichromis* (and other sand
466 sifters) can interrupt foraging by *Aulonocara* (which hover just above the sand searching for
467 prey) by just swimming nearby (M. Kidd, personal communication). Furthermore, the sand
468 plunging behavior of *Tramitichromis*, removes and likely disrupts other invertebrates in the sand,
469 altering the topography of the bottom sediments, which may prevent *Aulonocara* from detecting
470 prey by swimming just above sand surface. These two taxa also occupy different depth ranges
471 (*Tramitichromis* spp.:<15 m, Konings, 2007; *Aulonocara* spp.: 5–120 m, Konings, 1990, 2007).
472 Species of *Aulonocara* may escape competition in shallower waters by foraging in deeper water.
473 Genner and Turner (2012) assigned several species of *Aulonocara* to an assemblage of "deep
474 benthic feeders" and suggested that these fishes have sensory adaptations (including

475 modification of the cranial lateral line canal system) that should enable them to detect prey at the
476 depth at which they are found. This is supported by experimental work that demonstrated that *A.*
477 *stuartgranti* uses its lateral line system in prey detection, especially in the dark (Schwalbe et al.,
478 2012). Furthermore, the ability of species of *Aulonocara* to detect prey non-visually may allow
479 them to forage crepuscularly and/or nocturnally (not yet documented in the field), thus
480 facilitating spatial and temporal segregation between *Aulonocara* species and other cichlids that
481 feed on benthic invertebrates in the sand, including species of *Tramitichromis*.

482 Future studies that involve the integration of the analysis of laboratory-based sensory
483 biology with field-based ecological studies will allow tests of hypotheses that: 1) evolutionary
484 changes in the morphology and physiological capabilities of a sensory system (such as widened
485 canals) are adaptations that allow species to occupy novel trophic niches, and 2) that species use
486 different combinations of sensory cues in the same sensory environment to spatially or
487 temporally partition similar resources in a common habitat.

488

489 **Acknowledgements**

490

491 We thank Emily Becker and Rebecca Scott who contributed Fig. 1A, B, and Douglas Moore
492 (Orthopedics Research Lab, Rhode Island Hospital) and Timothy Alberg, who generated and
493 analyzed μCT data in Fig. 1C, D. Dr. Nathan Bird provided comments that improved earlier
494 versions of the manuscript. Edward Baker (Facilities Manager, RI NSF EPSCoR Marine Life
495 Science Facility), Emily Becker, Joshua Hower, Brandon Fuller, Callie Veelenturf, and Rebecca
496 Scott were responsible for fish husbandry. This research was funded by the University of Rhode
497 Island College of the Environment and Life Sciences and the National Science Foundation (NSF)

498 grant IOS 0843307 to JFW, and was supported in part by NSF EPSCoR Cooperative Agreement
499 EPS-1004057.

500

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Table 1. Generalized linear mixed model (GLMM) results for *Tramitichromis* feeding on live and dead prey during light and dark trials (Experiment I) comparing number of prey strikes, detection distance, and swimming phase during prey detection (pause vs. glide).

	Number of Prey Strikes			**Detection Distance**			**Pause vs. Glide**		
Source	*F*	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Light/Dark	273.28	1, 68	<0.001	40.89	1, 213	<0.001	10.39	1, 213	0.001
Prey	3.83	1, 68	n.s.	2.52	1, 213	n.s.	1.29	1, 213	n.s.
Light/Dark × Prey	4.68	1, 68	0.034	0.25	1, 213	n.s.	0.003	1, 213	n.s.

651 **Table 2**. Mean prey preference scores for *Tramitichromis* (Experiments I and II) and *A.*

652 *stuartgranti* (Experiment I only, data from Schwalbe et al., 2012) feeding on live and dead prey

653 in light and dark (Experiment I only) trials following Taplin (2007).

			Light Trials		Dark Trials	
Species	Experiment		Live	Dead	Live	Dead
Tramitichromis			5.74***	7.26	6.54	6.46
Aulonocara stuartgranti	Experiment I		5.49**	7.52	4.78**	8.22
		Pre-Cobalt	5.25*	7.75		
Tramitichromis	Experiment II	Cobalt	6.08	6.92		
		Post-Cobalt	6.67	6.33		

654

655 If the fish demonstrated a preference for a type of prey (indicated by a significant lower

656 preference score), it was always for live prey (paired *t*- test, **P* < 0.05, ***P* <0.01, ****P* <0.001).

657

Table 3. Generalized linear mixed model (GLMM) results for *Tramitichromis* feeding on live and dead prey during light trials after cobalt chloride treatment (Experiment II) comparing number of prey strikes, detection distance, and swimming phase during prey detection (pause vs. glide).

	Number of Prey Strikes			**Detection Distance**			**Pause vs. Glide**		
Source	*F*	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Trial	1.38	2, 12	n.s.	2.24	2, 76	n.s.	0.000	2, 75	n.s.
Prey	2.87	1, 12	n.s.	0.07	1, 76	n.s.	0.001	1, 75	n.s.
Trial × Prey	0.96	2, 12	n.s.	1.95	2, 76	n.s.	0.000	2, 75	n.s.

Table 4. Generalized linear mixed model (GLMM) results for *Tramitichromis* (this study) and *A. stuartgranti* (data from Schwalbe et al., 2012) feeding on live and dead prey during light and dark trials (Experiment I) comparing number of prey strikes, detection distance, and swimming phase during prey detection (pause vs. glide).

	Number of Prey Strikes			**Detection Distance**			**Pause vs. Glide**		
Source	*F*	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Species	0.38	1, 136	n.s.	2.34	1, 444	n.s.	0.000	1, 432	n.s.
Light/Dark	352.89	1, 136	<0.001	156.46	1, 444	<0.001	0.000	1, 432	n.s.
Prey	12.46	1, 136	0.001	6.24	1, 444	0.013	0.003	1, 432	n.s.
Light/Dark × Prey	0.40	1, 136	n.s.	0.12	1, 444	n.s.	0.000	1, 432	n.s.
Species × Light/Dark	7.69	1, 136	0.006	23.17	1, 444	<0.001	0.000	1, 432	n.s.
Species × Prey	1.29	1, 136	n.s.	4.45	1, 444	0.036	0.003	1, 432	n.s.
Species × Light/Dark × Prey	4.07	1, 136	0.046	2.11	1, 444	n.s.	0.000	1, 432	n.s.

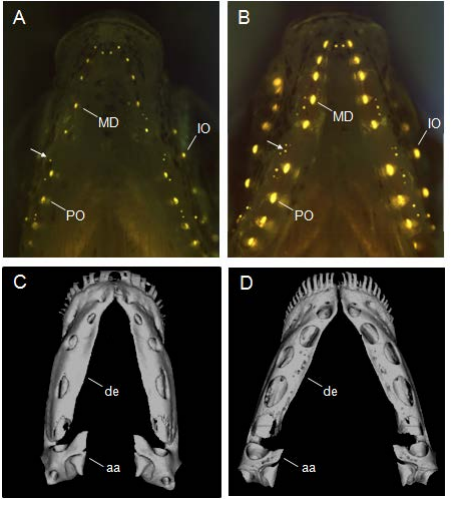
Figure Legends

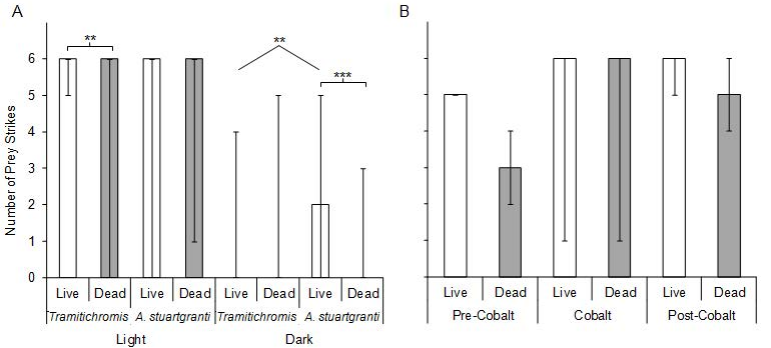
Fig. 1. Ventral view of the mandible of *Tramitichromis* sp. and *Aulonocara* spp. illustrating the canal and superficial neuromasts and mandibular lateral line canals. (A) Ventral view of a juvenile *Tramitichromis* sp. (standard length [SL] =18 mm) and (B) *A. stuartgranti* (SL = 16 mm) fluorescently stained with 4-Di-2-ASP (63 μM, 5 min) to reveal the hair cells in the sensory strip in superficial neuromasts (lines and clusters [arrows]) and larger canal neuromasts in the mandibular (MD), preopercular (PO), and infraorbital (IO) canals. MicroCT 3-D reconstruction of the mandible [dentary (de) and angulo-articular (aa) bones] of (C) *Tramitichromis* sp. (SL = 29 mm) showing the bony pores of the MD canal and (D) *A. baenschi* (SL = 87 mm).

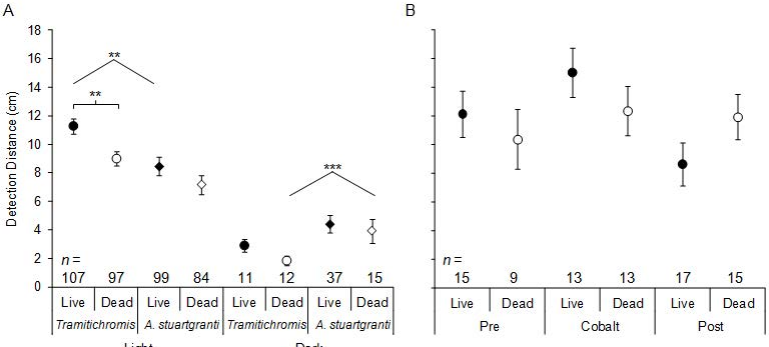
Fig. 2. Number of prey strikes (median ± min/max) on live and dead prey for (A) *Tramitichromis* (Experiment I) and *A. stuartgranti* (data from Schwalbe et al., 2012) in light and dark trials, and (B) *Tramitichromis* (Experiment II, light trials only). LSD, $^{**}P < 0.01$, $^{***}P < 0.001$. See text for additional details.

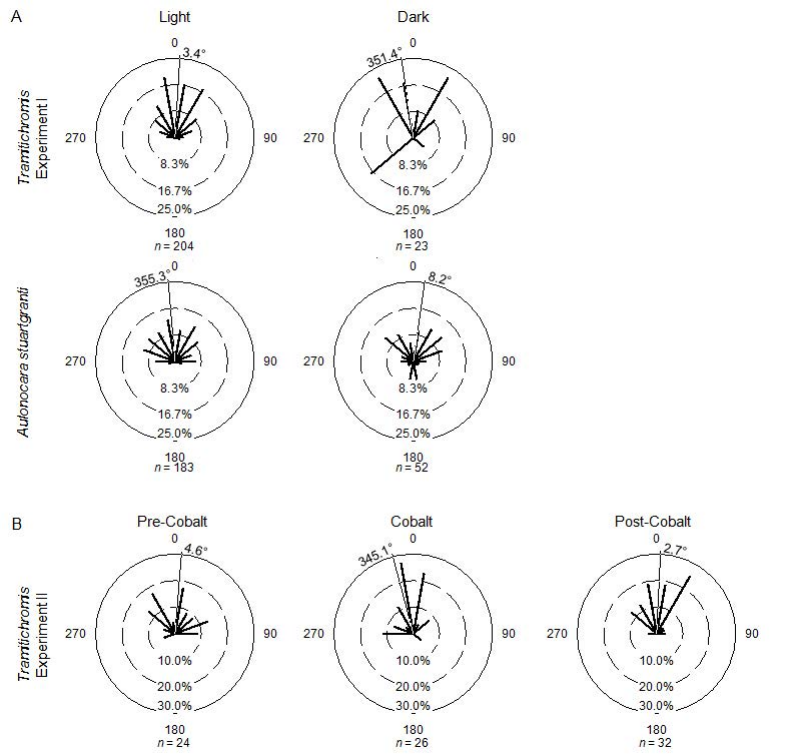
Fig. 3. Detection distance (mean ± SE) for live and dead prey for (A) *Tramitichromis* (Experiment I) and *A. stuartgranti* (data from Schwalbe et al., 2012) in light and dark trials, and (B) *Tramitichromis* sp. (Experiment II, light trials only). Non-transformed data are illustrated here (which are biologically relevant), but statistics were carried out on log-transformed data, as appropriate. LSD, $^{**}P < 0.01$, $^{***}P < 0.001$. See text for additional details.

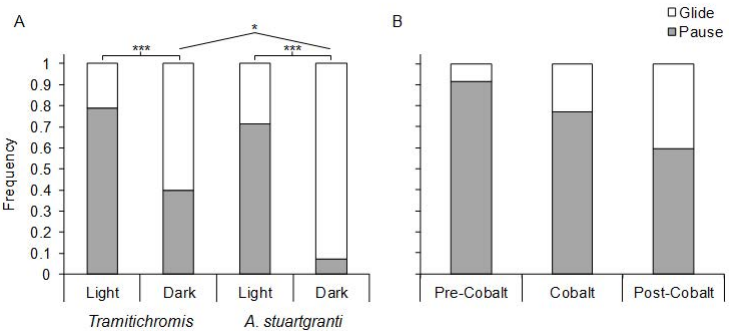
693 **Fig. 4.** Orientation to prey (live and dead combined) at time of detection for (A) *Tramitichromis*
694 (Experiment I) and *A. stuartgranti* (data from Schwalbe et al., 2012) light and dark trials and (B)
695 *Tramitichromis (*Experiment II, light trials only). Bars represent the proportion of the total
696 number of detection events grouped into 20° intervals. The narrow line represents mean angle.
697 The center of the polar plot (facing 0°) represents the location of the midpoint between the eyes.
698 See text for additional details.
699
700 **Fig. 5.** Frequency of prey detections that occurred during the glide or pause phase of swimming
701 leading to prey strikes in (A) *Tramitichromis* (Experiment I) and *A. stuartgranti* (data from
702 Schwalbe et al., 2012) light and dark trials, and (B) *Tramtichromis* (Experiment II, light trials
703 only). LSD, $*P < 0.05$, $***P < 0.001$. See text for additional details.


A
MD
IO
PO
B
MD
IO
PO
C
de
aa
D
de
aa


A
B
Number of Prey Strikes
0
1
2
3
4
5
6
**
**
***
Live
Dead
Live
Dead
Live
Dead
Live
Dead
Tramitichromis
A. stuartgranti
Tramitichromis
A. stuartgranti
Light
Dark
Live
Dead
Live
Dead
Live
Dead
Pre-Cobalt
Cobalt
Post-Cobalt


A
B
Detection Distance (cm)
18
16
14
12
10
8
6
4
2
0
**
**
***
n =
107
97
99
84
11
12
37
15
Live
Dead
Live
Dead
Live
Dead
Live
Dead
Tramitichromis
A. stuartgranti
Tramitichromis
A. stuartgranti
Light
Dark
n =
15
9
13
13
17
15
Live
Dead
Live
Dead
Live
Dead
Pre
Cobalt
Post


A
Light
Dark
Tramitichromis Experiment I
Aulonocara stuartgranti
0
90
180
270
3.4°
351.4°
355.3°
8.2°
8.3%
16.7%
25.0%
n = 204
n = 23
n = 183
n = 52
B
Pre-Cobalt
Cobalt
Post-Cobalt
Tramitichromis Experiment II
4.6°
345.1°
2.7°
10.0%
20.0%
30.0%
n = 24
n = 26
n = 32


A
B
Glide
Pause
Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
*
***
Light
Dark
Light
Dark
Tramitichromis
A. stuartgranti
Pre-Cobalt
Cobalt
Post-Cobalt

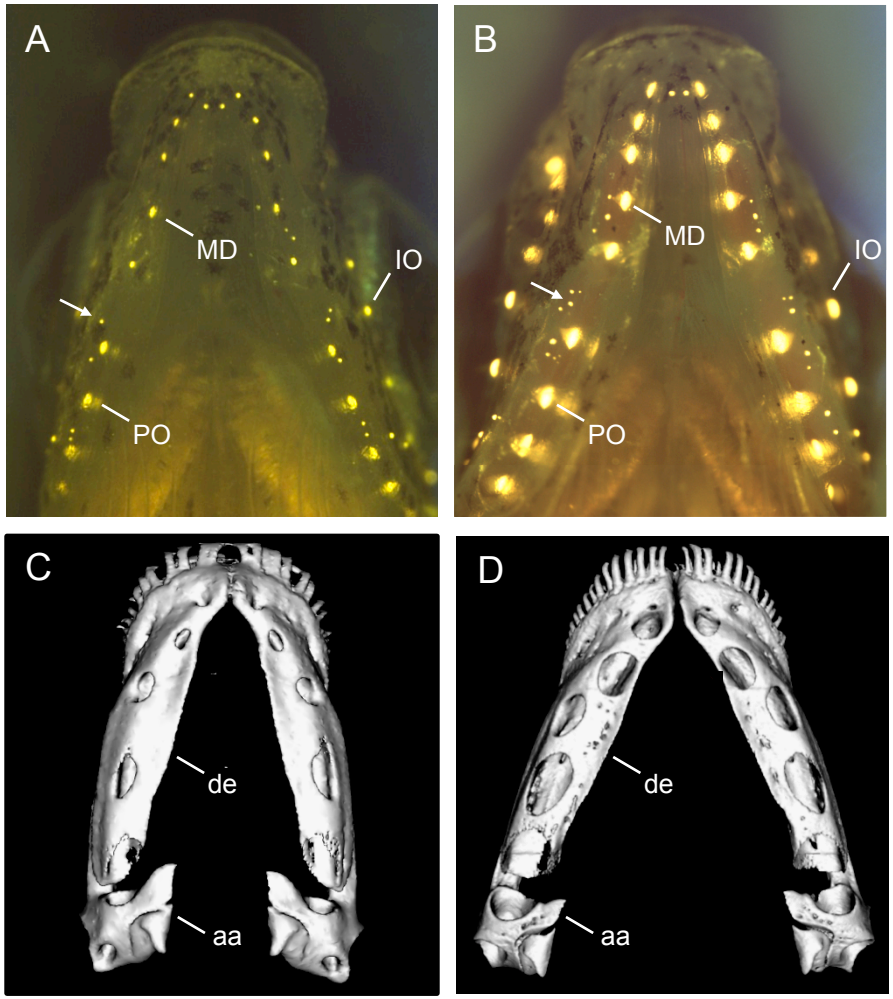


Figure 1. Color

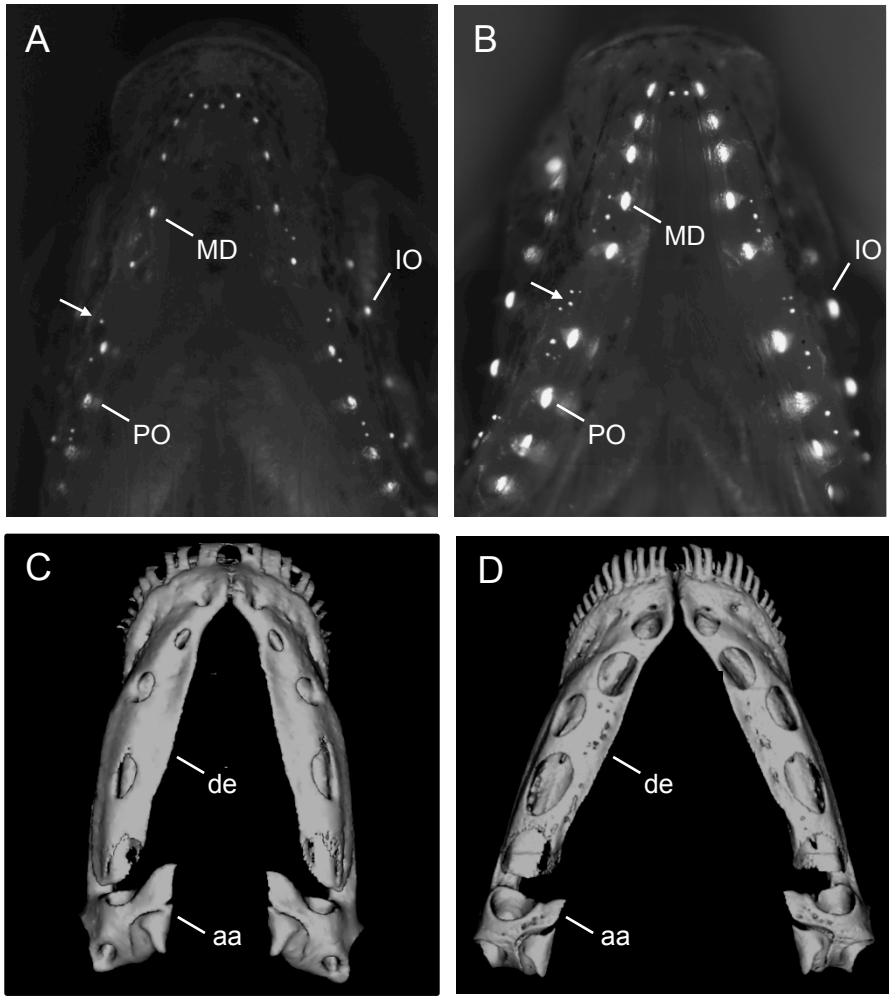


Figure 1. Grayscale

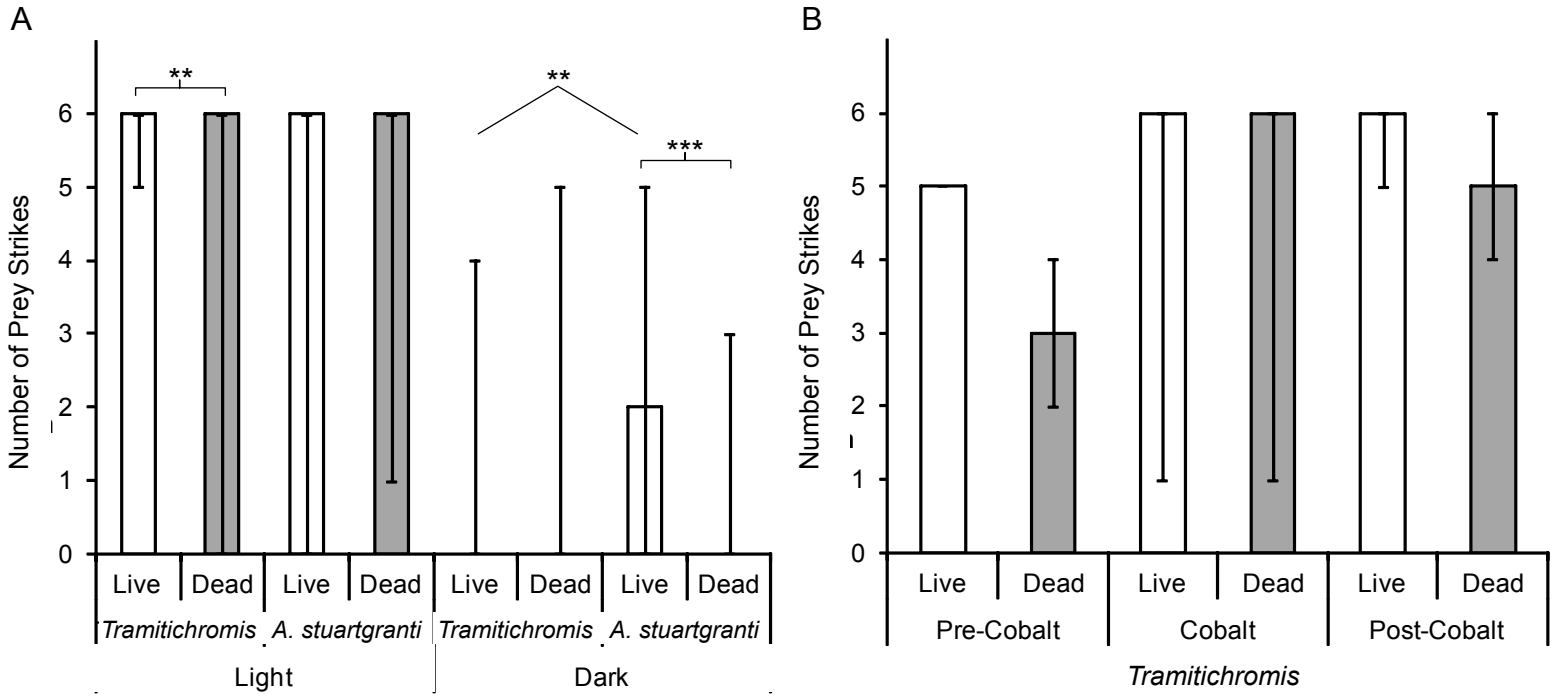

A
B
Number of Prey Strikes
0
1
2
3
4
5
6
**
**
***
Live
Dead
Tramitichromis
A. stuartgranti
Light
Dark
Pre-Cobalt
Cobalt
Post-Cobalt
Tramitichromis

Fig. 2

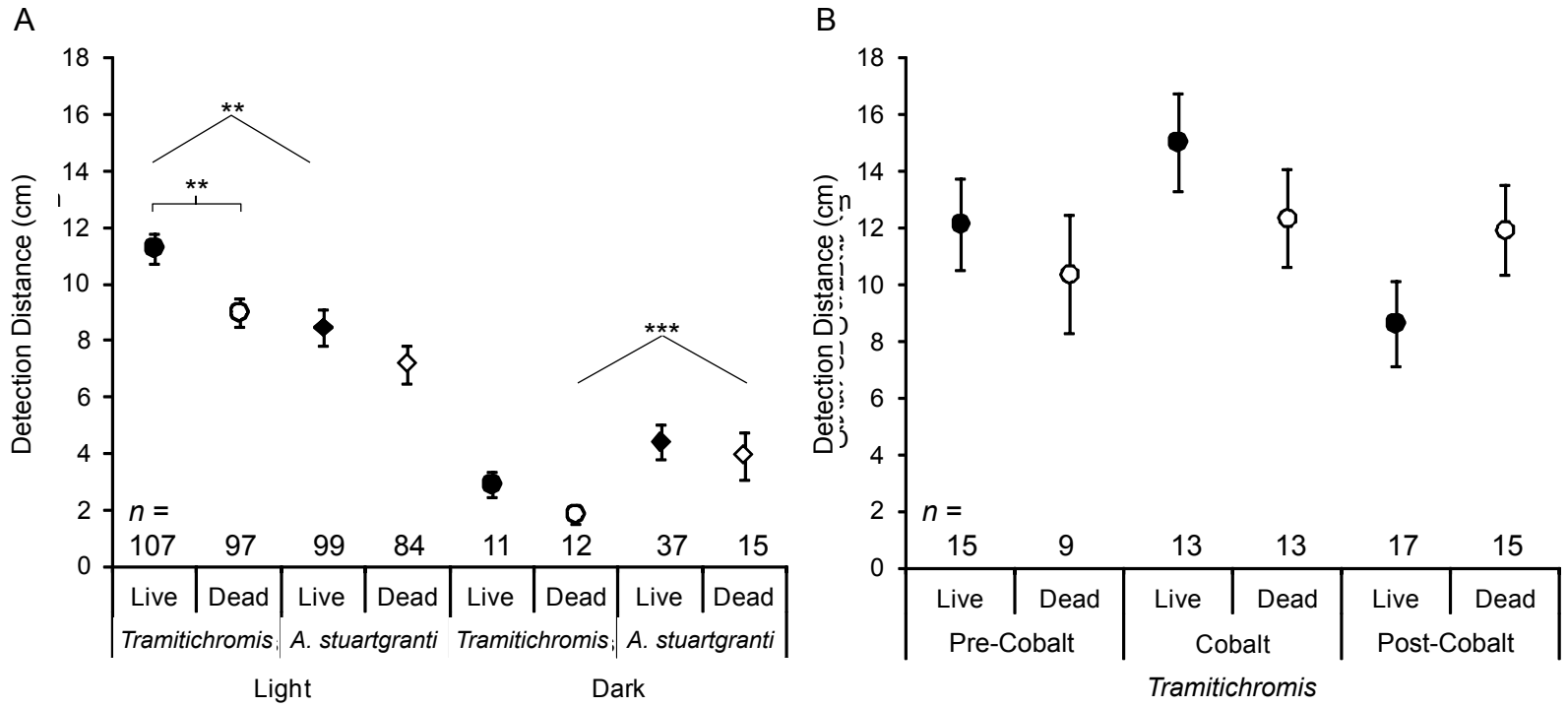

A
Detection Distance (cm)
18
16
14
12
10
8
6
4
2
0
**
**
***
n =
107
97
99
84
11
12
37
15
Live
Dead
Live
Dead
Live
Dead
Live
Dead
Tramitichromis
A. stuartgranti
Tramitichromis
A. stuartgranti
Light
Dark
B
Detection Distance (cm)
n =
15
9
13
13
17
15
Live
Dead
Live
Dead
Live
Dead
Pre-Cobalt
Cobalt
Post-Cobalt
Tramitichromis

Fig. 3

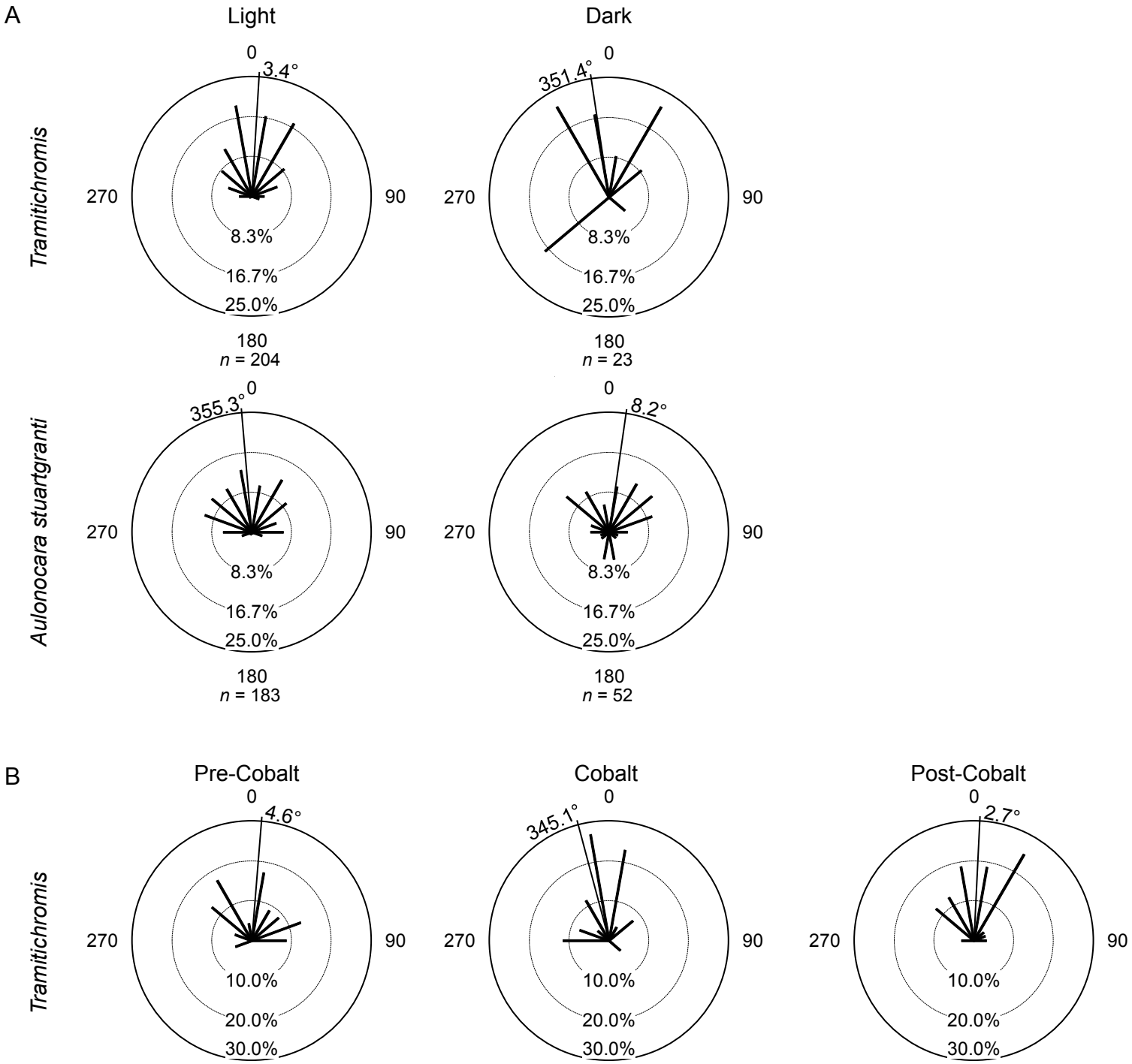

A
Light
Dark
Tramitichromis
Aulonocara stuartgranti
0
90
180
270
3.4°
351.4°
355.3°
8.2°
8.3%
16.7%
25.0%
n = 204
n = 23
n = 183
n = 52
B
Pre-Cobalt
Cobalt
Post-Cobalt
4.6°
345.1°
2.7°
10.0%
20.0%
30.0%

Fig. 4

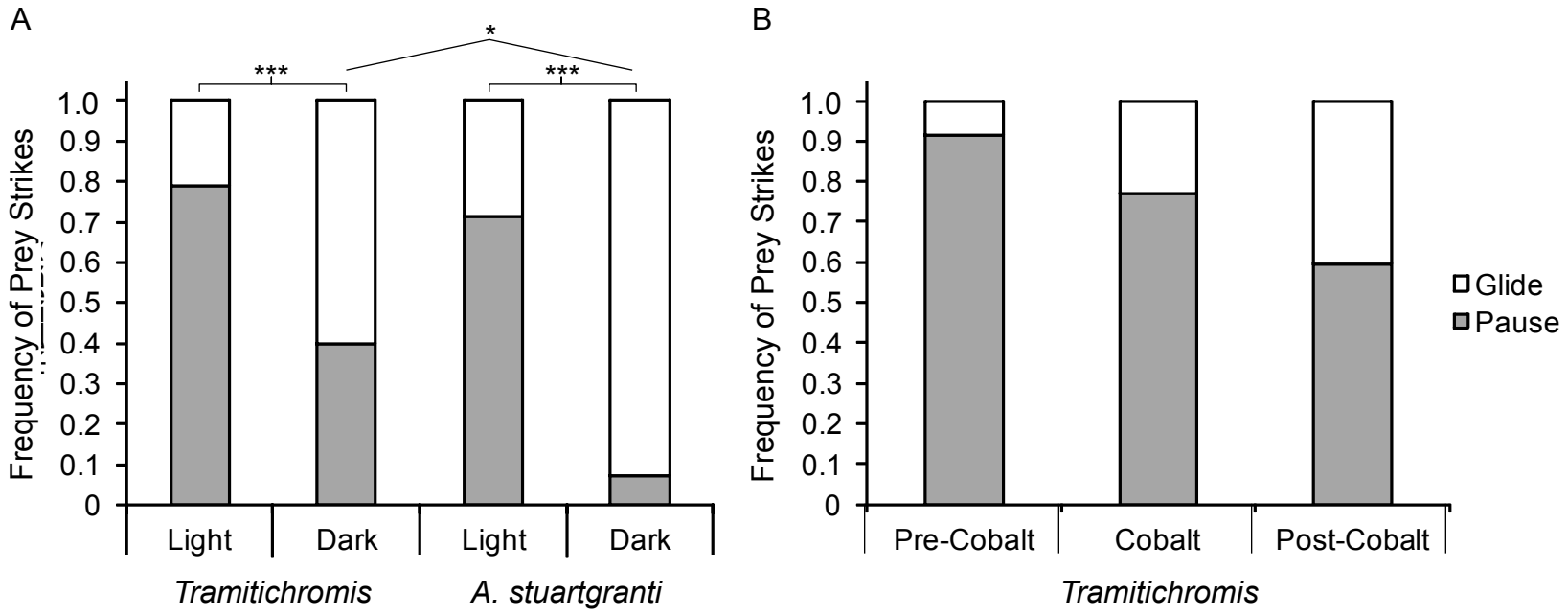

A
*
***
***
Frequency of Prey Strikes
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Light
Dark
Light
Dark
Tramitichromis
A. stuartgranti
B
Frequency of Prey Strikes
Pre-Cobalt
Cobalt
Post-Cobalt
Tramitichromis
Glide
Pause

Fig. 5