Colour Vision in Stomatopod Crustaceans: more questions than answers

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Abstract

Stomatopod crustaceans, or mantis shrimps, are known for their extensive range of spectral sensitivities but relatively poor spectral discrimination. Instead of the colour-opponent mechanism of other colour vision systems, the 12 narrow-band colour channels they possess may underlie a different method of colour processing. We investigated one hypothesis, in which the photoreceptors are proposed to act as individual wave-band detectors, interpreting colour as a parallel pattern of photoreceptor activation, rather than a ratiometric comparison of individual signals. This different form of colour detection has been used to explain previous behavioural tests in which low saturation blue was not discriminated from grey potentially because of similar activation patterns. Results here, however, indicate that the stomatopod, *Haptosquilla trispinosa* was able to easily distinguish several colours, including blue of both high and low saturation, from greys. The animals did show a decrease in performance over time in an artificially lit environment, indicating plasticity in colour discrimination ability. This rapid plasticity, most likely the result of a change in opsin (visual pigment) expression, has now been noted in several animal lineages (both invertebrate and vertebrate) and is a factor we suggest needing care and potential re-examination in any colour-based behavioural tests. As for stomatopods, it remains unclear why they achieve poor colour discrimination using the most comprehensive set of spectral sensitivities in the animal kingdom and also what form of colour processing they may utilise.

Introduction

Mantis shrimp, or stomatopods, possess perhaps the most complex retina of all visual systems known (Marshall, 1988; Cronin and Marshall, 1989; Marshall et al., 2007; Marshall and Arikawa, 2014). With 12 spectral photoreceptors (and others for polarisation and intensity detection bringing the total of input-channels to 20), they outnumber, with the possible exception of butterflies (Arikawa, 2003; Chen et al., 2016; Marshall and Arikawa, 2014), the receptor diversity of other animals, which commonly have between two and four spectral sensitivities (Barlow, 1982; Kelber and Osorio, 2010). The 12 colour receptors are spread evenly through the spectrum sampling from just below 300 nm to above 700 nm but most likely do not construct the dodecahedral colour space they are capable of, as there are no known colour tasks in nature requiring this degree of scrutiny (Barlow, 1982; Kelber and Osorio, 2010; Marshall and Arikawa, 2014). Their sharply tuned photoreceptor set has been proposed as effective in achieving colour constancy in the spectrally challenging marine environment (Osorio et al., 1997), but this idea remains hypothetical.

While early behavioural tests demonstrated colour vision in stomatopods based on the von Frisch colour from greys paradigm (von Frisch, 1974; Marshall et al., 1996), more recent and more detailed wavelength discrimination experiments suggest that stomatopods lack fine spectral discrimination (Thoen et al., 2014). Based largely on anatomical evidence (Marshall, 1988; Marshall et al., 1991a,b) a four-spectral window opponent comparison was originally proposed in which different eye regions (rows of ommatidia in the mid-band region of the eye) analysed discrete zones of the 400-700nm spectrum. This would still enable very fine spectral analysis, in particular due to the sharp sensitivities the eye achieves with serial filtering mechanisms (Marshall, 1988; Cronin and Marshall, 1989). Contrary to this, the results of Thoen and colleagues clearly showed that, at least in a two-choice food reward paradigm, spectral discrimination, or Δλ, was unexpectedly poor, indeed around ten times worse than other animals tested in similar ways, including goldfish, butterflies, birds and humans (Thoen et al., 2014; **Figure 1B**).

Although surprising, these results did potentially provide an explanation for one of the observations made in the original colours from grey behavioural assay. In this

59　experiment, the peacock mantis shrimp, *Odontodactylus scyllarus*, could not

60　discriminate a light blue feeding container from greys (Marshall et al., 1996). In an

61　attempt to explain this failure in choice, it was suggested that mantis shrimp may

62　analyse colour as a pattern of 12 excitations across the spectrum, rather than with

63　any comparison of spectral sensitivities. This idea is congruent with their scanning

64　eye-movements and way of examining coloured objects (Land et al., 1990; Land,

65　1999).

66　The reason stomatopods may have evolved such an apparently different visual

67　system is unknown, but evolutionarily they have been out on a limb for 400 million

68　years (Ahyong and Harling, 2000), hinting that they have been 'trying out' something

69　different to other animals for a while. It is possible they have a neural mechanism

70　that interprets colour somehow very differently to the typical opponent systems

71　(Thoen et al., 2014) but current investigations into their sub-retinal wiring and

72　potential colour-information processing, at least at the anatomical level, seems to

73　argue against this (Kleinlogel and Marshall, 2005; Templin, 2017; Streets, 2021).

74　There is no totally novel reorganisation in basic neural patterns beneath individual

75　photoreceptors at the lamina, medulla and lobula stages of information processing,

76　either between eye regions or indeed in comparison to other arthropods (Strausfeld

77　and Andrew, 2011; Thoen et al., 2017, 2018).

78　Stomatopod compound eyes are composed of two peripheral hemisphere regions on

79　either side of a central midband. While the morphology of the ommatidia in the

80　hemispheres is much like other crustaceans, the six rows of ommatidia in the

81　midband are modified in a number of ways. It is in the top four midband rows that the

82　specialisations for colour vision are found, each row sensitive to three wavelength

83　zones. The colour photoreceptors are sharpened and shifted in their spectral

84　sensitivity by a number of filtering mechanisms including short wavelength filtering

85　(in the UV) by the dioptric crystalline cone elements, photoreceptor tiering and, in

86　rows 2 and 3, photostable colour filters (Bok et al., 2014; Cronin and Marshall, 1989;

87　Marshall, 1988; Marshall et al., 2007) (**Fig. 1A,C**). Beneath the retina, information

88　from the midband rows initially remains separated from the hemispheres, and

89　through the eye-stalk neuropils, lamina, medulla and lobula, there are anatomically

90　segregated zones that receive input from midband and each of the hemispheres.

91   While, as noted already, the basic arrangement here appears similar to other

92   arthropods, in fact centripetally there is increasing complexity and some cross-talk

93   between retinal zones, including between the midband rows themselves (Thoen et

94   al., 2018). Until effective electrophysiological recordings are made at these levels,

95   further speculation is just that. Nonetheless, a brief review of past ideas, placed in

96   the context of knowledge level of the time, contributes to the background and

97   motivation for the behavioural data presented here.

98   There are several hypotheses previously suggested to explain how mantis shrimps

99   may process colour information. Based on initial anatomical evidence, it was

100   originally proposed that photoreceptor signals outside the ultraviolet (UV) range, may

101   be compared within each of the four colour-sensitive midband rows (1-4) (**Fig. 1C**),

102   delivering a possible "dichromatic" opponent system, each row examining a limited

103   spectral zone from 400-700 nm. Compelling evidence here is in the fact that the

104   rhabdomeric cells in each tier of these rows are the same as those that, in other

105   crustaceans and in the stomatopod hemispheres, are set up as polarisation

106   opponent sub-populations, comparing, for example, horizontally polarised light to

107   vertical (Glantz and Miller, 2002; Marshall et al 1991a; Strausfeld and Nassel, 1981).

108   This means that, without the need to reorganise existing wiring beneath the retina, a

109   polarisation opponency becomes colour opponency.

110   The UV sensitive rhabdomeric cells, called R8 by convention, in common with many

111   arthropods, possess long visual fibre axonal connection that penetrates through the

112   lamina to terminate in the medulla. In order for any form of intra-UV opponency to be

113   possible (a possibility as supported by the recent work of Bok et al., 2018), an inter-

114   row comparison must be made between R8s (Kleinlogel and Marshall, 2005;

115   Marshall, 1988; Marshall et al., 2007). UV sensitivity in many other animals seems

116   designated to a specific task, such as phototaxis towards UV in flying insects, as it

117   typically represents open sky (Baden and Osorio, 2019; Kelber et al., 2003;

118   Schnaitmann et al., 2020). While this may also be the case for stomatopods (Bok et

119   al., 2018), there is potential for the more complex intra-UV opponency, as they are

120   equipped with 4 separate UV visual sensitivities, peaking from as low as 315 nm. As

121   previously noted, recent behavioural tests support this intra-UV opponency

122   hypothesis (Bok et al., 2018).

123   An alternative idea is that stomatopods may analyse colour information in a manner

124   similar to the processing of auditory information by the cochlea, examining the

125   chromaticity of light within each spectral band as a continuum of frequency, rather

126   than using opponent processing (Marshall et al., 2007). Instead of comparing

127   spectral signals, downstream computation centres would determine colours by the

128   pattern, or placement in the frequency continuum, of photoreceptor activation. This

129   idea, sometimes called the 'bar-code' hypothesis, likening the scan over objects to

130   bar-code readers or indeed QR-codes. The idea has been used to explain the limited

131   colour discrimination ability and argue for a system in some ways more like a colour

132   categorising system based on which photoreceptors are activated. Supporting

133   evidence here comes in two forms. Firstly, there are striking similarities to the way

134   colours are processed in the inferior temporal cortex of primates (Zaidi et al., 2014)

135   and may facilitate faster processing at the cost of poorer colour discrimination

136   between similar wavelengths (Thoen et al., 2014). Secondly, the compressed optics

137   of the stomatopod eye (Marshall and Land, 1993) drive the system to sample the

138   world with slow scanning eye movements (Land et al., 1990). The stomatopod eye

139   therefore can be thought of as operating like a satellite-born push-broom detector or

140   indeed any line-scan device such as a photocopier (Wolpert, 2011).

141   The bar-code hypothesis also explains the apparent over-proliferation of spectral

142   sensitivities and their narrow-band tuning, as this set of 12 is needed to cover the

143   available spectrum from 300-700 nm (Marshall and Arikawa, 2014). As shown by

144   Barlow and others (Barlow, 1982), an opponent system only requires around 4

145   spectral sensitivities over this range to decode almost all colour information present

146   on earth.

147   There is in fact no reason why both opponency and some sort of pattern, barcode,

148   categorical of frequency analysis system might not operate simultaneously. If

149   stomatopods have divided up the spectrum into dichromatic bins, it may be that each

150   of these is used to solve some sort of relevant task while an overall sense of colour

151   is provided by bar-code scanning. Having, in some ways an over-precise opponent

152   process, has been suggested as a way to solve the problems of colour constancy

153   under water (Osorio et al., 1997). In order to further explore the various hypotheses,

154   we conducted a series of experiments using a different species of stomatopod,

155   *Haptosquilla trispinosa*, to determine whether all low saturation colours, not just blue

156   (Marshall et al., 1996), were more difficult to learn than high saturation colours

157   (Experiment 1). Based on initial results, we also set out to examine any

158   inconsistencies in performance, such as a change in visual performance after being

159   kept in captivity (Experiment 2). Potential species difference led us to examine innate

160   preferences for specific colours or colour types (Experiment 3).

161   # Methods

162   ## Animal Care

163   Stomatopods were collected on the shallow reefs around Lizard Island Research

164   Station (LIRS) (GBRMPA Permit no. G17/38160.1) during August 2018, 2019, and

165   2020. They were individually housed in aquaria with small PVC tubes to use as

166   burrows and fed small pieces of raw shrimp. Experiments requiring training were

167   conducted, either at the Lizard Island research Station, under shaded but natural

168   daylight, or at the University of Queensland (UQ), on a 12:12 h light:dark cycle with

169   salinity between 32-35 ppm. The UQ aquarium lights consist of fluorescent tubes

170   combined to provide illumination as close as possible to natural daylight (**Fig. 5**).

171   ## Stimulus Design

172   The stimuli were made of small white cable ties (2.5mm width, 10cm length), the

173   ratchet-end of the cable tie making a convenient small feeding dish and flat end on

174   which to attach various coloured or neutral density filters (Lee Filters, Andover, UK;

175   see Templin, 2017 for further details). Filters were: high and low saturation red (Lee

176   Filters 182/035, respectively), orange (287/162), green (124/725), blue (195/725),

177   and neutral density filters (Lee Filters: ND 0, 0.15, 0.6, 0.9). The low-saturation blue

178   filter (725) is the same one used in Marshall et al. (1996). ND filters were also the

179   same in Marshall et al. (1996) and other experiments (review: Kelber et al., 2003) to

180   ensure the animals made choice based on colour, not brightness.

181   **Fig. 2** shows spectral measurements of each stimulus filter attached to a cable tie. In

182   order to make relevant comparisons, spectra were normalised to the maximum

183   reflectance of the transparent filter ND 0. Photoreceptor responses were calculated

184   using a modified quantum catch calculation (Kelber et al., 2003; Marshall et al.,

185   1996). Individual spectral sensitivities for *H. trispinosa* were taken from Thoen et al.,

186   2014 (**Fig. 1A**) and downwelling light measured with an Ocean Optics USB2000

187   spectrophotometer (**Fig. 5**, "Experiment 1"). These values were normalised by the

188   individual spectra of the filters (**Fig. 2**) to give the final photoreceptor response (**Fig.**

189   **3**) as per Marshall and Vorobyev (2003).

Experimental Procedure

191   Feeding choice tests were used to compare the ability of *H. trispinosa* to discern

192   either high or low saturation colours from neutral greys. Each stomatopod was

193   assigned a single colour high or low saturation red, orange, green, or blue. Animals

194   readily emerge from their burrow to pick up the cable tie and retreat back into their

195   home to consume the food (**Fig. 4**). A successful choice was recorded if they took

196   the correct cable tie first and pulled it toward their burrow.

197   Animals were introduced to their stimulus during a "priming" week, where they were

198   given a single cable tie, coloured according to their assignment, with food in the

199   cavity created by the front of the cable tie. During the second week, they were

200   "trained" with a priming cable tie (containing food), plus the distractor(s) (ND 0, 0.15,

201   0.6, 0.9) presented in a pseudo-random order. After the second week, the animals

202   which reached approximately a 70% rate of cable-tie feeding were used for testing.

203   Animals that did not participate were considered untrainable and were removed. This

204   method is further described in Templin, 2017.

205   **Fig. 4** shows the experimental setup. During a trial, each stomatopod was presented

206   with their assigned colour and one (Experiment 2) or two (Experiment 1) neutral

207   density distractors. Cable ties were held loosely in a holder so they could be easily

208   removed. The cable ties were presented approximately 3 cm from the entrance to

209   their burrow as a set. In training trials, a small piece of food was placed inside the

210   cavity of the target cable tie. During tests, the target cable tie and all neutral density

211   distractors were newly fabricated and had never come into contact with food in order

212   to eliminate any residual olfactory cues.

213 Tests in which cable ties were not in contact with food were conducted once a day,
214 five times a week. Additionally, mantis shrimp were trained once a week with food
215 present to reinforce the behaviour. The stomatopods were given approximately three
216 minutes to make a decision or were considered to not have participated. A trial
217 began when the barrier inserted between the burrow and the stimulus (**Fig. 4**) was
218 lifted. If the mantis shrimp chose correctly, it was rewarded with a small piece of
219 food. If it chose the unrewarded colour, the cable ties were removed, and no food
220 was awarded.

221 The combination of coloured cable ties and neutral density filters was made using a
222 random number generator. Enough experiments were conducted so that each
223 combination occurred at least once during all testing trials but not more than three
224 times during both training and testing. The trials were ordered such that the target
225 cable tie was not in the same location for two (for 3-choice, Experiment 1) or three
226 (for 2-choice, Experiment 2) consecutive trials. If a mantis shrimp participated 2
227 times or less per week, for more than one week, it was considered untrainable, and
228 therefore replaced.

229 <u>Individual Experiments</u>

230 *Experiment 1: High/Low Saturation*

231 In Experiment 1, we investigated the original "barcode" hypothesis, the possible
232 expectation being that they would fail or find more difficult all low-saturation colours.
233 Animals were tested across 8 colour types (four hues: red, orange, green, and blue,
234 each at two saturation levels: high and low). Stomatopods were given a three-choice
235 test, with two neutral density distractors. However, after a while it was noted that
236 animals significantly preferred the middle position of the target cable tie ($p < 0.001$).
237 Only one distractor was used in later experiments to prevent this bias, and in three-
238 way choices, results were adjusted to account for this bias. These tests were
239 exclusively conducted in captivity at UQ, beginning at least 3 months post-capture.

240   *Experiment 2: High saturation discrimination over time*

241   In the second experiment, we tested whether the length of time in captivity affected

242   the ability of the mantis shrimp to discriminate colour. Tests consisted only of highly

243   saturated red, green, and blue versus one neutral density distractor. Tests were

244   divided into three time points to judge abilities at different lengths of time spent in

245   captivity: (1) One week at LIRS; (2) weeks 1-10, at UQ under artificial lighting; and

246   (3) at the final weeks of testing (weeks 10-20) at UQ. Animals at LIRS were trained

247   and tested for one week, three times a day.

248   *Experiment 3: Naïve-Choice Tests*

249   All naïve-choice tests were conducted in the LIRS aquarium system under natural

250   lighting in which freshly caught animals were given 24 hours to acclimate. Two-

251   choice tests were conducted across all colours (red, orange, green, blue): (A) high

252   saturation preference, (B) low saturation preference, (C) preference of colour versus

253   neutral density grey, and (D) preference of high or low saturation. In high and low

254   saturation tests (A and B), individuals were given a random pair of red, orange,

255   green, or blue stimuli in the same saturation type. In the saturation preference test

256   (C), individuals were given a high and low saturation stimulus of a single colour.

257   Finally, for colour versus grey preference (D), animals were given a randomised

258   colour (red, orange, green, or blue) and neutral grey (ND 0, 0.15, 0.6, and 0.9) pair.

259   Each individual was only used once in each experiment type; some were used in two

260   different experiments due to collection restrictions. Individuals were given 30 minutes

261   to make a choice. If an individual did not participate, it was tested again with the

262   same choice for up to two additional sessions, and then replaced.

263   ## Data Analysis

264   Ability to learn the task was evaluated with a simple binomial test (BINOM.DIST,

265   Microsoft Excel). This approach compares the number of times they chose a

266   particular stimulus with the number of times they would be expected to choose it by

267   chance. Individuals were included in analysis only if they were successful more often

268   than expected by chance during training trials, as well as those which participated in

269   at least 10 trials in Experiment 1, at least 3 trials at LIRS, or 5 trials at UQ in

270   Experiment 2. Animals were assumed to have learned the task if they selected the

271   target stimulus more often than chance. Experiment 3 analysis was also performed

272   with a binomial test to determine significance between the two choices.

273   Data from Experiments 1 and 2 were analysed using the general linear mixed effects

274   model package in R (lme4 package, R version 3.5.3; Bates, Martin and Bolker,

275   2011). Success for each experiment was used for the response variable in all

276   analysis. Depending on the experiment, the colour (red, orange, green, blue), colour

277   type (high or low saturation), time period, and neutral density distractors were treated

278   as fixed factors. The individual ID of each animal was treated as a random factor in

279   all analyses.

280   To understand interacting effects, including the effect of the neutral density filter

281   types and cable tie position, a post-hoc test was performed for individual colours and

282   colour types using Tukey's multiple comparisons test (Hothorn et al., 2015). Only

283   data from "test" trials were used, and only when the stomatopod performed the task.

Results

Experiment 1: High/Low Saturation

286   *H. trispinosa* were able to learn to distinguish all colours from grey (binomial test:

287   highly saturated orange $p < 0.05$ and highly saturated blue $p < 0.01$, $p < 0.0001$ for

288   all others). There was an overall trend towards better performance in less saturated

289   colours (**Fig. 6A**). This was significant for red (GLMM, $p < 0.05$) and blue ($p <$

290   0.0001), but not orange ($p > 0.05$) or green ($p > 0.1$) (**Table 1**).

291   An average of 9±1 individuals per colour type participated in an average of 36 trials

292   (range 10-71, depending on trainability and mortality). There was no significant

293   variation between individuals (model variance < 0.1). Overall, many animals were

294   less successful on trials when at least one of the distractors was ND 0.15 and/or 0.6,

295   especially in less saturated red and highly saturated green (**Fig. S1.1, Table S1.1**).

296   These neutral density distractors were similar in brightness to the target stimuli (**Fig.**

297   **2**). There was no significant effect of sex or size on the overall success, therefore

298   these factors were not included in further analysis ($p > 0.5$).

Experiment 2: Discrimination over time

299

300   *H. trispinosa* were trained to discern red, green, and blue high saturation stimuli from

301   a neutral grey distractor, at LIRS and UQ. Over the first set of ten weeks in captivity,

302   all animals were able to learn the task successfully ($p < 0.001$). In the second set of

303   ten weeks, accuracy was severely diminished. During this time, animals were not

304   able to choose the coloured stimulus significantly more than chance for all colours (p

305   > 0.05) (**Table 2**).

306   For all colours, *H. trispinosa* became significantly less successful at selecting the

307   correct stimuli after spending time in artificial light (GLMM $p < 0.001$). After the first

308   10 weeks in captivity, animals became less effective at selecting red and blue high

309   saturation stimuli ($p < 0.05$). In addition, animals learning highly saturated blue were

310   more successful at LIRS in natural light than in the artificial lab light ($p < 0.05$) (**Fig.**

311   **6B, Table S1.2**).

312   Individual variance in the linear mixed model was less than 0.2, and lower for most

313   colours and sets, except for highly saturated blue and at LIRS (approximately 0.5).

314   On average, 6 individuals were used per colour with an average of 5 trials each at

315   LIRS; 7 individuals per colour with 17 trials each during the first ten weeks at UQ;

316   and 5 individuals with 10 trials each during the second ten weeks at UQ (**Table 2**).

317   Animals did significantly better in trials with neutral density filter ND 0 in all trials

318   (GLMM $p < 0.0001$), when trained to green and blue ($p < 0.01$ and $p < 0.05$,

319   respectively) and on LIRS and the first 10 weeks at UQ ($p < 0.05$ and $p < 0.0001$,

320   respectively) (**Table S1.2**).

Experiment 3: Naïve-Choice Tests

321

322   *H. trispinosa* were given a two-choice test to determine if there was an innate

323   preference for any of the colours or colour types. There was a significant preference

324   for red (binomial test, $p < 0.01$) and aversion to green ($p < 0.05$) among highly

325   saturated colours (**Fig. 7A**). No preference was found for any low saturation colours

326   (**Fig. 7B, Table S2.1**).

327 Other individuals were given a choice between the high and low saturation stimuli of

328 each colour. Animals preferred low saturation to high saturation blue (p < 0.05) but

329 had no preference between saturation types for the other colours (**Fig. 7B, Table**

330 **S2.1**). When animals were given a choice between a colour filter and neutral density

331 filter, stomatopods preferred the neutral density to all colours (p < 0.05), but this was

332 not significant when colours were analysed separately (**Fig. 7C**). However, they

333 significantly avoided the brightest neutral density filter (ND 0, p < 0.05) (**Fig. S1.2B**).

334 # Discussion

335 The aim of this study was to examine colour processing in *H. trispinosa*, by exploring

336 the two current hypotheses, opponent processing and pattern activation (also called

337 barcode or cochlea-vision). Our initial hypotheses were that stomatopods may: a) fail

338 at all low-saturations colours (mirroring and extending the results of Marshall et al.,

339 1996 and Thoen et al., 2014) or b) that they may just fail with low-saturation blue,

340 suggesting some behavioural relevance or significance for this colour. This would

341 have provided evidence, albeit circumstantial, that their colour processing was

342 somehow different and potentially bar-code-like. It should be noted that Marshall et

343 al. (1996) did not exclude opponent processing for colour vision, pointing out that the

344 chromatic signal differential was lowest for the low-saturation blue which that species

345 (*O. scyllarus*) failed on.

346 Results of our study did not clarify this debate in stomatopod vision (Marshall and

347 Arikawa, 2014; Marshall et al., 1996, 1997; Thoen et al., 2014). Interestingly, some

348 of our findings raise questions regarding the methodologies in previous and current

349 experiments regarding animal colour vision capabilities. Hence the reason for

350 Experiments 2 and 3 conducted here as discussed below.

351 We found that *H. trispinosa* could learn to discriminate both high and low saturation

352 colours from grey. This was an unexpected result as we originally proposed all low

353 saturation colours would be harder to discriminate based on the results of Marshall

354 et al., 1996 and Thoen et al., 2014 (**Fig. 6A**). H. *trispinosa* demonstrated relatively

355 poor discrimination of high saturation orange and blue colours, compared to other

356 colours, however we suggest this was due to length of time spent in captivity, rather

357 than an accurate reflection of their visual capabilities. Extended time in captivity may

358   reduce visual capacity due to the light spectrum being more limited than natural

359   daylight, or possibly other influences such as restricted diet (**Fig. 6B**). Most notably,

360   stomatopod require carotenoids for the formation of red filters in the retina, and these

361   must be obtained through their diet (Cronin and Caldwell, 2002; Marshall et al,

362   1991b). In light of this, the previous finding that *O. scyllarus* could not discriminate

363   low saturation blue may be a product of spending time in captivity. In particular, due

364   to the assumption that if humans can see the colour, another animal with apparently

365   superior colour vision must be able to, little effort was made to imitate natural

366   daylight. Animals were kept in an inside room with no window and whatever

367   fluorescent strip-light present in the room at the time. Furthermore, as animals were

368   sourced from a tropical marine supplier, the amount of time in captivity prior to

369   experiments starting was unknown. An alternative explanation for the apparently

370   contradictory results we obtained is one of species specificity. Blue and indeed other

371   low-saturation colours may be more salient and important for *H. trispinosa* than *O.*

372   *scyllarus*.

373   Since 1996, it has been demonstrated that the spectral sensitivities of stomatopods,

374   and indeed other animals, are remarkably plastic, being influenced by both depth

375   and light environment, as well as changing seasonally or on even shorter timescales,

376   such as days or within a single day (crustaceans: Cronin et al., 2002; Jessop et al.,

377   2020; fishes review: Carleton et al., 2020; Musilova et al., 2021). Our results with *H.*

378   *trispinosa* further support this visual plasticity, and suggest it is possible, that the

379   animals trained in 1996 had a short-term-modified colour sense significantly different

380   to the one ordinarily present under natural lighting conditions (**Fig. 6**). Even in the

381   wild, stomatopods are known to modify and tune spectral sensitivities depending on

382   the spectral envelope of ambient light they live in, according to the depth of habitat

383   they settle into as post-larvae (Cronin and Caldwell, 2002; Cronin et al., 2000, 2001;

384   Cheroske et al., 2006; Osorio et al., 1997).

385   There are other historical problems with the study of Marshall et al. (1996). For

386   example, at that stage the relative sensitivity of each row based on optics were

387   unknown, thus non-normalised spectral sensitivities were used to calculate and

388   compare quantum catch between photoreceptors (Cronin et al., 2000). This resulted

389   in an apparent closer match between quantum catch pattern looking at ND filters and

390   the unsaturated blue (Marshall et al., 1996 Fig. 4). Quantum catch calculations

391   performed here (**Figs 2F, G**) where the same blue filter was used but with more

392   uniform absolute sensitivities in each row, may be more accurate.

393   The results of Experiment 1, led to the conception of Experiment 2, to determine if

394   the previous findings of Marshall et al (1996) could be explained by time in captivity

395   and/or lighting conditions. Interestingly, stomatopods had trouble distinguishing

396   highly saturated red and blue after three months in captivity (**Fig. 6B**), supporting the

397   idea that the negative result of Marshall et al. (1996) needs to be viewed with caution

398   or may indeed be wrong. More recently we have discovered that mantis shrimps are

399   able to shift their spectral sensitivity under different light environments, both natural

400   and unnatural, and do so on the same timescales seen here (Cheroske et al., 2003,

401   2006; Cronin et al., 2000). This shift is usually towards shorter wavelengths in the

402   more red-sensitive row 3, an adaptative response to the reduced long wavelengths

403   in deeper or bluer habitats (Cheroske et al., 2006; Cronin et al., 2002; Cronin and

404   Caldwell, 2002). The result here suggests that the stomatopods used in Marshall et

405   al. (1996) had a change in colour discrimination ability and that the failure to

406   discriminate unsaturated blue from grey may have been a result of this short-term

407   adaptation process.

408   In response to the results from Experiment 1 - where *H. trispinosa* displayed a

409   reduced ability to learn saturated orange and blue - we also conducted Experiment 3

410   to test if *H. trispinosa* has an innate avoidance or preference for colours used in this

411   experiment. *H. trispinosa* display bright blue markings on the carapace and

412   maxillipeds in aggressive and mating contexts (Chiou et al., 2005) making it possible

413   that blue may have attached to it a specific significance. These and other species

414   have been found to spontaneously avoid UV markings, also a feature of the frontal

415   displays of stomatopods when they meet or contest ownership of cavities within

416   which to live on the reef (Bok et al., 2018). Our results indicate that *H. trispinosa* did

417   not show any specific avoidance of highly saturated blue but did display a preference

418   for red and an avoidance of green (**Figs 7A, S2.1A**). The preference for longer

419   wavelengths is similar to the results from Daly et al. (2017) where a colour

420   preference for yellow and an avoidance of red was found in naïve choice tests in the

421   peacock mantis shrimp, *O. scyllarus*. The nature of the tests conducted was

422   different, but this comparison highlights a potential difference in colour behaviour

423   between species. In addition, we found that *H. trispinosa* had no preference among

424   low saturation colours (**Fig. 7B**), but did prefer low over high saturation blue (**Fig.**

425   **7C**). This may account for the difference in success rate between high and low

426   saturation blue in the first experiment and may indicate an underlying avoidance of

427   blue in this species (**Figure 6A**)

428   Different specific colour preference in naïve choice tests have been found in other

429   crustaceans. For example, male blue crabs, *Callinectes sapidus*, show a preference

430   for red over orange claws in females, as mature females have red claws while those

431   of prepubertal females are orange (Baldwin and Johnsen, 2012). On the other end of

432   the spectrum, fiddler crab (*Uca mjoebergi*) females prefer males whose yellow claws

433   are also UV reflective (Detto and Backwell, 2009). Among the insects, innate long-

434   wavelength preference also occurs in some species of butterflies (Kinoshita and

435   Arikawa, 2014; Swihart and Swihart, 1970; Weiss, 1997) and flower-pollinating flies

436   (An et al., 2018; Lunau et al., 2018).

437   Interestingly, naïve-choice tests showed that *H. trispinosa* had a preference for

438   medium-brightness grey over high saturation colours (**Figure 7D**). Conversely,

439   innate preference tests in crabs show that they prefer colours over grey, which is

440   suggested due to use of colour in sexual selection. Female fiddler crabs prefer

441   yellow over grey, similar to male claw colouration (Detto, 2007), while male blue

442   crabs prefer the red claws, which mature females exhibit, over white and black claws

443   (Baldwin and Johnsen, 2009).

444   To find if the ability to distinguish both low and high saturated colours is more

445   widespread among stomatopods, we repeated experiments 1 and 2 with *G. smithii.*

446   In common with *H. trispinosa*, there was a trend towards learning low saturation

447   colours, and degradation of results over time kept in captivity (**Fig. S3.1, Table**

448   **S3.1**).

449   Although the set of experiments described here have further explored the colour

450   discrimination of stomatopods, even the basics of the colour processing mechanism

451   remain unclear. It is possible that stomatopods use a combination of multiple

452   mechanisms to process colour information in different behavioural contexts,

453   including opponency and photoreceptor activation comparisons, or bar-code
454   analysis. In addition, although the retinal design and underlying structures are largely
455   similar in all mantis shrimp species with six-row midbands, different species may
456   process colour differently (Marshall et al., 2007; Thoen et al., 2017).

457   Given that mantis shrimps display species-specific colour markings during
458   encounters between and within species on the reef (Caldwell and Dingle, 1975) it is
459   worth considering if this aspect of behaviour plays a stronger part in stomatopod
460   colour vision and that the colour of food, or food containers, is irrelevant in normal
461   life. During aggression sequences, often both mantis shrimp spread their front
462   raptors to show the species-specific colour of their meral spot to evaluate their
463   opponent; an act which may either lead to fighting or submission (Caldwell and
464   Dingle, 1975; Dingle and Caldwell, 1969; Green and Patek, 2015, 2018). Both
465   intensity and chromaticity of the meral spot is a signal of aggression in some
466   species: a darker meral spot indicates a stronger strike force, and a lighter meral
467   spot often leads to the receiver increasing antagonism (Caldwell and Dingle, 1975;
468   Franklin et al., 2017, 2019; Hazlett, 1979).

469   While the results of experiments here have not led to firm conclusions, what is clear
470   is that a number of previous experimental protocols in colour vision experimentation
471   may need adjusting. The fact that vision changes over evolutionarily short time
472   spans (reviewed in: Land and Nilsson, 2012; Nilsson, 2013; Kelber and Osorio,
473   2010; Marshall et al., 2015) and apparently colour vision is remarkably plastic in both
474   vertebrates (Carleton et al., 2020; Kelber et al., 2003; Musilova et al., 2021) and
475   invertebrates (Cronin et al., 2002; Jessop et al., 2020; Strausfeld and Andrew, 2011;
476   van der Kooi et al., 2020) on very short time scales presents a fascinating area for
477   further study. Does a change in visual pigment expression level or photoreceptor
478   complement lead to a change in colour detection or discrimination? What degree of
479   colour constancy underlies these changes? Is a food reward-based behavioural
480   experiment sufficiently basal that other colour-based behaviours, such as mate
481   choice or aggressive interaction, simply follow suit; or are there different levels of
482   discrimination for different behavioural tasks? As usual with the stomatopods, we
483   have found more questions than answers.

Competing Interests

The authors declare no competing or financial interests.

Author Contributions

AS and JM conceived and designed the experiment; AS and HE collected data; AS analysed data and AS and JM wrote the manuscript; all authors edited the manuscript.

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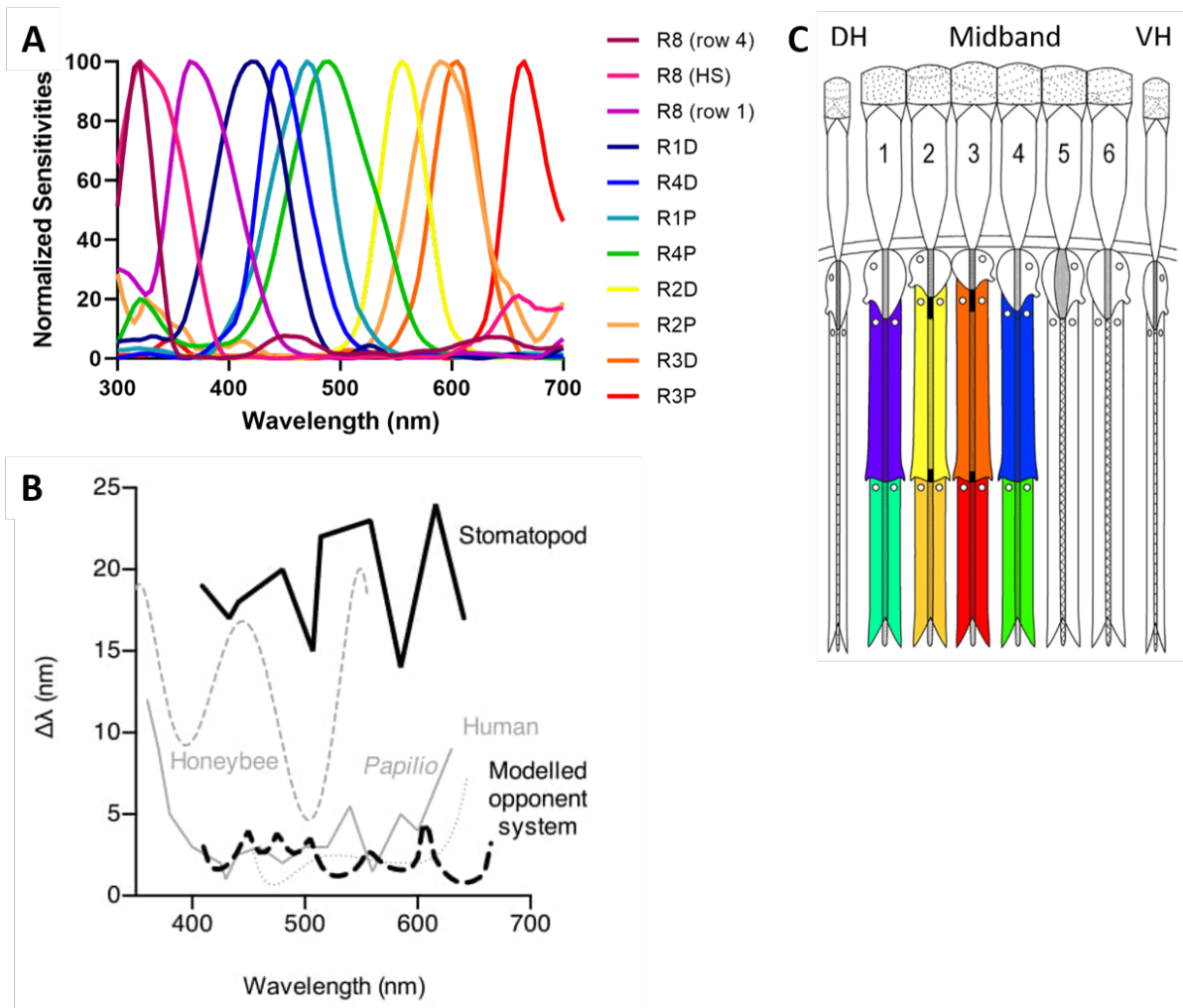
Figure 1: Model of stomatopod (*H. trispinosa*) visual system. A) Normalized electrophysiological response of each type of photoreceptor in first 4 midband rows. **B)** Spectral discrimination ability at different wavelengths for several animals. See text for discussion and reference. **C)** Stylized midband showing approximate photoreceptor type sensitivity as colours. The distally placed UV sensitive R8 cells are not coloured. Numbers refer to the respective midband rows and the colours the approximate spectral sensitivity of each tier. **A** and **B** are from Thoen et al., 2014; **C** adapted from Marshall et al., 2007.
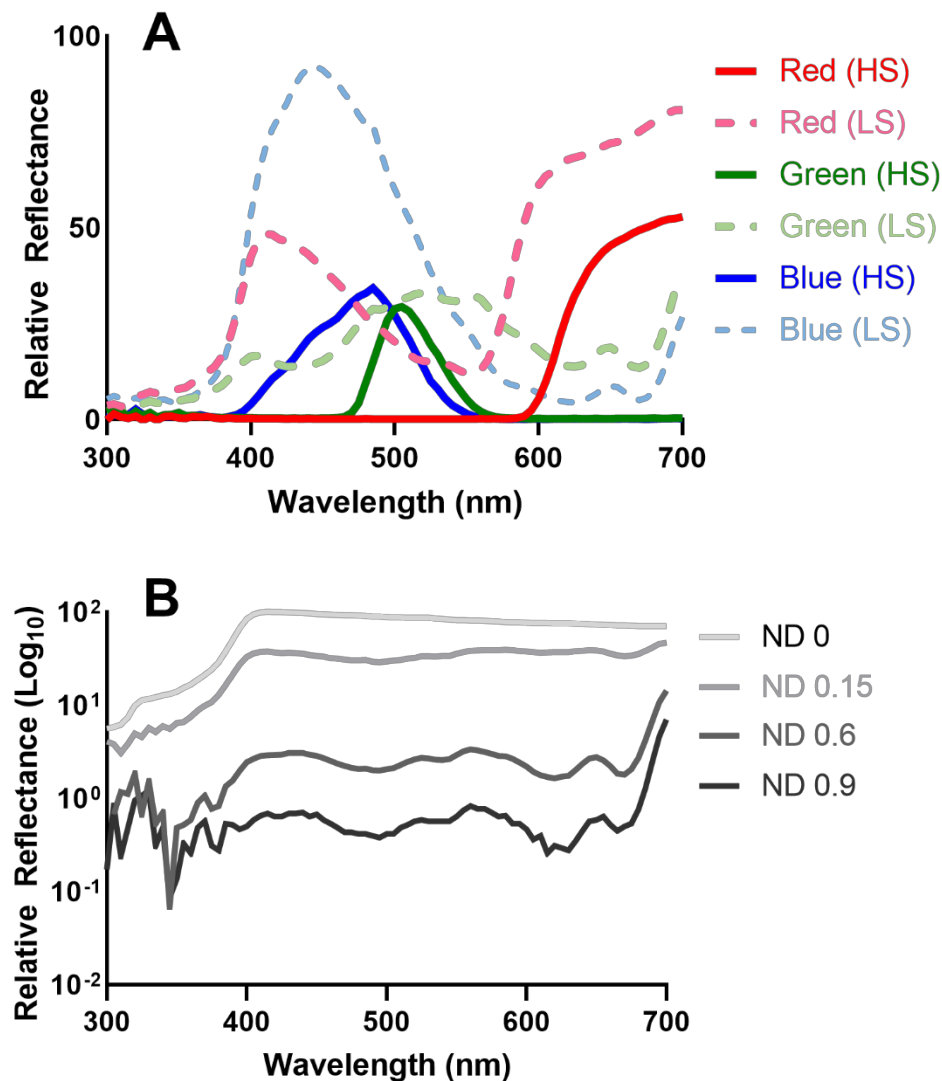
Figure 2: Spectral reflectance curves of stimulus filters. Spectral reflectance measurements of **A)** coloured filters and **B)** neutral density filters. All curves have been normalized to ND 0 in order to demonstrate relative brightness. Highly saturated (HS) coloured filters are solid lines, and less saturated (LS) coloured filters are dashed in **A**.
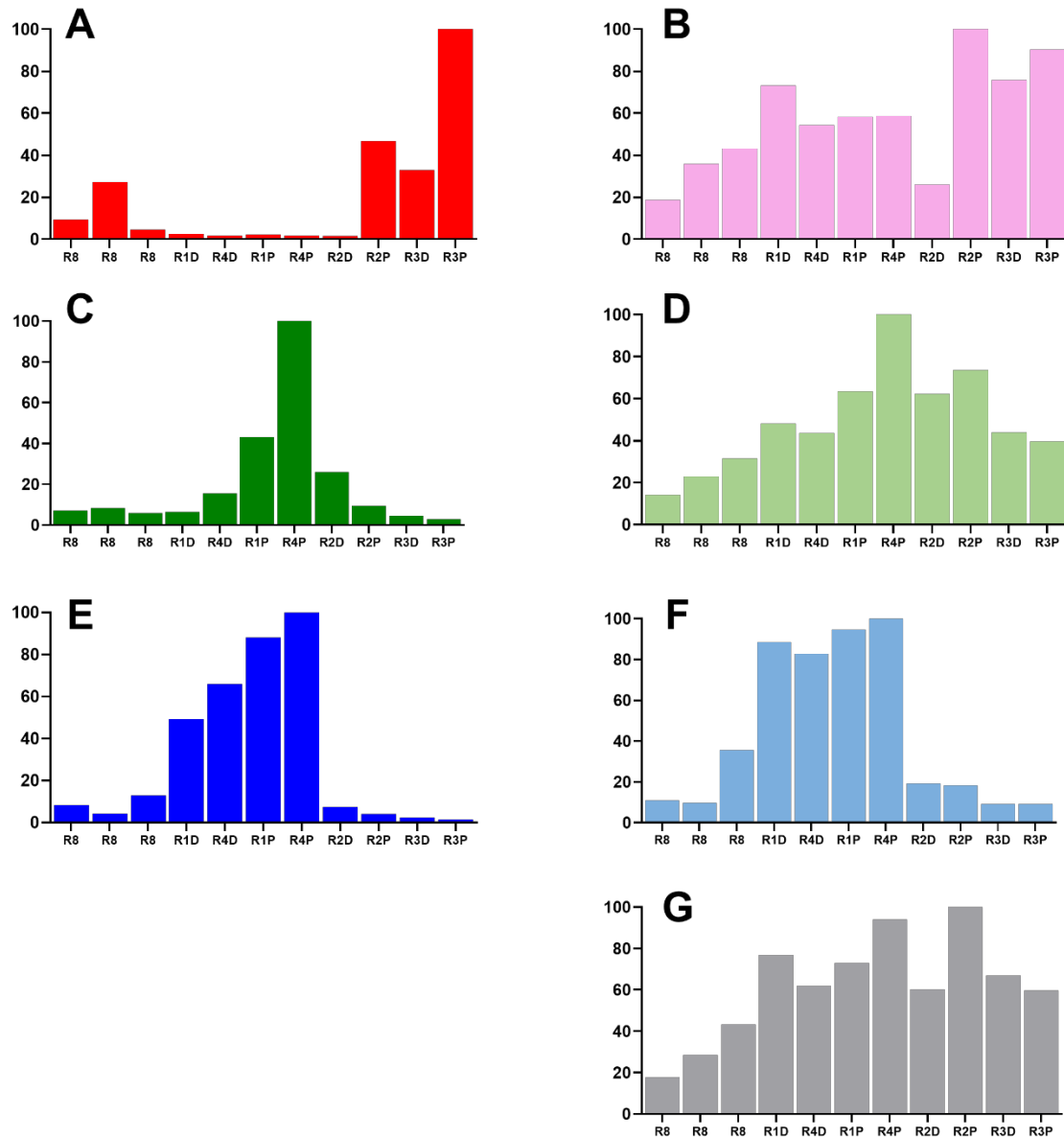
Figure 3: Photoreceptor responses to each filter type. Calculated relative quantum catch of each photoreceptor type to each coloured filter. **A)** highly saturated red **B)** less saturated red **C)** highly saturated green **D)** less saturated green **E)** highly saturated blue **F)** less saturated blue (the same used in Marshall et al., 1996) **G)** neutral density 0.15. Horizontal axis gives photoreceptor types (see **Fig. 1** for types).
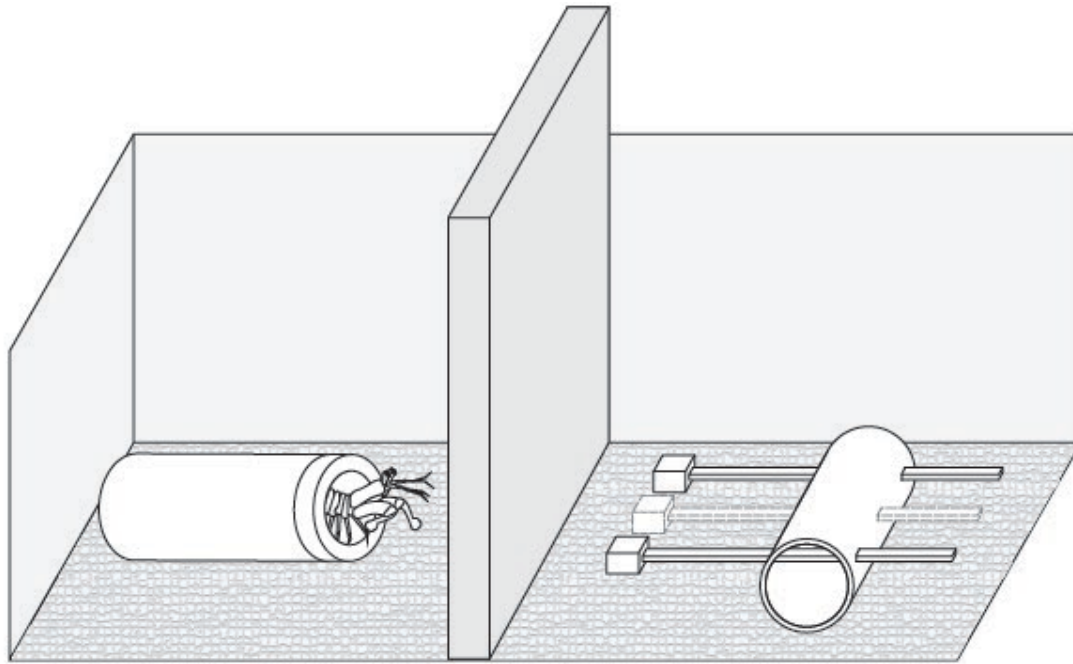
Figure 4: Behaviour setup. Setup just before an experiment begins. Stomatopod is in its burrow when a barrier is inserted and the cable ties placed (either two or three, see methods). The removal of the barrier signals the start of a trial, and the stomatopod will pull one cable tie into its burrow.
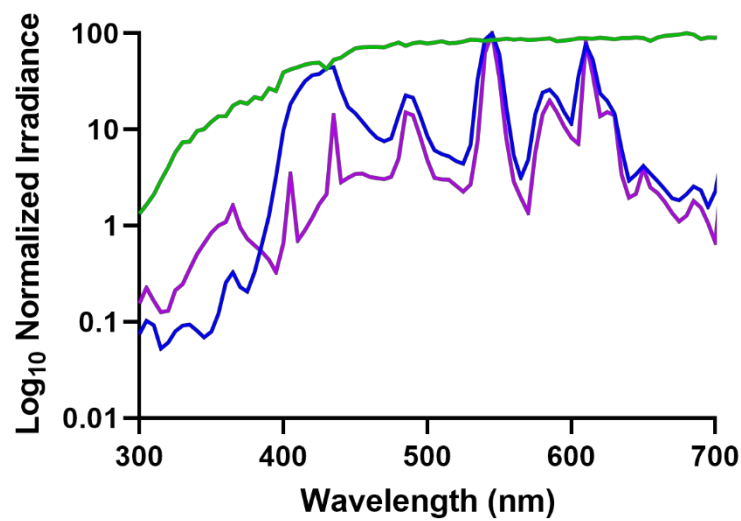
Figure 5: Available light spectra. Available light in natural environment at LIRS (green) and overhead lights in UQ Lab: Experiment 1 (blue) and Experiment 2 (purple). Irradiance is normalized for each measurement to show relative spectral availability.
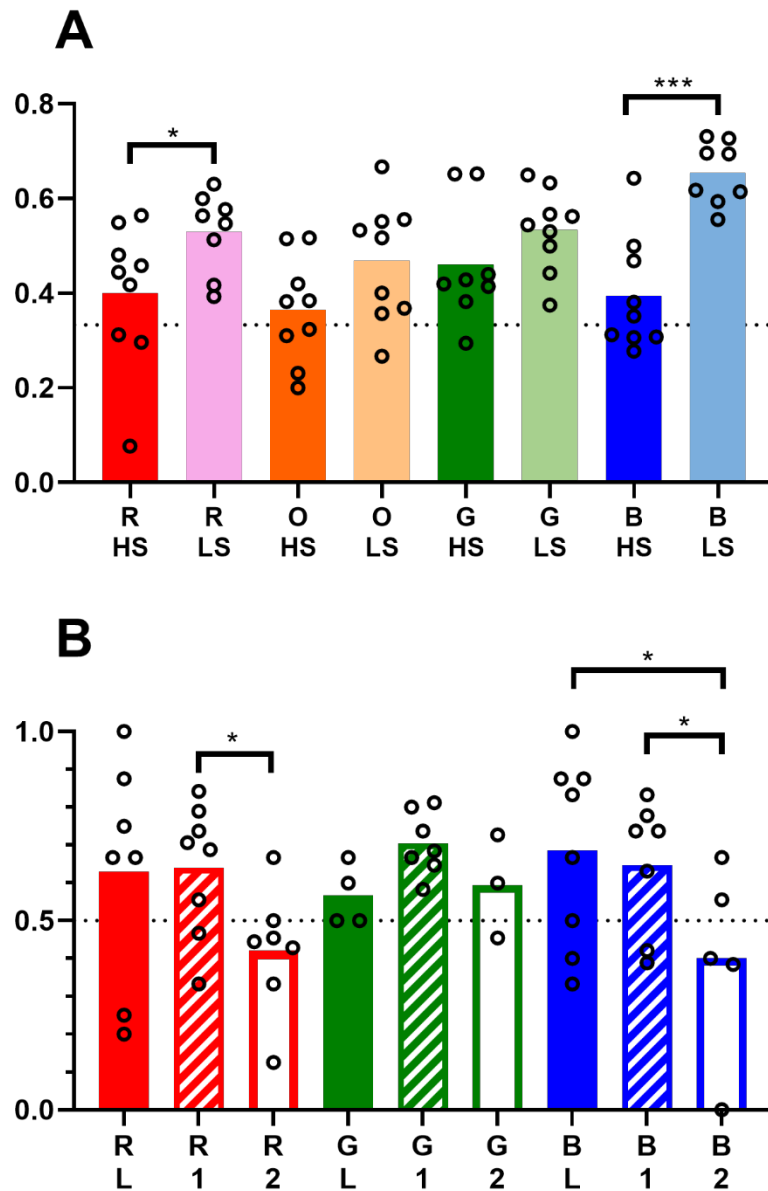
Figure 6: *H. trispinosa* performance in captivity. Average performance of individuals in each category. **A)** Experiment 1 results, comparison of high saturation (HS) and low saturation (LS) stimuli. **B)** Experiment 2 results, performance over time in captivity, at LIRS ("L"), first 10 weeks ("1"), and second 10 weeks ("2"). Dotted black line indicates the level of chance. Circles indicate each individual's average.
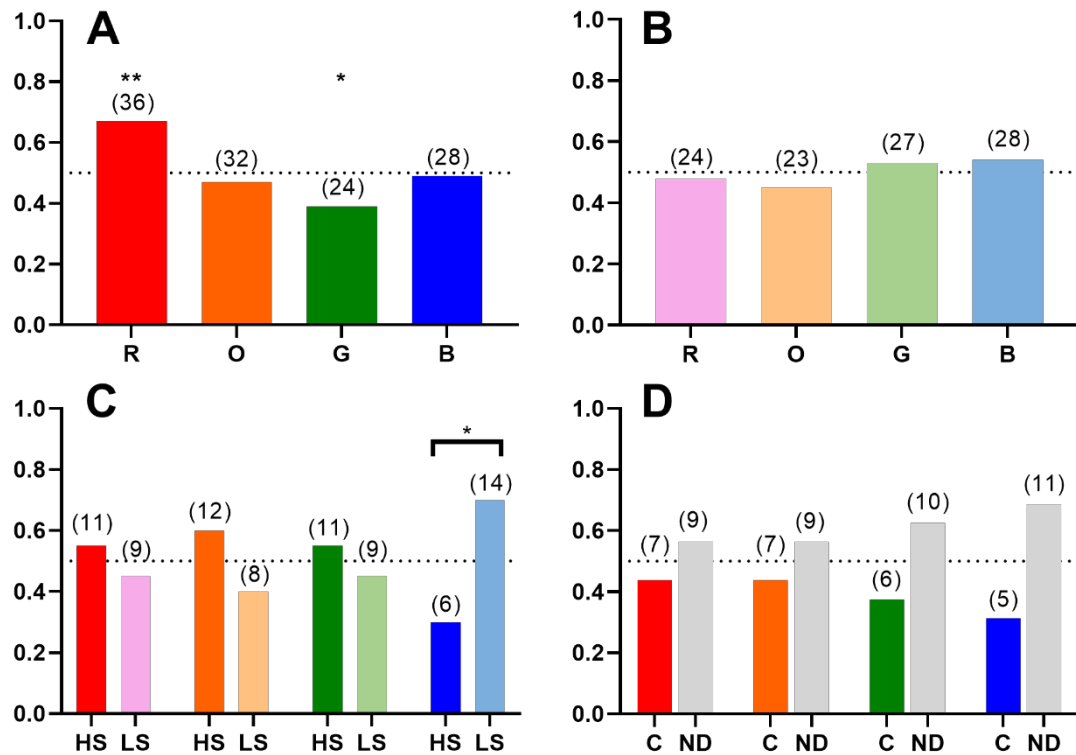
Figure 7: H. trispinosa naïve-choice tests. **A, B**) Results from a two-choice test between each colour in **A**) high saturation and **B**) low saturation colours (i.e. R/O, R/G, R/B, O/G, O/B, G/B). **C**) Choice between high saturation (HS) and low saturation (LS) stimuli of the same colour. **D**) Choice between a high saturation colour (R, O, G, B) and neutral-density grey (ND 0, 0.15, 0.6, 0.9). Black line refers to the level of chance. Numbers refer to number of individuals with the respective preference.

Colour	Type	Individuals	Participated	Average Success	Learned (BINOM)	*p-value* HS vs LS
R	HS	10	40	0.437	9.79e-6	0.0183*
	LS	9	43	0.539	1.94e-15	
O	HS	10	25	0.386	0.01353	0.0909
	LS	9	25	0.493	2.29e-7	
G	HS	8	41	0.502	1.06e-10	0.4254
	LS	9	41	0.529	6.48e-15	
B	HS	10	31	0.402	0.002656	1.94E-6***
	LS	9	42	0.632	5.93e-29	

Table 1: High and low saturation learning. Number of individuals, average number of trials, average success, learning ability, and difference between high (HS) and low (LS) saturation for each colour and colour type. The trials included 3 choices, suggesting that an average success rate over 0.333 corresponds to learning the task.

A	1 vs 2	1 vs 3	2 vs 3
All	0.9482	0.0288*	<0.001***
R	0.9858	0.2449	0.0368*
G	0.524	0.999	0.374
B	0.9240	0.0431*	0.0207*

B	R v G	R v B	G v B
All	0.461	0.899	0.722
Set 1	0.966	0.748	0.640
Set 2	0.736	0.994	0.685
Set 3	0.273	1.000	0.314

C	Colour	Individuals	Participated	Average Success	Learning (BINOM)
	R	7	4.3	0.633	0.0509
Set 1	G	4	4.75	0.579	0.1442
	B	8	5.6	0.717	0.00145**
	R	8	17.25	0.652	1.08e-4***
Set 2	G	7	16.6	0.704	2.91e-6***
	B	7	18.6	0.646	2.61e-4***
	R	7	8.7	0.443	0.0685
Set 3	G	3	12.3	0.595	0.0681
	B	5	9	0.444	0.0901

Table 2: Discrimination over time. A, **B)** show *p-values* of mixed models using GLMM (success ~ set + colour + ND + [1|individual]) and further Tukey tests on the factors. **A)** Effect of set, by colour. **B)** Effect of colour, by set. **C)** Number of individuals and average participation per colour per set, along with learning performance (see methods). Average success is in a 2-choice trial, and thus a success rate of 0.5 would correspond to chance.