1   **The adaptive value of camouflage and colour change in a polymorphic prawn**

2   Rafael Campos Duarte[a,b,*], Martin Stevens[c], and Augusto Alberto Valero Flores[a]

3   a – Centro de Biologia Marinha, Universidade de São Paulo. Rod. Manoel Hypólito do Rego, km 131.5, São Sebastião, SP, Brazil.

4   11612-109.

5   b – Programa de Pós-Graduação em Biologia Comparada, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade

6   de São Paulo, Ribeirão Preto, Brazil.

7   c – Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Penryn. TR10 9FE, UK.

8   * Corresponding author. E-mail: rafaduarte87@usp.br

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Abstract

Camouflage has been a textbook example of natural selection and adaptation since the time of the earliest evolutionists. However, aside from correlational evidence and studies using artificial dummy prey, experiments directly showing that better camouflaged prey to predator vision are at reduced risk of attack are lacking. Here, we show that the level of camouflage achieved through colour adjustments towards the appearance of seaweed habitats is adaptive in reducing predation pressure in the prawn *Hippolyte obliquimanus*. Digital image analysis and visual modelling of a fish predator (seahorse) predicted that brown prawns would be imperfectly concealed against both brown and red seaweed respectively, whereas pink prawns should be well camouflaged only in red weed. Predation trials with captive seahorses (*Hippocampus reidi*), coupled with high-speed video analyses, closely matched model predictions: predation rates were similar for brown prawns between seaweed types, but pink individuals were attacked significantly less on red than brown weed. Our work provides some of the clearest direct evidence to date that colour polymorphism and colour change provides a clear adaptive advantage for camouflage, and also highlights how this can be asymmetric across morphs and habitats (i.e. dependent on the specific background-morph combination).

Introduction

The study of animal coloration has fascinated evolutionary biologists for centuries and provided important evidence of adaptation and natural selection[1,2]. Colour attributes may modulate individual fitness in many different ways, playing a crucial role in behavioural processes ranging from courtship and mate selection to predator deterrence through visual warning cues[3]. Furthermore, many animals spanning a wide array of taxonomic groups take advantage of their colour patterns for concealment against the surrounding environment[3,4], mainly by adopting a camouflage strategy known as background matching[4]. In this type of camouflage, better concealed individuals are less frequently detected by visual predators and therefore their survival chances are higher[5]. This is a fundamental prediction of camouflage theory but, despite several emblematic cases consensually considered key examples of natural selection[6–9], appropriate experimental evidence of the adaptive function of camouflage remains remarkably rare.

A substantial body of previous work has used artificial dummy prey[10,11] or computer-generated stimuli[12,13] to test the survival advantage of camouflage in the laboratory or in the field. Other studies, such as the classic example of camouflage and industrial melanism in the peppered moth (*Biston betularia*)[6], have used correlational evidence, often based on morph-specific recapture rates, or artifical prey targets[9] to support the hypothesis that better camouflaged individuals are less frequently attacked by predators. More recently, with a better understanding of the anatomy of predator eyes, spectral sensitivity and visual modelling, different studies have estimated how individuals are camouflaged based to the view of predators through the use of spectrometry[14–18] or digital imagery[19,20]. However, while all these studies comprise important evidence that individuals are efficiently concealed against the substrate, no study has directly quantified how closely differently coloured individuals match their background to predator eyes, and then how matching effectively reduces predation rates in natural conditions. As such, the most basic, yet fundamental prediction of camouflage theory, is still poorly validated[21].

Camouflage through colour change is commonplace in the animal kingdom and may be achieved over different time scales; from responses of less than a minute, when individuals are moving through a patchy background, to ontogenetic shifts over months or years, accompanying the transition between nursery and adult habitats[21]. In general terms, colour change is basically mediated by different endocrine and cellular

67 processes, usually guided by vision, promoting modifications on the state and abundance of pigment-

68 containing chromatophore cells[21,22]. Physiological colour changes refer to the dispersal or aggregation of

69 pigments within chromatophores and determines patterns of fast colour change, within seconds or

70 minutes, such as those observed in cephalopods[23] or chameleons[24]. Slower morphological changes over

71 days, weeks or months[21,22] are more common and imply alterations in the quantity and proportion of

72 chromatophore types and pigment content. Colour-changing species make ideal systems to investigate the

73 adaptive value of camouflage[21], because they allow testing the importance of colour adjustments of

74 immigrant individuals upon contact with novel habitat, and also whether survival advantages of adjusted

75 individuals are symmetrical between habitats. Despite its potential to unravel important ecological and

76 evolutionary processes, suitable tests of the survival advantage of camouflage in colour-changing species

77 are still rare. Some studies have used vision models to assess changes in concealment[20,25], but they have

78 not confirmed modelling outcomes with predation trials. Other studies include tethering or predator-

79 exclusion experiments, but they have not modelled prey camouflage to the vision of predators[26,27].

80   The marine prawn *Hippolyte obliquimanus* (Decapoda: Caridae) is a common seaweed-dwelling species

81 found in shallow vegetated areas along the western Atlantic coast, from the Caribbean to Southern Brazil[28].

82 This species is polymorphic in colour, with individuals presenting homogeneous coloration that can be

83 brown, yellow, green, red or pink, or comprising partially or fully transparent forms marked with stripes or

84 spots[29]. Prawn polymorphism has been thought to function as protective coloration and to provide

85 camouflage against the seaweed types where prawns live. Optimal concealment should be important in

86 reducing both the detection and consumption of prawns by visual fish predators, especially those living in

87 close association with seaweed, such as seahorses, gobies and blennies[30,31]. In Southeast Brazil, prawns

88 exhibiting solid colour patterns on a range of brown to pink tones are commonly found associated with the

89 brown seaweed *Sargassum furcatum* and the red weed *Galaxaura marginata*[29] (Fig. 1). Both morphs are

90 capable of changing their colour in the direction of their host substrates over a period of a few days[32], but

91 changes are more remarkable and prawns obtained better concealment when kept in the less intricate red

92 seaweed *Galaxaura*[32]. Although based on colour reflectance alone, holding no relationship with any specific

93 visual system[33], those results are consistent with the hypothesis that camouflage through colour change is

94   more important in the less structured habitat where shelter is limited (*Galaxaura*), compared to the

95   physically more complex habitat (*Sargassum*) where refuges are more abundant and background matching

96   probably less critical[32]. Although this species is widely distributed along the Central and South America[28],

97   there are no studies testing whether prawns from other regions and living on substrates of different

98   coloration are also capable of changes to their colour and camouflage against variable backgrounds.

99   Here we tested the adaptive value of colour change and camouflage in *H. obliquimanus* prawns. First,

100   we described colour variation within and between morphs to test whether 'pink' and 'brown' individuals

101   can actually be viewed as distinct categories, and compare the colour of prawns and their host seaweed

102   habitats to verify how closely they resemble their background. Based on their likelihood to remain

103   unnoticed by a seahorse predator, which exhibits colour vision and detects prey primarily using visual

104   cues[31,34], we next quantified the camouflage of prawn morphs on both the host and the alternative

105   seaweed habitat using image analyses and visual modelling. This translates in nature to the capacity of

106   individuals to conceal in primary habitat, where they have remained long enough for colour adjustments to

107   take place, and in secondary habitat shortly upon arrival. Finally, we tested model predictions in a

108   manipulative experiment using real prey and predators. Considering previous results on habitat-specific

109   prawn camouflage based on general colour reflectance[32], we tested the hypothesis that the survival

110   advantage of camouflage through colour change is dependent on the seaweed habitat, with much reduced

111   detection and predation rates on individuals adjusting their coloration to the red seaweed *Galaxaura*

112   compared to those shifting towards the brown seaweed *Sargassum*.

113   **Results and discussion**

114   Our results validate the distinction of brown and pink prawns and their segregation between habitats,

115   reinforcing the need to examine the adaptive value of camouflage separately in brown and red seaweed

116   canopies. Principal component analyses applied on standardised seahorse cone catch values of prawns and

117   seaweed indicate that 'pink' and 'brown' morphs of the prawn *Hippolyte obliquimanus* are clearly discrete

118   colour entities to both the vision of humans and seahorses, and confirm that prawns tend to adjust their

119   colour to the host seaweed since prawns categorized as pink and brown cohesively clustered with the

120   seaweeds *Galaxaura* and *Sargassum* respectively (Fig. 2). Discriminant function analyses further validated

121   the prawn classification, as all individuals were correctly reassigned to their morphs, and further supported

122   the correspondence of prawn morphs to seaweed species, as 55 out of 60 prawns (92%) were correctly

123   linked to their host weed. The few exceptions were invariably 'brown' prawns lying closer to the red

124   *Galaxaura* than to the brown *Sargassum* pattern (crosses in Fig. 2). In fact, the wider spread of brown

125   individuals in Fig. 2 indicates an overall less precise physiological response of prawns acclimating to

126   *Sargassum*, and provides first evidence for less effective camouflage in these individuals compared to

127   prawns established in *Galaxaura*.

128   Predator discrimination of prawn morphs further suggests that any advantages of camouflage through

129   colour change may be modest in *Sargassum*, but important in *Galaxaura*. Here, we used the discrimination

130   model of Vorobyev and Osorio[35] for colour and luminance and infer prey detectability based on "Just-

131   Noticeable Differences" (JNDs) to seahorse vision. Briefly, prey are predicted to be discriminated from the

132   background for JND values higher than 1, with detection chances increasing beyond that threshold level,

133   even under unfavourable viewing conditions[36]. Contrasts of colour JNDs between prawns and background

134   habitats are morph-specific, as indicated by the significant interaction term in Table 1. Namely, the colour

135   discrimination of pink prawns in *Galaxaura* (mean JND ± SE; 1.99 ± 0.17) is much lower than in *Sargassum*

136   (7.57 ± 0.28; Fig. 3a), while brown prawns were similarly discriminated in both algal habitats, above the

137   colour detection threshold (3.24 ± 0.40; Fig. 3a). In other words, colour change should lead to superior

138   camouflage and lower detection rates in *Galaxaura* but not in *Sargassum* (see how both prawn morphs and

139   seaweed look like in the view of seahorses in the Supplementary figure S1). It is important to note that JND

140   variation was lowest for pink prawns in *Galaxaura* and highest for brown morphs in *Sargassum*, further

141   indicating that improved concealment to seaweed background relies on a precise physiological response

142   leading to a standardised colour pattern. The markedly right-skewed distribution of JND values for brown

143   prawns in *Sargassum* suggests that the poorer camouflage in this habitat is due to the relatively few

144   individuals attaining exceedingly high JNDs (Fig. 3a). Results on luminance contrasts were less informative

145   because they were consistently much higher than detection thresholds across level combinations of factors

146   'prawn morph' and 'seaweed habitat' (mean JND ± SE; 6.63 ± 0.62), and therefore are not likely to

147   modulate any predator effects. The significant *p*-level of the interaction term (*p* = 0.046, Table 1) is

148   attributed to morph-dependent habitat differences, with brown prawns showing lower JNDs in *Sargassum*

149   (6.08 ± 1.20) than in *Galaxaura* (9.65 ± 1.41), and pink prawns showing similar JNDs between seaweed

150   habitats (5.41 ± 0.74). Although being consistently lower for pink prawns on both habitats, all luminance

151   contrasts were much higher than the putative threshold for detection, indicating that seahorses probably

152   did not use this channel for detecting their prey and primarily base their hunting behaviour on colour

153   cues[34]. However, we note that the achromatic version of the receptor noise model is much less tested than

154   the chromatic model (the original model originally disregarded achromatic information altogether[35]), and

155   the mechanism of achromatic perception in fish is often poorly known and variable. Therefore, caution

156   should be used with interpreting the overall magnitude of the luminance JND values. Additional

157   behavioural experiments are necessary to understand the importance of both chromatic and achromatic

158   signals in the visual repertoire of this predator[37].

159   Results of predation trials closely corresponded to colour JND modelling, thus supporting the adaptive

160   value of camouflage through colour change as a mechanism to reduce predation rates on the prawn

161   *Hippolyte obliquimanus* (Fig. 3). Habitat-dependent predation on prawn morphs is backed by the

162   significance of the interaction term of the linear model examined (Table 2): seahorses *Hippocampus reidi*

163   equally preyed on brown prawns held at the two seaweed habitats (mean ± SE; *Sargassum*: 46.1 ± 3.7%;

164   *Galaxaura*: 50.1 ± 5.2%), but predation rates on pink individuals were reduced to almost 35% in *Galaxaura*

165   compared to *Sargassum* (*Sargassum*: 49.4 ± 4.8%; *Galaxaura*: 35.6 ± 3.9%), indicating that colour change

166   towards the background was efficient in the red but not in the brown seaweed environment (Fig. 3b). It is

167   important to note that in spite of their much higher JNDs (Fig. 3a) pink prawns on *Sargassum* were eaten at

168   similar rates than brown prawns on either habitat (Fig. 3b), suggesting that detection and predation rates

169   would be high and fairly constant at JNDs over 3 or 4 (i.e. beyond the detection threshold). Interestingly,

170   consumption rates were very consistent among seahorse individuals, as indicated by the non-significant

171   random factor 'seahorse ID' nested in the morph*habitat interaction (Table 2). Positive effects of colour

172   adjustments on prey survival may thus be pervasive, dampening any potential behavioural syndromes

173   underlying individual-based differences among predators[38,39]. Consistent results among individual predators

174 probably reflect specialized hunting techniques, involving a very specific pattern of prey spotting,

175 approaching and striking common to all seahorse individuals (Fig. 4). High-speed video recordings (480 fps)

176 taken during experimental trials confirmed that seahorses use primarily visual cues for prey detection,

177 taking on average 4.28 ± 0.82 s (mean ± SD) to strike after first visual contact (Supplementary video S1).

178 Once detected, seahorses move slowly without losing eye contact until they reach a distance to prey that

179 can be covered during a very fast strike (less than 0.063 s; Fig. 4). Still, our observations show that strikes

180 involve an upward rotation of the head (frame 2 to 3), slightly increasing the path travelled by the mouth as

181 revealed by models of seahorse feeding biomechanics[40]. According to these authors, an extended strike

182 distance allows seahorses to probe a larger volume of water and hence locate prey more precisely, which

183 could explain the very high percentage of successful attacks (90%) observed in our trials.

184   In this study we present novel evidence showing the adaptive value of camouflage through colour

185 change. A wide range of recent studies have tested how types and levels of camouflage affect detection,

186 but predominantly using artificial (human-made) 'prey' presented to human and other animal

187 observers[10,41]. Furthermore, while iconic studies of the peppered moth quantified morph-specific survival

188 advantage in different habitats[6], and recent studies of wild birds have shown that camouflage level

189 correlates with survival in the field[19], no study has yet directly demonstrated that camouflage level, to

190 predator vision, directly influences individuals' survival chances. Here, the visual model we used closely

191 predicted specific camouflage success for each *H. obliquimanus* colour morph on each seaweed background

192 in terms of colour discrimination / detection to a seahorse predator. Therefore, our study is the first to

193 quantitatively demonstrate that predation risk in an animal is directly related to predator-perceived levels

194 of camouflage, and concurrently validates widely used but seldom tested models of visual discrimination.

195 Although focusing in a specific seahorse predator, which exhibits colour vision[34] and uses visual cues to

196 detect prey[31] (Fig. 4 , Supplementary video S1), our results should be generalizable to other fish potentially

197 hunting *H. obliquimanus*, including gobies, blennies and pinfish species which are frequently found

198 associated with *Sargassum* and *Galaxaura* canopies[30,42]. There is no specific information regarding the

199 visual system or the existence of colour vision in these alternative predators, but studies on similar species

200   have suggested that most of them use colour cues to detect prey[37,43–46] and therefore would likely exhibit

201   similar behaviour to seahorses and be potentially deceived by prawn camouflage.

202   In our study we found that the survival advantage of camouflage through colour change is asymmetric

203   across different habitats. Colour concealment was shown to be adaptive for prawns shifting to pink in

204   *Galaxaura* but not for prawns changing to shades of brown in *Sargassum*; a result consistent to our initial

205   predictions. Adequate shelter and extensive foraging grounds provided by the more intricate architecture

206   of *Sargassum* and accompanying epiphytic algae[47,48] may be more important than concealing coloration to

207   maintain high prawn densities in the brown weed habitat[29]. Differently, lower prey density and reduced

208   shelter supply - two conditions known to increase per-capita predation pressure[49,50] - make lower prey

209   detection rates critical in the less complex *Galaxaura* canopy. In summertime, *Sargassum* blooms, becomes

210   primary habitat and hosts very large prawn aggregations[29], but by winter time the brown-weed have

211   decayed[51,52] and the perennial *Galaxaura* becomes a more important habitat. Fast colour change allowing

212   camouflage in the red weed canopy[32] may be therefore of paramount importance by increasing survival

213   rates of overwintering individuals and hence ensuring population stability through time.

214   In conclusion, by integrating the more recent area of image analysis and visual modelling with classical

215   behavioural experiments our study highlights an important future avenue of research in both sensory and

216   behavioural ecology. The results we obtained represent a fundamental starting point for understanding the

217   adaptive value of camouflage – one of the most common anti-predator strategies observed in nature – for

218   many different species. In addition, colour change for camouflage is widespread in nature, being common

219   in animals from both terrestrial and aquatic habitats[21], which permits the generalization of our findings to

220   different species living on heterogeneous habitats, such as many insects[53], crabs[54–56], fish[25,57] and lizards[58]. It

221   is important to appreciate, however, that both colour change and camouflage may differentially affect the

222   survival of individuals in each of the different habitats where they live, since each background type will

223   exhibit specific requirements that may change the close relationship between animal and substrate

224   coloration.

225   **Methods**

226   **Field sampling.** Seaweeds *Sargassum furcatum* and *Galaxaura marginata* were collected by skin diving in

227   the vicinities of the Centre for Marine Biology, São Sebastião, SP, Brazil (23°49'40"S; 45°25'22"W) during

228   the spring of 2015 and summer of 2016. Prawns were sorted from seaweeds (as in[29]) and visually classified

229   as brown or pink morphs, which proved to be a simple method for an accurate assignment[32] (Fig. 2). Before

230   being used in experiments, prawns and seaweeds were kept in indoor tanks (30 X 20 X 10 cm) at ambient

231   temperature (~ 27° C), supplied filtered running seawater and aeration. A random set of prawn and

232   seaweed samples was separated for image analyses and visual modelling to measure the potential of

233   prawn camouflage against algal habitats. Another set was used for predation experiments to test

234   predictions of modelling results.

235   **Prawn camouflage.** Pieces of seaweed and living prawns (*n* = 30 for each seaweed species and prawn

236   morph) were photographed in an acrylic chamber (5 x 5 cm) using a Nikon D80 digital camera, coupled with

237   a Nikkor 60 mm lens and a UV-blocker filter (62 mm, Tiffen, USA). The camera was set up to capture only

238   visible light (400 to 700 nm) because objects exhibited low overall UV reflection (as observed in images

239   acquired with a UV-sensitive camera), and because fish preying on prawns are likely less sensitive to UV

240   light[43,59]. Images were taken in RAW format, with manual white balancing and fixed aperture settings to

241   avoid over-exposure[60], and included black (7.5%) and white (91%) Spectralon reflectance targets

242   (Labsphere, Congleton, UK) following the current standard procedure[61]. Illumination was provided by one

243   human visible Colour Arc Lamp (70W, 6500K Iwasaki), coupled to a polytetrafluoroethylene diffuser

244   cylinder around the photography chamber to ensure even lightning. Images were successfully linearized (R^2

245   ≥ 0.997 for all camera channels), using the curves modelled from eight Spectralon reflectance standards

246   (from 2 to 99% reflectance) to correct for camera non-linear pixel responses to light intensity[60,61].

247   Photographs were equalized for changes in light conditions using 7.5% and 91% standards and saved as 32-

248   bit multispectral images. All routines were performed using customized functions in the ImageJ

249   software[61,62].

250   Prawn and seaweed colour was analysed based on a seahorse vision model, since seahorses are

251   abundant in seaweed beds[63] and commonly prey on caridean prawns[64], including *H. obliquimanus*[31]. Since

252   there is no information on the visual system of the local seahorse predator *Hippocampus reidi*, the spectral

253   sensitivities of the closely related species *Hippocampus subelongatus*[59] were used for modelling. We

254   assumed the visual capacity of the two seahorse species are similar as they both live in similar green-water

255   vegetated habitats[59,65]. *H. subelongatus* has spectral peaks for single cones at 467 nm (shortwave

256   sensitivity, SWS), and for double cones at 522 nm (mediumwave, MWS), 537 nm (medium-longwave, M-

257   LWS), and 560 nm (longwave, LWS)[59]. A 50% light transmission cut-off at 425 nm was incorporated[59], and a

258   D65 standard irradiance spectrum was used as a measure of incident illumination[66], compatible to the

259   restricted shallow-water environment, of only a few cm, where predator-prey visual interactions take place

260   (Fig. 4). We assumed that colour vision is encoded by independent spectral channels in double cones (DCs),

261   as reported for the reef fish *Rhinecanthus aculeatus*[45]. Compared to other fish which have only one or two

262   pigments in their DCs[45,67], the seahorse *H. subelongatus* exhibits an unusual DC configuration, with three

263   different cone types accounting for the medium-long wave sensitivity[59]. We thus assumed that *H.*

264   *subelongatus* has a trichromatic visual system, but still modelled colour vision as encoded by SWS single

265   cones plus LWS DCs, and either MWS DCs ('Model 1') or M-LWS DCs ('Model 2'). We only report results for

266   'Model 1' because outputs of both models were very similar (Supplementary table S1). Tetrachromatic

267   vision was discarded because similar MWS and M-LWS cone types were present in DCs, strongly suggesting

268   that one of them is used for luminance (i.e. achromatic) contrast[59]. Polynomial mapping was used to

269   convert images from the camera colour space[60,68] into values of seahorse cone catches, closely

270   corresponding to spectrometry techniques[19,20,25,61]. Before building the model, we calculated the spectral

271   sensitivity curves of our equipment[20,69], and obtained the following sensitivity range and spectral peaks:

272   SW; 400 – 550 nm (peak 472 nm), MW; 420 – 620 nm (peak 534 nm), LW; 550 – 700 nm (peak 596 nm).

273   Visual modelling resulted in multispectral images that were used to estimate photon catch values for

274   each colour channel in the selected regions of interest (ROIs; prawn carapace and abdomen, from the

275   region behind the eyes to the end of the third abdominal somite, avoiding the stomach region, and

276   seaweed fronds). A principal component analysis (PCA) on the covariance matrix of the standardized cone

277   data was used to visualise colour differences between morphs and backgrounds and to determine the

278   channels responsible for clustering. Prawn and seaweed PC scores (PC1 and PC2) were used to create

279   discriminate functions to, respectively, confirm morph classifications and validate the correspondence of

280 morphs to seaweed species. The 'lda' function from the MASS library in R[70] was used to run discriminant

281 function analyses. A widely implemented log-linear form of colour discrimination model[35], which assumes

282 limitation by receptor noise, was used to predict chromatic and achromatic perception as "just noticeable

283 differences" (JNDs). Since behavioural data backing visual discrimination is lacking for *H. subelongatus*, we

284 used a conservative and frequently adopted Weber fraction value (0.05) for the most abundant cone

285 type[66], and set cone proportions to LWS = 0.44, MWS = 1.00, M-LWS = 0.89 and SWS = 0.56[59]. Colour

286 detection by predators is expected at JNDs higher than 1.00[36]. We then calculated colour and luminance

287 contrasts in single prawn-seaweed random pairings, resulting in 15 independent JNDs for each prawn

288 morph / seaweed species combination. Colour and luminance JNDs were analysed separately using a 2-way

289 ANOVA, in which factors 'prawn morph' (brown or pink) and 'seaweed type' (*Sargassum* or *Galaxaura*)

290 were fixed and orthogonal. Variances remained heterogeneous for colour JNDs even after log

291 transformation. Still, we proceed with the analysis using raw data because this was a balanced design with

292 a large sample size (n = 15), which makes the test robust to variance heterogeneity[71]. The Student-

293 Newman-Keuls (SNK) procedure was used for *a posteriori* comparisons.

294 **Laboratory predation trials.** There were different reasons to select seahorses as ideal model predators in

295 this study. First, seahorses are specialised consumers of seaweed-dwelling invertebrates, curling their tail

296 around weed thalli or holdfasts and ambushing prey upon visual detection[63]. Second, caridean prawns have

297 been ranked first or second in seahorse diet[72,73]. Regarding our focal species, the prawn *Hippolyte*

298 *obliquimanus* is heavily consumed by *Hippocampus reidi*, preferring this prey to amphipods and brine

299 shrimp[31]. Third, *H. obliquimanus* and *H. reidi* are common species in our study region[29,74] and therefore the

300 predator-prey interaction addressed here should be quite frequent at the sampling area.

301 A set of ten cubic aquaria (25 x 25 x 25 cm), supplied a thin layer of natural sand covering the bottom

302 and constant flow of 5-µm filtered seawater, was maintained in natural temperature (26.5°C ± 1.1) and

303 salinity (31.1 ± 0.7) conditions. Five of these aquaria were used to maintain stocks of freshly collected

304 seaweeds, prawns and seahorses, and the other five were used for experimental trials. Prawn stocks were

305 kept with their original plant hosts ('brown' prawns on *Sargassum* and 'pink' prawns on *Galaxaura*). Three

306 non-reproductive *H. reidi* individuals (S1: female, height 11.4 cm; S2: female, height 10.6 cm; S3: male,

height 11.4 cm) were collected by snorkelling from seaweed meadows in the same area (ICMBio-approved

license #55633-1) and kept in individual tanks where they were fed *ad libitum* a variety of seaweed-

dwelling invertebrates. Predation trials were carried out under natural daylight in aquaria provided with a

clump of either *Sargassum* or *Galaxaura* (approx. 50 ml), devoid of any mobile epifauna after brief

immersion in freshwater. In each tank, 20 individuals of either the brown or pink morph were included and

left to acclimatize for 10 minutes before the addition of a single seahorse, initially caged in a 5 mm mesh-

bag. After 20 minutes, when all prawns had settled on seaweed, the predator was released and left in tanks

for 2 hours. Predation rate was calculated as the proportion of prawns that were consumed until the end of

the trial. A maximum of two experimental aquaria were run at the same time and combinations among

levels of factors 'prawn morph', 'seaweed habitat' and 'seahorse ID' were randomly replicated in time,

three times, summing 36 trials over 1.5 months. The tank used in each trial was also randomly chosen to

avoid potential artefacts due to uncontrolled spatial variation of any physical variables within laboratory

space. We also certified that seahorses were left without food for at least 20 hours before their use in

trials, ensuring that complete gastric evacuation has occurred[31]. In some trials (n = 10) we used a high-

speed camera (Sony NX-FS700R, coupled with a Nikkor 60 mm lens, capturing images at 480 fps) to record

seahorse hunting behaviour. All experimental procedures complied with Brazilian ethical standards.

Predation rate was analysed using a specific ANOVA model in which the factors 'prawn morph' (brown

and pink) and 'seaweed habitat' (*Sargassum* and *Galaxaura*) were considered fixed and orthogonal.

'Seahorse ID' (S1, S2 and S3) was included as a random factor, nested in the interaction of main factors,

allowing proper replication and a test for the generality of predation effects. As for JND comparisons, we

used the SNK post hoc test to further examine significant sources of variation.

Ethics. Collection of seahorses and their maintenance in the laboratory together with their use in the

experiments complied with Brazilian ethical standards and were licensed accordingly ['Instituto Chico

Mendes de Conservação da Biodiversidade' (ICMBio), license number #55633-1].

Data availability. The data generated and analysed during the current study are available from the

corresponding author on request.

Acknowledgements

Author Contributions

R.C.D., M.S. and A.A.V.F. designed the study. R.C.D. obtained the digital images and conducted the predation experiment. R.C.D., M.S. and A.A.V.F. designed the analyses. R.C.D. conducted analyses and together with M.S. and A.A.V.F. wrote the first draft and revised the manuscript.

Additional Information

Supplementary information accompanies this paper at

Competing interests: the authors declare there are no competing interests.

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Table 1. Summary results of prawn camouflage against seaweed backgrounds based on seahorse vision.

Results of two-way analyses of variance testing differences in "just-noticeable differences" (JNDs) for colour

and luminance measurements, according to combinations of *Hippolyte obliquimanus* colour morphs

('brown', 'pink') and seaweed backgrounds (*Galaxaura marginata*, *Sargassum furcatum*). Cochran's *C*:

Cochran statistic testing variance heterogeneity.

Source of variation	*df*	Colour JNDs			Luminance JNDs		
		MS	*F*	*p*	*MS*	*F*	*p*
Prawn morph – M	1	35.7	21.50	< 0.001	92.0	4.42	0.040
Seaweed habitat – S	1	86.6	52.08	< 0.001	20.4	0.98	0.327
M x S	1	150.4	90.43	< 0.001	86.4	4.15	0.046
Error	56	1.7			20.8		
		Cochran's *C* = 0.541; *p* < 0.01			Cochran's *C* = 0.356; *p* > 0.05		

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535 **Table 2. Summary results of seahorse predation on prawn colour morphs.** Results of mixed-model analysis

536 of variance testing contrasts of seahorse *Hippocampus reidi* predation rates on prawn *Hippolyte*

537 *obliquimanus* colour morphs maintained in different seaweed habitats (as percentage of individuals

538 consumed by seahorses over 2h trials). The factors 'prawn morph' and 'seaweed habitat' are fixed, while

539 'seahorse ID' is random and nested in the interaction of main factors. Cochran's *C*: Cochran statistic testing

540 variance heterogeneity.

Source of variation	Predation rate			
	df	*MS*	*F*	*p*
Prawn morph – M	1	0.031	3.39	0.103
Seaweed habitat – S	1	0.020	2.22	0.174
Seahorse ID (M x S)	8	0.009	0.43	0.888
M x S	1	0.076	8.38	0.020
Error	24	0.021		
Cochran's *C* = 0.324; *p* > 0.05				

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Figure 1. *Hippolyte obliquimanus* colour morphs and seaweed habitats. (**a**) Brown (left) and pink (right) prawns resembling the colour of the brown seaweed *Sargassum furcatum* and the red-pink seaweed *Galaxaura marginata*, respectively. (**b**) *Sargassum* and *Galaxaura* canopies as commonly observed in shallow rocky reefs along the South-eastern Brazilian coast.

Figure 2. Background resemblance of prawn morphs against seaweeds. Principal Component Analysis applied to seahorse *Hippocampus subelongatus* cone catches showing colour variation of *Hippolyte obliquimanus* colour morphs ('pink' and 'brown' to the human vision) and seaweed habitats (red *Galaxaura marginata* and brown *Sargassum furcatum*). Percentage values correspond to the total variation explained by each component. The upper-right indent panel indicates that the shortwave colour channel (sws) is the main responsible for the segregation of groups. Brown crosses indicate the few ($n = 5$) cases in which prawn colour resemblance was closer to the alternative rather than to the host habitat colour (all 'brown' individuals which were actually closer to *G. marginata*). Sws, mws and lws stand for short, medium and long-wave sensitivity channels.

Figure 3. The adaptive value of camouflage in *Hippolyte obliquimanus* prawns. (**a**) Seahorse vision discrimination (as 'just noticeable differences'; JNDs) of prawn morphs against seaweed habitats. Boxes display medians and inter-quartile ranges (IQRs), whiskers represent lowest and highest values within $1.5*$IQRs, and black filled circles represent outliers. The dashed line (JND = 1) indicates the threshold for visual discrimination of prawns by seahorses. ns: not significant; *** $p < 0.001$. (**b**) Seahorse predation rates, as the percentage of individuals consumed in 2h trials (mean ± SE), on brown and pink prawn morphs when placed in *Galaxaura* and *Sargassum* habitats. ns: not significant; * $p < 0.05$.

Figure 4. Sequence of still images from high-speed video footage (480 fps), over less than 1.5 s, showing a seahorse preying on a prawn camouflaged on brown seaweed *Sargassum furcatum*. The yellow arrow indicates the prawn position in the first frame. Note that the attack took shorter than 0.06 s (frame 2 to 3).