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Aprendizado e atenção em diferentes
contextos ecológicos em opiliões e aranhas
(Arachnida)

Learning and attention in different ecological
contexts in harvesters and spiders
(Arachnida)

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It is what it is.

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Introdução Geral

Há registros de estudos de comportamento animal sistemáticos no mínimo desde o século XIX por fisiologistas que estudavam mecanismos que explicassem determinados comportamentos, seguindo linha mecanista já seguida por René Descartes no século XVII. A América do Norte seguiu, de maneira geral, uma linha mecanística de estudos com a Psicologia Experimental nos séculos XIX e XX, enquanto a Europa seguiu para uma linha mais naturalística, comparativa e evolutiva nessa mesma época (Alcock 2009). A partir da metade do século XX, estudos derivados destes comparativos ganharam força, e adição de modelos teóricos como modelos econômicos, estratégias evolutivamente estáveis, gene egoísta e aptidão inclusiva fizeram nascer a Ecologia Comportamental (Krebs & Davies 1997). Esta foi muito influente e mudou o direcionamento dado pela maioria dos pesquisadores, que passaram a focar em questões evolutivas em detrimento de mecanísticas (Willemart, no prelo). Entretanto, com o passar das décadas se percebeu que estudos de mecanismos são fundamentais para se compreender o comportamento animal. Não é possível entender, por exemplo, a evolução do aposematismo sem se compreender os mecanismos de percepção de cores pelos predadores, o sistema de acasalamento de uma espécie sem entender como machos e fêmeas se encontram e se avaliam ou a evolução e manutenção de determinada estrutura utilizada em determinado comportamento sem se compreender por quais mecanismos genéticos e de desenvolvimento ela se forma. Logo, nas últimas décadas existe uma visão mais integrada do estudo do comportamento animal: estudar porque determinado comportamento é adaptativo ou como se deu sua evolução na filogenia do grupo são relevantes, mas não mais do que estudar os mecanismos sensoriais, fisiológicos e de desenvolvimento que influenciam tais comportamentos (Krebs & Davies 1997; Alcock 2009).

Nesta tese, questões mecanísticas são o foco. Particularmente, estudamos o aprendizado e a atenção, temas relevantes em diversos contextos ecológicos e que pode ser estudado com diversas abordagens e táxons. Há inúmeras maneiras de se definir aprendizado somente na área de comportamento animal. Entendemos aprendizado como uma mudança em um determinado comportamento após uma experiência prévia, tipicamente adaptativa, mas não necessariamente (Costa et al

34 2006; Alcock 2009), havendo nuances que não são relevantes para este texto (Hogan
35 2017). Há amplas evidências de que diferentes formas de aprendizado influenciam de
36 maneira decisiva o comportamento de animais (Barnard 2004). Estes podem parar de
37 reagir ao aprenderem que determinado estímulo não é perigoso (eg Liu et al 2023),
38 mudar suas preferências sexuais e alimentares (Hebets 2003; Russell et al 2011),
39 evitar predadores (Randler e Kalb 2020), encontrar abrigos mais rápido (Santos et al
40 2013) e realizar uma série de tarefas com maior rapidez e eficiência por conta de
41 aprendizado (Barnard 2004). No capítulo 1, estudamos uma das formas mais
42 elementares de aprendizado, a habituação. Na habituação, um animal diminui sua
43 resposta a determinado estímulo após ser estimulado sucessivas vezes,
44 desconsiderando fadiga motor ou sensorial (Rankin et al. 2009). Perguntamos se um
45 animal deveria deixar de reagir a um estímulo que, embora não estivesse causando
46 danos, fosse um estímulo potencialmente letal. No capítulo 2, perguntamos se a
47 maneira pela qual um estímulo foi aprendido influenciaria no aprendizado e no tempo
48 de retenção deste aprendizado. Para tanto, desenvolvemos um aparato para realizar os
49 testes em artrópodes e trabalhamos com aprendizado aversivo.

50 Diferentes animais não atendem igualmente a um mesmo estímulo. Esta atenção
51 diferenciada pode enviesar a capacidade de diferentes estímulos de formarem
52 determinadas associações entre estímulo e resposta (Barnard 2004). A atenção pode
53 ser abordada de um ponto de vista fisiológico, como o efeito de neurotransmissores
54 como endorfina, a dopamina ou a serotonina. Pode ainda ser estudada do ponto de
55 vista neuronal, correlacionando determinados neurônios com a orientação para o
56 estímulo, desencadeamento e manutenção da atenção (Barnard 2004). Do ponto de
57 vista comportamental, o entendimento de para onde a atenção está voltada tem
58 importantes implicações para se entender evolução do comportamento. Os clássicos
59 trabalhos de Basolo sobre exploração sensorial em peixes poecilídeos (caudas longas
60 atraem a atenção da fêmea) (Basolo 1990, 1995) ou de cortejo em ácaros aquáticos
61 (vibrações causadas por machos que simulam presas atraem fêmeas) (Proctor 1991,
62 1992) são exemplos de estudos evolutivo apoiados em causas mecânicas, em
63 particular referentes à atenção. Da mesma maneira que pode se estudar quais fatores
64 influenciam a atenção, pode se investigar como melhor estudá-la e como obter
65 informações sobre a maneira pela qual a atenção influencia o comportamento animal.
66 Dois capítulos desta tese se prestam estas duas áreas.

67 No capítulo 3, estudamos o direcionamento do olhar como um *proxy* de para onde a
68 atenção está voltada. Este é um campo relativamente bem estudado em vertebrados
69 incluindo os seres humanos, mas pouco explorado em invertebrados. Primeiramente
70 revisamos a literatura sobre o assunto, publicando uma revisão que compila e explora
71 como podemos utilizar o direcionamento do olhar (*gaze direction*) para entender
72 atenção e processos cognitivos, com foco em invertebrados. Já no capítulo 4,
73 utilizamos um aparelho exclusivo, o *Eyetracker*, para estudar o direcionamento do
74 olhar e a atenção. Este aparelho permite que consigamos acessar para onde uma
75 aranha visual de menos de 1 cm está olhando, e assim para onde está voltada sua
76 atenção. Perguntamos se a exposição a um estímulo de um parceiro sexual afeta a
77 velocidade para iniciar um cortejo, mas também se afeta a atenção dada a distratores
78 ambientais e predadores.

79 O trabalho de revisão teve foco em invertebrados, por conta da escassez de dados
80 nestes animais no que diz respeito à atenção e direcionamento do olhar, além da
81 própria familiaridade dos autores do estudo com invertebrados. Os capítulos 1 e 2
82 foram feitos com opiliões no LESCA, o Laboratório de Ecologia Sensorial e
83 Comportamento de Artrópodes da EACH USP. Opiliões são aracnídeos da ordem
84 Opiliones, grupo com quase 7000 espécies descritas, a terceira maior ordem de
85 Arachnida depois de Araneae e “Acari”. Já o capítulo 4 foi desenvolvido durante
86 estágio sanduíche nos EUA, no laboratório da Prof Elizabeth Jakob, da University of
87 Massachusetts. Utilizamos aranhas da família Salticidae, conhecidas como aranhas
88 papa moscas, ou *jumping spiders*. O arcabouço de história natural suficiente para se
89 compreender cada capítulo é dado nas próprias introduções dos trabalhos. O capítulo
90 1 está resubmetido após a primeira revisão para o periódico *Animal Cognition*, o
91 capítulo 2 está submetido para o *Journal of Arachnology*, o capítulo 3 está publicado
92 em *Biochemical and Biophysical Research Communications* e o capítulo 4 está em
93 preparação para submissão.

94

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129

130 **CHAPTER 1**

131

132

133 **HABITUATION TO A PREDATORY STIMULUS IN A HARVESTER**
134 **(ARACHNIDA, OPILIONES)**

135

136 **HABITUAÇÃO A UM ESTÍMULO PREDATÓRIO EM UM OPILIÃO**
137 **(ARACHNIDA, OPILIONES)**

138

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148

149 **ABSTRACT**

150

151 Several studies have investigated habituation in a defensive context, but few have
152 addressed responses to dangerous stimuli. In such cases, animals should not habituate
153 since this could cost their lives. Here we have stimulated individuals of the
154 harvestman *Mischonyx squalidus* with a predatory stimulus (squeezing with tweezers)
155 in repeated trials within and between days, and measured the occurrence and
156 magnitude of nipping, a defensive behavior. Contrary to our expectations, they did
157 habituate to this stimulus. The probability and magnitude of response declined over
158 trials each day in a typical habituation pattern, but overall responding was lower on
159 the second and third days of testing. During the trials we also observed other
160 defensive behaviors. We discuss our results mainly considering alternative defensive

161 responses. Our data show how we lack information on (1) the role played by the
162 ambiguity of stimuli, (2) the role played by subsequent stimuli and (3) the importance
163 of the array of defensive behaviors of a species in understanding habituation.
164 Although ubiquitous across animals and therefore expected, habituation is described
165 for the first time in the order Opiliones.

166 Key words: defensive behavior, experience, non-associative learning, retention

167

168 **RESUMO**

169

170 Vários trabalhos investigaram a habituação em um contexto defensivo, mas poucos
171 abordaram as respostas a estímulos perigosos. Nesses casos, os animais não devem
172 habituar-se, pois isso poderia custar suas vidas. Aqui estimulamos indivíduos de
173 opiliões da espécie *Mischonyx squalidus* com um estímulo predatório (apertando com
174 pinças) em experimentos com repetições de estímulos em sequência e entre dias, e
175 medimos a ocorrência e magnitude de *Nipping*, um comportamento defensivo.
176 Contrariamente às nossas expectativas, eles habituaram-se a esse estímulo. A
177 probabilidade e magnitude de resposta diminuíram ao longo das repetições a cada dia,
178 em um padrão típico de habituação, mas a resposta geral foi menor no segundo e
179 terceiro dia de teste. Durante o experimento, também observamos outros
180 comportamentos defensivos. Discutimos nossos resultados principalmente
181 considerando respostas defensivas alternativas. Nossos dados mostram como falta
182 informação sobre (1) o papel desempenhado pela ambiguidade dos estímulos, (2) o
183 papel desempenhado pelos estímulos subsequentes e (3) a importância do conjunto de
184 comportamentos defensivos de uma espécie na compreensão da habituação. Embora
185 seja ubíquo em animais e, portanto, esperado, a habituação é descrita pela primeira
186 vez na ordem Opiliones.

187

188 **1. INTRODUCTION**

189

190 Habituation is a non-associative learning process defined as a decrease of a
191 response that results from the repetition of a stimulus if there is no motor or sensory
192 fatigue (Rankin et al. 2009). It is important because it allows animals to distinguish
193 between irrelevant and relevant stimuli, saving energy by ceasing responding to the

194 former (Eisenstein et al. 2001; Bell and Peeke 2012). However, some stimuli may be
195 ambiguous from the perspective of the prey (Liang et al. 2019). To respond optimally
196 to stimuli, animals require reliable information and should respond differently to
197 ambiguous and to actual high-risk predatory stimuli (e.g., Raderschall et al. 2011;
198 Wolfe et al. 2016). Responding repeatedly to harmless shadows, for example, would
199 probably deplete energy reserves (Rodríguez-Prieto et al. 2006). This distinction
200 becomes even more important when the effects of habituation last for more than 24h
201 (Rankin et al. 2009).

202 If animals can remember information about a dangerous event, such learning
203 may positively influence future behavior (Shettleworth 2010). After experiencing a
204 predatory attack, being able to maintain a long-term memory could improve not only
205 the defensive behavior itself, but also help avoiding future encounters with such
206 predators by using cues released by the predator in the environment (Pueta et al.
207 2021).

208 When facing unambiguously dangerous stimuli, animals should not habituate
209 since this could cost them their lives. Different non-associative learning theories
210 could help understanding those situations, such as sensitization or the dual-process
211 theory in cases where the stimulus is highly arousing/sensitizing. However,
212 irrespective of the explanation, the unambiguity of the stimulus *per se* has not been
213 specifically addressed or studied. Previous papers have reported the absence of
214 habituation (and/or sensitization) in cases where the stimuli used were unambiguous
215 (Prestrude and Crawford 1970; Zangrossi and File 1992; Masini et al. 2006; but see:
216 Pueta et al. 2021).

217 Another important factor is the level of threat imposed by a predatory
218 stimulus. According to the threat-sensitive hypothesis, animals are expected to
219 modulate their defensive response according to how dangerous the stimulus is
220 (Helfman 1989). Animals are expected to adjust their antipredator behavior to match
221 the actual level of predation risk, therefore avoiding the cost of unnecessary predator
222 avoidance behaviors (Rodríguez-Pietro et al. 2010).

223 Both short-term and long-term habituation have been reported with
224 unambiguous stimuli (predator model at a distance and predator sound - Long et al.
225 1989; May and Hoy 1991) but both these stimuli are at the “less dangerous” end of

226 the continuum. In contrast, being handled by the predator resides at the opposite and
227 most threatening end of the continuum.

228 Here we looked at habituation to an unambiguous stimulus that is highly
229 dangerous to the prey. We therefore expected to observe no short-term habituation
230 and that consequently there would be no long-term habituation of the behavior
231 involved. We tested this hypothesis using the armored harvestman *Mischonyx*
232 *squalidus* (Roewer, 1913) (Arachnida, Opiliones). To our knowledge, this is the first
233 study to investigate habituation and long-term habituation in the order Opiliones
234 using a predatory context.

235

236 2. MATERIAL AND METHODS

237

238 (a) Study animal, collection, and maintenance

239 We collected 25 adult males of *M. squalidus* at the Parque Ecológico do Tietê
240 (-23.507722, -46.547899), in the city and State of São Paulo in July 2022. We
241 maintained them in individual terraria (10x5x5 cm height) and fed them twice a week
242 with damp dog food. Water was provided in a damp cotton ball. We kept the room
243 temperature at 24 ° C and the light was ambient.

244 (b) Eliciting nipping behavior

245 Nipping is a well -known defensive behavior in harvestmen. Mainly males can
246 nip an aggressor by rapidly flexing both legs IV. Particularly in *Mischonyx squalidus*,
247 nipping has been documented to pierce, cut and repel predators (Dias & Willemart,
248 2013; Segovia et al 2015; Silva et al. 2018) and pierce human skin (GFP personal
249 observation). This behavior can be reliably elicited by holding the harvestman's body
250 dorso-ventrally with tweezers, a procedure that mimics capture by a bird (Gnaspini
251 and Hara 2007 and references therein). Tweezers also allow control to the force
252 applied and can be compared with the actions of birds, a known predator of
253 harvestmen (Cokendolpher and Mitov 2007).

254 In our experiments, we have used tweezers with a drop of melted hot glue on
255 its tips to avoid damaging the animal's cuticle. To minimize differences of strength
256 between trials, we used a peg connected to the tweezers to apply a pressure of 20 N
257 (measured with a dynamometer). This pressure is in the range of the biting force of
258 several birds (Herrel et al. 2005). If there was no response after the first stimulus, we

259 reapplied it two more times and the test resumed after the first nipping. We applied 10
260 sequential stimuli with an interval of 3s between each stimulus. This interval time
261 was chosen from a previous experiment for another study, in which we have observed
262 hens biting harvestmen repeatedly with intervals of 2 or 3 seconds (GFP personal
263 observation). To test for dishabituation, we used an 11th stimulus that consisted of
264 stimulating the chelicerae with a brush (that usually causes the harvestman to respond
265 trying to grab the brush with the chelicerae and/or pedipalps) and after 3s we applied
266 the regular pressure stimulus again. To test for long-term habituation, we repeated the
267 exact same procedure with the same animals 24h and 48h after the first test making
268 ten trials (plus one dishabituation trial) on each of the 3 days (n=25 males)

269 We noted if the animals nipped (flexing the legs so that the coxa-trochanter
270 area articulates) or did not nip (legs motionless) and we also checked the videos for
271 the magnitude of the behavior, looking at the difference between the angles of the
272 legs IV before and after the nipping movement (Fig. 1). The nipping behavior is a
273 quick (usually 1s or less of duration) pinching movement that causes the spined femur
274 of both legs IV to cross each other hitting the target between the legs (Fig. 1B). After
275 pinching, the legs return to their normal posture (Fig. 1A). The animal can perform
276 several nipping behaviors in a sequence. During the tests we also scored other
277 defensive behaviors that can be elicited with repeated stimulation (Rankin et al.
278 2009).

279 (c) Statistical analyses

280 To analyze the responses during trials, days, and possible interaction between
281 the two, we applied a generalized linear mixed model (GLMM, package lme4) with a
282 binomial structure (logit-link). All the response variables were either 1 or 0 (if the
283 responses occurred or not, respectively). Days and trials were included as fixed
284 effects and individual ID as a random effect to account for repeated measures. To
285 analyze the dishabituation phase, we applied a McNemar's test between trial 10 and
286 trial 11 (dishabituation) for each day.

287 We also measured the magnitude of response of the nipping behavior as the
288 difference between the angles of the legs IV before and after the nipping movement.
289 To analyze that response during trials, days, and possible interaction between the two
290 we also applied a generalized linear mixed model (GLMM, package glmmTMB) with
291 Gamma structure for inflated-zero model, to guarantee that all the trials and responses

292 were included in the analysis. Days and trials were included as fixed effects and
293 individual ID as a random effect to account for repeated measures. All statistical
294 analyses were performed using “R” software, version 3.5.3 (www.r-project.org; R
295 Development Core Team, 2008).

296

297 **3. RESULTS**

298 The results for the habituation trials are shown in Figure 2. The probability
299 and magnitude of response declined over trials each day in a typical habituation
300 pattern, and overall responding was lower on the second and third days of testing. For
301 the probability of respond to the stimulus we found differences between trials
302 (GLMM, CI = 0.46-0.67; $z = -6.043$; $p < 0.001$), and between days, (GLMM, CI =
303 0.34-0.92; $z = -2.298$; $p < 0.022$), but no positive interaction between days/trials,
304 (GLMM, CI = 1.00-1.18; $z = 1.879$; $p = 0.060$).

305 The results for the magnitude of response are also similar. We found
306 differences between trials (GLMM, CI 1.24-1.83; $z = -5.740$; $p < 0.001$) and days
307 (GLMM, CI = 1.60-4.83; $z = 3.621$; $p < 0.001$) but no positive interaction between
308 trial/day (GLMM, CI = 0.86-1.02; $z = -1.399$; $p = 0.162$).

309 The stimulus used for dishabituation (trial 11) elicited more intense responses
310 than in the last trial of each day for magnitude and probability. For magnitude the
311 median of the angle between legs IV on trial 11 was 65 ° (max = 93 °; min = 0 °)
312 while the trial 10 was 0 ° (max = 33 °; min = 0 °). On the second day the median of
313 the angle between legs IV was 59 ° (max = 94 °; min = 0 °) and for trial 10 the median
314 was 0 ° (max = 52 °; min = 0 °). Finally, on the third day the median of the angle
315 between legs IV on trial 11 was 44 ° (max = 93 °; min = 0 °) and for trial 10 the
316 median was 0 ° (max = 52 °; min = 0 °). We also found differences in the probability
317 of response. On day one the probability of response on trial 11 was 0.88% and for trial
318 10 it was 0.2% ($\chi^2 = 7.1$; $df = 1$; $p = 0.007$). On the second day the probability of
319 response on trial 11 was 0.64% and for trial 10 it was 0 ($\chi^2 = 7.1 = df = 1$; $p = 0.007$).
320 Finally on the third day the probability of response on trial 11 was 0.64% and for trial
321 10 it was 0.2 ($\chi^2 = 12$; $df = 1$; $p = 0.005$). Showing that the animals did not decrease
322 their response due to sensory or motor fatigue.

323 We also observed another defensive behavior, namely the release of chemicals
324 ($n = 16$ in 750 observations), a typical defense in harvestmen (Gnaspini and Hara

325 2007). Most of these observations (n=14) happened on the 1st day of trials and the
326 other two on the second day. They all occurred after the 5th trial and only a single
327 animal released the chemical twice (on different days). Although this strategy
328 occurred infrequently, it was used by more than 50% of the animals (15 of 25) across
329 the experiment. We also observed two cases of tanatosis (playing dead, cf. Segovia
330 et al., 2018).

331

332 4. DISCUSSION

333

334 We have shown that habituation of a defensive response occurs to an
335 unambiguously threatening stimulus in individuals of *M. squalidus*. Animals also
336 showed a decrease in their responsiveness, but with a similar habituation pattern, over
337 consecutive days. We used an unambiguous, highly threatening stimulus that
338 resembled a predator, since no stimulus other than a predator would pinch and lift the
339 harvestman's body. Moreover, as explained above, we applied a force similar to a
340 bird bite. We were not expecting habituation to occur based on examples from
341 previous work (Prestrude and Crawford 1970; Zangrossi and File 1992; Masini et al.
342 2006; Ardiel et al. 2017; Pueta et al. 2021).

343 Proximately, it has been shown that repeated stimulation of specific
344 nociceptive neurons decreases the magnitude of a given response causing habituation
345 (Ardiel et al. 2016). However, repeated activation of such neurons may also have
346 other effects, including behavioral responses. For example, other defensive strategies
347 could be evoked (Ardiel et al. 2017; McDiarmid et al. 2019). In only two cases have
348 we observed the animals playing death (legs are flexed and close to the body).
349 Although this behavior is common in predatory situation (Segovia et al 2018), in our
350 experiment playing death was rarely observed. It is possible that maintaining the legs
351 extended in this situation makes it harder for the harvestman to be swallowed by the
352 predator. The use of defensive mechanisms other than nipping might be associated
353 with a switching of the defensive strategy. This might be a result of habituation in one
354 defensive mechanism and sensitization in another defensive mechanism (Rushford et
355 al. 1963; Evans 1969; Ardiel et al. 2017). It is noteworthy that the release of
356 chemicals, when it occurred, happened after the animals had stopped responding with
357 nipping. The composition of this defensive chemical is a blend of several

358 components including benzoquinones and phenols among other chemicals (for more
359 details see Hara et al. 2005). The release of this compound, though efficient
360 (Machado et al. 2005; Silva et al. 2018), is costly (Nazareth and Machado 2015;
361 Nazareth et al. 2016), which may justify why none of the animals released chemicals
362 more than once on the same day.

363 Another interesting point is that animals have been reported to habituate the
364 magnitude of the response but not the probability of responding (Ardiel et al. 2017).
365 However, our data show habituation in both variables. Because the defensive nipping
366 did not prevent stimulus from occurring, we can only speculate that the animal would
367 stop reacting with nipping and instead rely on other defensive mechanisms were it
368 subsequently manipulated in the predator's mouth. In such a case, heavy armor,
369 spines, or chemical defenses would serve this purpose (see references in Gnaspini and
370 Hara 2007; Silva et al. 2018).

371 We also found a difference in the probability of responsiveness on successive
372 days. Individual differences are indeed expected according to the Behavioral
373 Homeostasis Model (Eisenstein et al. 2001) since some individuals have lower
374 sensory thresholds. Still, the animals in our study might have learned that the stimulus
375 is not dangerous because both the context and the inter-stimulus interval (ISI) were
376 precisely the same (see McDiarmid et al. 2019), which would be unusual in nature
377 (May and Hoy 1991). Another possibility is that we did not provide other putative
378 relevant sensory cues such as shades or olfactory cues of a predator (see Pueta et al.
379 2021). Furthermore, the interaction between trials/days was not significant. The
380 general habituation pattern was similar between days, but on days two and three there
381 was a slightly lower probability of responses, compared to day one (Fig. 2). In long-
382 term habituation, the responses typically do not return to naïve levels (Tomsic et al.
383 1993; Rankin et al. 2009; Randlett et al. 2019). Moreover, the maintenance of this
384 pattern of response with higher probability of response in the first trials may be an
385 active process of not ignoring the stimulus at all. This may be important to trigger the
386 possibility of switching to another defensive strategy, which could be of high survival
387 value when facing potential lethal stimuli (Ardiel et al. 2017; McDiarmid et al. 2019).
388 Learning by habituation might be an optimization of behavioral strategies in
389 accordance with particular internal (physiological) and external (environmental
390 context) factors.

391 Finally, our sample size does not allow us to make any statement about
392 whether or not we found support for the "behavioral homeostasis model" (Eisenstein
393 et al 2001), which predicts differences between individuals and a relation between
394 sensory thresholds and responsiveness to stimuli, sensitization and habitation.
395 However, our method could be used for this specific purpose in future studies.

396 In summary, we have shown that habituation occurs following an
397 unequivocally dangerous stimulus and that memory follows in this context. Our study
398 suggests we need a better understanding of the roles of ambiguity of the stimulus, the
399 influence of how the animal responds to subsequent behaviors, and the specific role
400 of each defensive behavior. We also present a new technique to test habituation and
401 dishabituation in a new model organism (Opiliones).

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497 **FIGURES**

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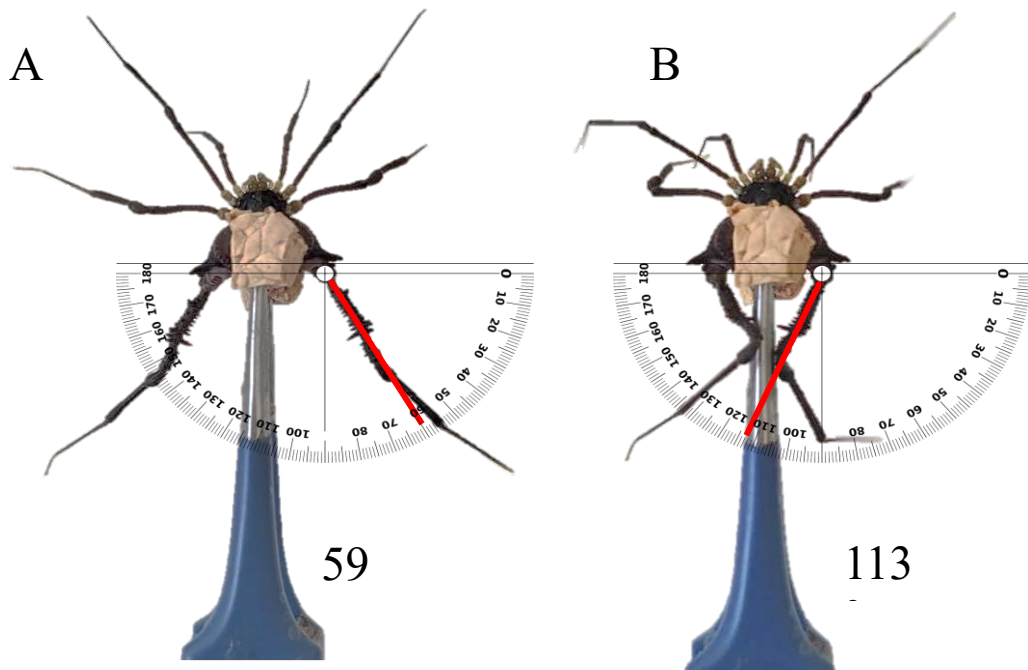
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511 **Fig.1** Method using tweezers for triggering nipping with legs IV in the harvestman
512 *Mischonyx squalidus*. The initial angle is shown on “A” and the final angle is shown
513 on “B”.

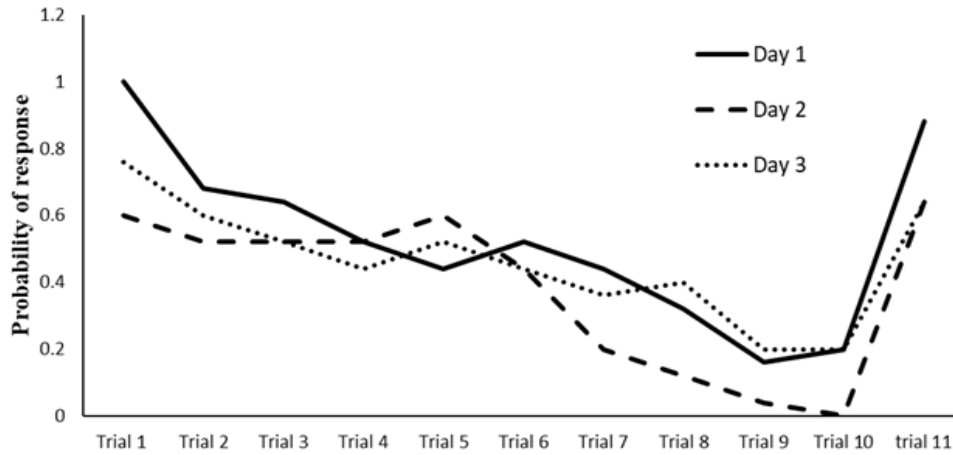
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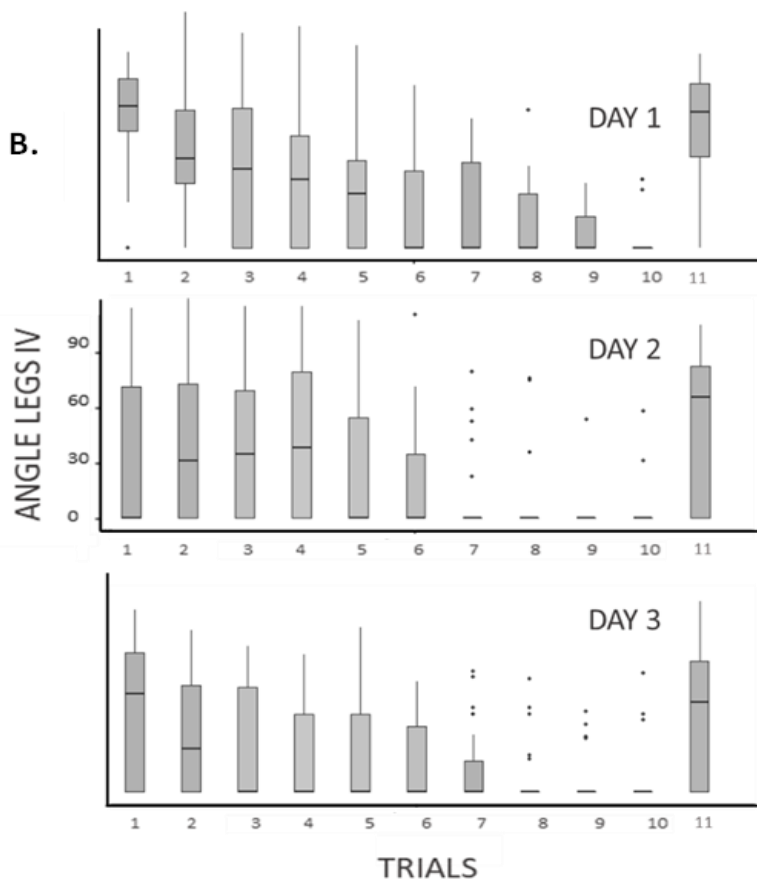


A.



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520 **Fig. 2** A – Probability of delivering a nip after being stimulated by tweezers in the
 521 harvestman *Mischonyx squalidus*, per trial and per day. B – Box plot of the magnitude
 522 of response. The angles are the difference between the same leg IV before and after
 523 being stimulated by tweezers in the harvestman *M. squalidus*. Trial 11 is showing the
 524 dishabituatation trial on both graphics.

525 **CHAPTER 2**

526

527

528 **AVERSIVE CONDITIONING AND MEMORY IN THE HARVESTER**
529 ***MISCHONYX SQUALIDUS* (ARACHNIDA: OPILIONES)**

530

531 **CONDICIONAMENTO AVERSIVO E MEMÓRIA NO OPILIÃO**
532 ***MISCHONYX SQUALIDUS* (ARACHNIDA: OPILIONES)**

533

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549

550

551 **ABSTRACT**

552 Aversive conditioning is a specific form of associative learning that can be
553 memorized depending on the learning process. We trained harvesters to associate an
554 aversive stimulus (shock) with a neutral stimulus (tea). We separated the subjects into
555 two groups, trained in short-term memory (STM) and long-term memory (LTM)
556 protocols. Each subject went through three consecutive trials, in which pairings
557 between the chemical and the shock lasted for 3s, with intervals of 1 (STM) and 30

558 (LTM) min. We then placed the subjects in a two-choice arena with blank and tea
559 areas, recording them for 10min. We did this test immediately after the last trial and
560 24h after it. Both protocols were successful for memory within the same day, but not
561 for memory in the following day. In summary, we provide a new method to train
562 aversive conditioning in harvesters and evidence for short-term memory.

563 Key-words: Learning, negative stimulus, shock platform,

564

565

566 **RESUMO**

567 O condicionamento aversivo é uma forma específica de aprendizagem associativa que
568 pode ser memorizada dependendo do processo de aprendizagem. Treinamos os
569 opiliões para associar um estímulo aversivo (choque) com um estímulo neutro (chá).
570 Separamos os indivíduos em dois grupos, treinados nos protocolos de memória de
571 curto prazo (STM) e de memória de longo prazo (LTM). Cada indivíduo passou por
572 três *trials* consecutivos, nos quais os emparelhamentos entre o químico e o choque
573 duraram 3s, com intervalos de 1 (STM) e 30 (LTM) min. Em seguida, colocamos os
574 indivíduos em uma arena com dois lados de escolha com áreas em branco e de chá, e
575 filmamos por 10min. Fizemos este teste imediatamente após o último *trial* e 24h após
576 último *trial*. Ambos os protocolos foram bem-sucedidos para formação de memória
577 de curto prazo, no teste do mesmo dia, mas não para memória de longo prazo, no
578 teste de 24h - dia seguinte. Em resumo, fornecemos um novo método para treinar
579 condicionamento aversivo em opiliões e evidências para memória a curto prazo.

580

581 Palavras-chave: Aprendizado, Estímulo negativo, Plataforma de choque,

582

583 **INTRODUCTION**

584

585 The study of cognition in arachnids has developed incredibly in the last
586 decade. Innumerable works have been published showing from simple forms of
587 learning abilities to complex problem solving (Peckmezian and Taylor 2015a; Jakob
588 and Long 2016; Cross and Jackson 2019; Rößler et al. 2021) Most of these papers
589 have dealt with spiders, whereas other arachnids have received less attention over the
590 years (but see Santer and Hebets 2009; Wiegmann et al. 2016; Gaffin et al. 2022;

591 Lehmann et al. 2022). The order Opiliones is the 3rd in number of described species in
592 the class Arachnida, with 6676 spp (Kury et al. 2021). Learning and memory have
593 been shown with experiments involving associative learning (Santos et al. 2013;
594 Costa et al. 2016), both studies using a positive stimulus and testing only for short-
595 term memory. However, being able to learn and memorize from a previous
596 threatening experience can be fundamental to survival (Gabriel and Golightly 2014;
597 Wystrach et al. 2020), so one could expect long-term memory. In some cases,
598 learning can occur faster and with higher durability when involving an aversive
599 situation (Perry et al. 2013). For example, aversive conditioning is a special form of
600 associative learning that includes an unconditioned stimulus (negative stimulus in this
601 case), associated with a neutral stimulus. Aversive conditioning protocols can be a
602 good option when investigating learning from a negative stimulus and for long-term
603 memory. Different protocols have been used to evoke short-term memory (STM) and
604 long-term memory (LTM) in invertebrates (Amano and Maruyama 2011). Typically,
605 STM protocols use massed training with no/or a very short interval between the trials
606 and it induces memories that are independent of protein synthesis. The LTM
607 protocols use spaced training with a longer interval between the trials. This interval is
608 fundamental for the occurrence of protein synthesis and memory consolidation
609 (Fulton et al. 2005; Mery and Kawecki 2005).

610 In this study we investigate the learning and memory capacity of the harvester
611 *Mischonyx squalidus* under an aversive conditioning protocol, using two different
612 protocols to test the formation of short and long-term memories.

613

614 MATERIAL & METHODS

615

616 *Mischonyx squalidus* (Roewer, 1913) appears as *Mischonyx cuspidatus* or
617 *Ilhaia cuspidata* in previous papers (see Gueratto et al. 2021). We collected males of
618 *M. squalidus* under rocks and trunks at the Parque Ecológico do Tietê (-23.507722, -
619 46.547899), in the city and State of São Paulo in July 2019. We used a single sex to
620 minimize confusion variables and because we found more males than females. We
621 maintained them in individual terraria (10 x 5 x 5 cm height), fed them twice a week
622 with damp dog food, and provided water *ad libitum* in a damp cotton ball. We
623 maintained the temperature at 24 °C with ambient light.

624 Shock is probably the most used and effective modality to produce a negative
625 stimulus (Amano and Maruyama 2011; Berdnarski et al. 2012). However, it is
626 common to have problems with the shock apparatus when it is not adequate to the
627 species being studied or in some machine models that had inconsistent voltage or
628 problems with conductivity (Long et al. 2015). For that reason, we built a specific
629 shock platform following Peckmezian and Taylor (2015b). We made several
630 adjustments and calibrations to adequate it to harvesters. For the neutral stimulus, we
631 chose to use chemicals since harvesters rely on chemicals in a great variety of
632 biological tasks (Willemart et al. 2009; Dias et al. 2020).

633 The experiment consisted in having a harvester tied to a shock platform + a
634 chemical (conditioning phase) and subsequently submitting the same individual to a
635 choice arena, where it could stay on the side with the same chemical previously
636 experienced in the shock platform or the control side (test phase). For the shock
637 platform, we used a copper base (10 x 5 cm) with parallel strips, spaced 1.5 mm from
638 each other, alternating a positive and a negative bar (Fig. 1). We designed the pattern
639 using Adobe Photoshop CS5.5 (Adobe Systems, San Jose, CA, U.S.A.) and printed
640 on the toner transfer film. The film was transferred using a hot iron to a blank copper
641 board. To prevent the animals from falling off the platform we used a rubber band to
642 tie the harvester to the platform with the abdomen and legs touching two neighboring
643 strips (therefore a positive and a negative), guaranteeing the electrical circuit was
644 completed (Fig. 1). Using a switch, we determined when the shock was released and
645 its duration. For this experiment we were interested in testing two different protocols:
646 one with a short interval time between the shocks (1 min) to test short-term memory
647 formation (STM) and a second one with a longer interval (30 min) between the
648 shocks to test long-term memory (LTM) (see Brembs 2003; Mery and Kawecki 2005;
649 Amano and Maruyama 2011 for similar protocols to test memory).

650

651 Conditioning phase

652

653 For the aversive conditioning protocols, we paired a chemical stimulus
654 (neutral stimulus) with a shock (aversive stimulus). For the chemical stimulus, we
655 used filter paper left 24 h in contact with mate tea dried leaves. These have been
656 previously used in experiments with harvesters because they detect it but are not

657 attracted or repelled by it (Santos et al 2013). To rest assured, we tested the neutrality
658 of the tea by offering a blank filter paper paired with a filter paper previously
659 impregnated for 24 h. The harvesters spent 2.7 min (median; min = 0 min, max = 13.3
660 min) in the half with tea and 1.9 min (median; min = 0 min, max = 11 min) in the
661 blank side ($p = 0.777$; $t = 48$; $n = 20$), a non-significant difference.

662 The procedure consisted in placing the animals with the ventral part of the
663 body in contact with the platform but with the pedipalps, legs I and II (sensory legs –
664 Gainett et al 2017) in contact with a sample of the filter paper with tea chemicals
665 (Fig. 1). To provide an aversive stimulus, we used 20-volt shock applied on the
666 copper platform described above. We tried higher voltage, but it hampered the
667 animal's movements. Lower voltages triggered no observable response by the
668 harvesters. Each animal went through 3 consecutive trials for the learning trials
669 with pairings between the chemical and the shock for 3s. We used 40 male
670 individuals, and 20 animals for each treatment that were randomly selected within our
671 sample.

672

673 Test phase

674

675 After training, we submitted the animals to a test phase. We placed the
676 animals in a circular arena (18 x 6 cm) in which the halves were covered with a filter
677 paper substrate with or without tea chemicals. The animals were acclimated for 2 min
678 under a transparent cup (8 cm diameter) and then released to move freely in the arena.
679 We recorded the animals for 10 min (plus the 2 minutes of acclimatization), and later
680 scored the time spent in each half. We tested animals of both treatments, STM and
681 LTM, in two stages: the first test was right after the conditioning phase (same-day
682 test); and the second test was in the following day, 24 h after the conditioning phase
683 (following day test).

684

685 **RESULTS**

686

687 When we moved the animals to the test arena, some animals ($n = 9$)
688 performed a freezing behavior that sometimes lasted more than 2 min of acclimation.

689 In these cases, we started counting the time spent in one half when the animal moved
690 any part of the body.

691 For the same-day test, individuals trained in STM remained for 120 s
692 (median) in the blank half (min = 0 s; max = 552 s) and 18 s (median) in the half with
693 tea chemicals (min = 0 s; max = 420 s), showing avoidance of the side with tea
694 chemicals ($t = 104$; $p = 0.02$; $n = 20$). Individuals trained in LTM spent 240 s
695 (median) in the blank half (min = 0 s; max = 540 s) and 0 s (median) in the half with
696 tea chemicals (min = 0 s; max = 300 s), also showing avoidance of the side with tea
697 chemicals ($t = 2$; $p < 0.001$; $n = 20$).

698 For the following day test, individuals trained in STM remained 168 s
699 (median) in the blank half (min = 0 s; max = 600 s) and 120 s (median) in the half
700 with tea chemicals (min = 0 s; max = 540 s), showing no avoidance of the side with
701 tea chemicals ($t = 135$; $p = 0.33$; $n = 20$). The same happened for individuals trained
702 in LTM that remained for 102 s (median) in the blank half (min = 0 s; max = 600 s)
703 and 204 s (median) in the half with tea chemicals (min = 0 s; max = 600 s), showing
704 no avoidance of the side with tea chemicals ($t = 121$; $p = 0.39$; $n = 20$).

705

706 **DISCUSSION**

707

708 We have shown associative learning using an aversive stimulus and short-term
709 memory irrespective of the time elapsed between the two given stimuli. However, we
710 did not observe long-term memory. Finally, we have developed a successful protocol
711 using shock that applies to Opiliones.

712 We found that the tested harvesters retain the information acquired
713 immediately previous to the training, both in STM and LTM protocols. Though
714 expected, most of the work on learning in invertebrates has been conducted in insects
715 (Mizunami et al. 2004; Giurfa 2013). However, mechanisms in insects and other taxa
716 are not necessarily the same, calling for the need for such studies in a broader range
717 of taxonomic groups. Harvesters learn to associate a stimulus with a shelter (Santos et
718 al. 2013), habituate to a predatory stimulus (Pagoti, Hogan, and Willemart,
719 unpublished data), and may alter their food choice because of learning (Costa et al.
720 2016). We are no one step further, showing that an aversive stimulus also triggers
721 associative learning but that the memory does not last.

722 We did not find evidence of long-lasting memory in our study neither in the
723 STM nor LTM protocols. Protocols for generating memory vary in the literature, with
724 respect to both the intervals between trials and the number of trials. We used a three-
725 trials design in all our tests and it is known that performance in acquiring memory
726 increases as the number of training trials increases (Yu et al. 2005). Moreover, it has
727 been previously reported that only one in six different training schedules has
728 produced LTM in flies (Yu et al. 2006). Therefore, future experiments could be
729 conducted with 5-10 trials (see references in Davis 2011) and maybe with different
730 schedules. Alternatively, differences at the molecular, cellular, and/or systems level
731 between harvesters and flies explain the absence of long-term memory (see
732 discussion in Davis 2011).

733 The aversive conditioning protocol used in this study is a cheap and easy way
734 to access learning and short-term memory in harvesters similar to what has been used
735 in other arachnids (Peckmezian and Taylor 2015b;2017). Since harvesters learned to
736 avoid the place with the conditioned chemical stimuli within just a few short trials
737 pairing shock and a chemical, such protocol has been successful and can be used in
738 future studies.

739 The present study was the first that used an aversive protocol to study learning
740 in harvester showing short-term memory. Also, we developed a shock platform that
741 might be adapted in future learning studies in Opiliones. Moreover, the use of a broad
742 methodology and protocols could support comparative studies with other *taxa*.
743 Finally, we hope that the described protocol and material can also incentive more
744 learning studies in harvesters as in jumping spiders (Long et al. 2015; Jakob and Long
745 2016; De Agrò 2020).

746

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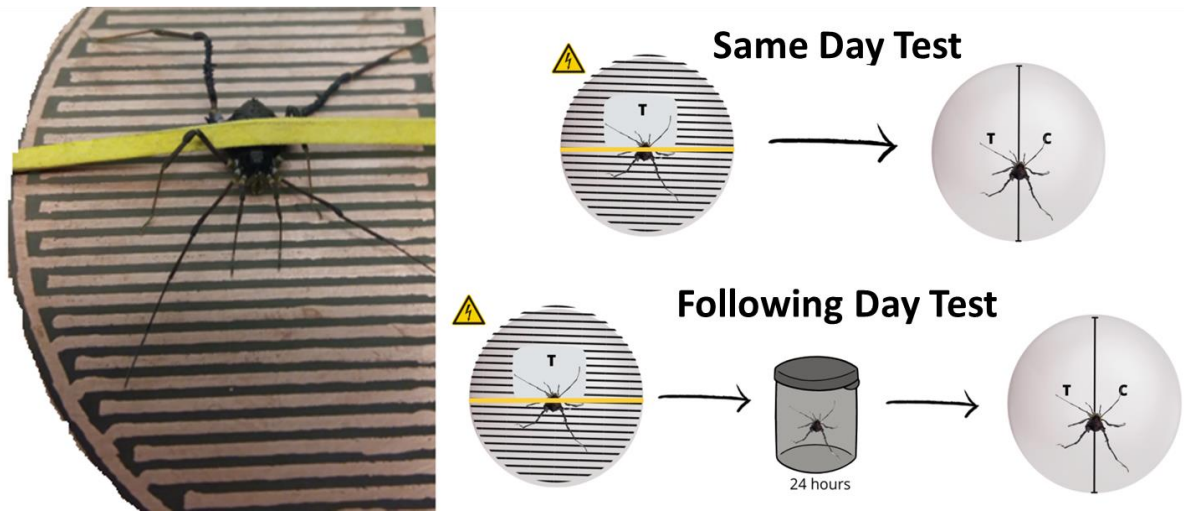
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840

841 **FIGURES.**

842



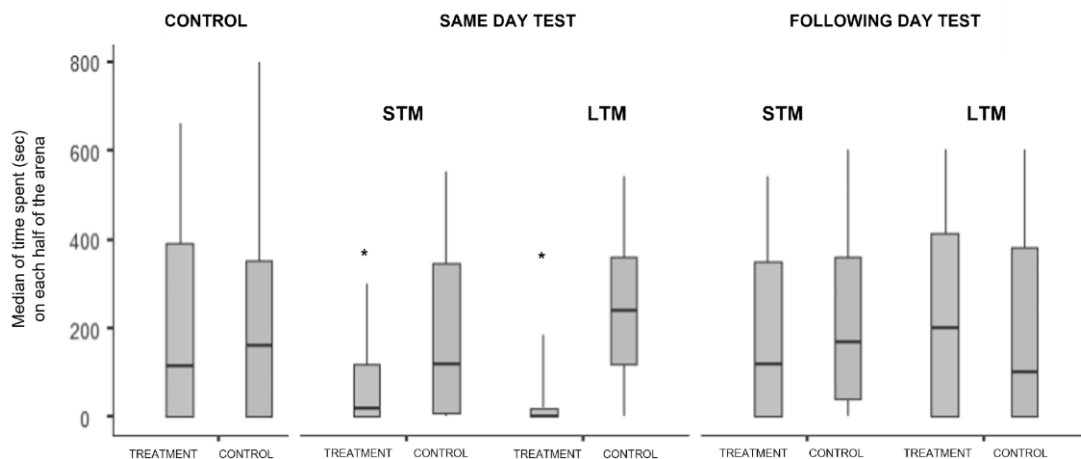
843

844 Figure 1. Set up of the shock platform, with its copper base with parallel negative and

845 positive strips (left) and basic procedure of the experiment (right). T = treatment

846 (tea); C = blank (control)

847



848

849 Figure 2. Time spent on the treatment and control halves in a two-choice arena after

850 the harvester *Mischonyx squalidus* (Arachnida, Opiliones) has been subjected to an

851 aversive stimulus (shock) associated with the treatment (tea). STM and LTM = Short-

852 term and long-term memory protocols, respectively.

853

854

855 **CHAPTER 3**

856

857

858 **WHAT GAZE DIRECTION CAN TELL US ABOUT COGNITIVE**
859 **PROCESSES IN INVERTEBRATES**

860

861 **O QUE A DIREÇÃO DO OLHAR PODE NOS DIZER SOBRE OS**
862 **PROCESSOS COGNITIVOS EM INVERTEBRADOS**

863

864

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878

879

880 **ABSTRACT**

881 Most visually guided animals shift their gaze using body movements, eye
882 movements, or both to gather information selectively from their environments.

883 Psychological studies of eye movements have advanced our understanding of
884 perceptual and cognitive processes that mediate visual attention in humans and other

885 vertebrates. However, much less is known about how these processes operate in other
886 organisms, particularly invertebrates. We here make the case that studies of

887 invertebrate cognition can benefit by adding precise measures of gaze direction. To
888 accomplish this, we briefly review the human visual attention literature and outline
889 four research themes and several experimental paradigms that could be extended to
890 invertebrates. We briefly review selected studies where the measurement of gaze
891 direction in invertebrates has provided new insights, and we suggest future areas of
892 exploration.

893 Keywords: Visual attention; Gaze direction; Cognition; Invertebrate

894

895 **RESUMO**

896 A maioria dos animais guiados visualmente mudam a direção de seu olhar usando
897 movimentos corporais, movimentos dos olhos, ou ambos para coletar informações
898 seletivamente de seus ambientes. Estudos psicológicos sobre movimentos oculares
899 avançaram nossa compreensão dos processos perceptuais e cognitivos que mediam a
900 atenção visual em humanos e outros vertebrados. No entanto, sabe-se muito menos
901 sobre como esses processos operam em outros organismos, particularmente
902 invertebrados. Nós argumentamos que estudos sobre a cognição de invertebrados
903 podem se beneficiar com a adição de medidas precisas da direção do olhar. Para isso,
904 revisamos brevemente a literatura sobre a atenção visual em humanos e apresentamos
905 quatro temas de pesquisa e vários paradigmas experimentais que poderiam ser
906 estendidos para invertebrados. Também revisamos brevemente estudos selecionados
907 nos quais a medição da direção do olhar em invertebrados forneceu novas
908 informações e sugerimos áreas futuras de exploração.

909

910 **1. INTRODUCTION**

911

912 How do animals acquire, evaluate, and process visual information? How do
913 they decide which parts of the rich visual environment to attend to? Animal
914 behaviorists typically study these questions by presenting animals with a variety of
915 different stimuli and observing their responses. This fruitful approach has led to an
916 understanding of male traits that are preferred by females (e.g., Ref. [1]), how animals
917 detect and assess threats (e.g., Ref. [2]), and how animals detect prey (e.g., Ref. [3])
918 or avoid aposematic prey (e.g., Ref. [4]), among many examples. This experimental
919 approach has been particularly productive since the advent of software that enables

920 researchers to manipulate videos and create animations to present stimuli (reviews in
921 Refs. [5-7]).

922 Here we advocate for increased use of a complementary approach: the precise
923 measurement of gaze direction. Eye movements have long been used to study
924 cognitive processing in humans (see Ref. [8], reviewed in Ref. [9]). Humans direct
925 their gaze and as such selectively gather visual information: only a small amount of
926 information that is present in the environment is processed by the high-resolution
927 foveal area of the eye. Thus, a person's gaze is a direct measurement of overt
928 selective attention that tells us something about cognitive processes underlying visual
929 attention. The development of increasingly accurate and easy-to-use eyetrackers has
930 generated thousands of papers addressing a range of questions on human visual
931 processing and cognition, including how humans recognize objects (e.g., Ref. [10]);
932 explore visual scenes (e.g., Ref. [11]); complete active tasks (e.g., Ref. [12]); and
933 adjust their gaze based on their goals, expectations, and prior knowledge (reviewed in
934 Refs. [13,14]).

935 Of course, gaze control is not restricted to humans: nearly all visually guided
936 animals control their gaze using coordinated body or eye movements [15]. While
937 much elegant comparative work has been done on the study of eye movements
938 (reviewed in Refs. [16,17]), most of the research questions have not been framed in
939 the context of cognition. Rather, many papers have focused on how animals use eye
940 movements to solve particular functional problems, such as stabilizing their vision or
941 pursuing moving objects (reviewed in Ref. [17]). Our goal in this paper is not to
942 revisit that body of literature, but rather to highlight some of the questions and
943 experimental approaches commonly used in psychology and ask whether they can be
944 applied to other animals. We focus on invertebrates and particularly on jumping
945 spiders, our own research organisms.

946

947 1.1. Common themes in visual attention research

948

949 The research on visual attention, particularly in humans, is vast. However,
950 there are some recurring ideas threading through the literature. First, the process of
951 visual attention must be inherently selective [18]. It is not possible to process all
952 incoming information, necessitating that relevant information be prioritized over

953 extraneous information [19]. Neural tissue required for computation is energetically
954 expensive both in its development [20] and use [21], which is thought to constrain
955 selective attention.

956 Second, visual attention can be influenced by bottom-up processes, in which
957 stimulus properties drive attentional shifts [19], or by top-down processes, in which
958 attentional shifts result from goals of the observer [22]. Top-down processing is
959 characteristic of higher-order processing as it relies on working memory to determine
960 which information is stored and attended to [23]. A large body of evidence in humans
961 and a smaller body in non-human animals suggests that both stimulus-driven and
962 goal-directed processes direct visual attention (see Ref. [24] for humans, [25] for
963 insects). Top-down and bottom-up processing can interact: topdown attentional states
964 can regulate attention to bottom-up cues [26], and bottom-up and top-down guidance
965 can even work in opposition to each other [27].

966 Here we focus on four areas where we think an expanded study of gaze
967 direction in invertebrates might prove profitable: object recognition (section 2), visual
968 search (section 3), learning (section 4), and navigation (section 5). For a detailed
969 discussion on why these processes are thought to be cognitive, see Shettleworth [28].
970 Before covering these areas, we first consider the methods by which gaze direction
971 can be measured.

972

973 1.2. The measurement of gaze direction and eye movement across species

974

975 Techniques for measuring gaze direction are, as one might expect, most
976 advanced for humans. Humans have foveated visual systems where the central area of
977 the retina has substantially higher acuity than the periphery. Eye movements typically
978 follow a pattern of saccades and fixations rapid shifts in gaze direction followed by
979 periods of stasis as people scan their environment and direct their foveas to areas of
980 interest [17]. It is these fixation points on a visual scene that are often the behavioral
981 variable of interest for cognitive research. A number of methods for tracking human
982 gaze direction have been developed (reviewed in Ref. [29]), including those based on
983 corneal reflection or on the measure of electrical currents on skin surrounding the
984 eyes. Whereas many eyetrackers require a person to be sitting in place, lightweight

985 head-mounted systems [30] have allowed researchers to track eye movements in
986 infants and in study participants that are moving freely about (see Ref. [29]).

987 Researchers have adapted human eyetracker technology to monitor gaze
988 direction in a small number of non-human vertebrate species. Vertebrates tend to scan
989 with saccade-and-fixation patterns, similar to those described in humans, which
990 makes the application of previously developed technologies relatively feasible [15].
991 Birds have flexible necks and light heads, which enable them to rotate their heads at
992 similar speeds to human saccades [17]. Eyetrackers have been successfully
993 implemented in non-human primates (e.g., Ref. [31]), birds (e.g., Refs. [32e37]), and
994 rodents (e.g., Ref. [38]).

995 By contrast, most invertebrate species, including insects, do not have movable
996 eyes and can only shift their gaze by shifting their body or head orientation. Precise
997 measures of head orientation (e.g., using high-speed cameras [39]), coupled with an
998 understanding of the visual field of view, can generate insight into how animals with
999 immovable eyes explore their visual environment. Some crustaceans have stalked
1000 eyes that can move independently of the body. By monitoring eye position, it is
1001 possible to reconstruct the panoramic visual field of fiddler crabs [40]. The
1002 independently moving eyes of stomatopods, marine crustaceans also known as mantis
1003 shrimp, can also be measured (see Ref. [41] for a review). For example, Marshall et
1004 al. [42] videoed stomatopod eyes, tracked their eye positions frame-by-frame, and
1005 fitted a three-dimensional outline of each eye to infer gaze angle of the fovea,
1006 revealing several distinct eye movement strategies. Cephalopods such as squid and
1007 octopuses have moveable eyes resembling those of vertebrates [17], and only recently
1008 have techniques to measure eye movement in these species been developed and
1009 deployed [43].

1010 Other invertebrate species have eyes that move only internally. Our lab works
1011 on jumping spiders (Family Salticidae), which are appealing subjects for visual
1012 attention research as they are highly visual in many aspects of their lives, possess
1013 high-acuity vision [44], demonstrate an ability to learn (reviewed in Refs. [45,46]),
1014 and exhibit complex decision-making behaviors [46]. The jumping spider visual
1015 system is modular, with a pair of principal eyes that are responsible for high spatial
1016 acuity and color vision, and three pairs of secondary eyes that are particularly attuned
1017 to detecting and processing movement. The retinas of the principal eyes are situated

1018 at the back of long tubes within the cephalothorax, with an immovable lens on the
1019 exterior of the spider. The most forwardfacing pair of secondary eyes direct the
1020 principal eye retinas to locate and track objects [47]. In our lab, we have conducted
1021 experiments in which we show spiders video stimuli while we monitor gaze direction
1022 with a specially designed eyetracker that captures infrared (IR) light reflected from
1023 the retinas [47,48] (Fig. 1)

1024

1025 2. GAZE DIRECTION AND OBJECT RECOGNITION

1026

1027 Recognition is the process by which animals perceive and classify objects in
1028 appropriate categories, such as food, predators, and conspecifics. The behavior of
1029 many invertebrate taxa can be driven by images of objects. There are a number of
1030 well-studied examples. Bees use many visual features to detect and recognize
1031 flowers, including color contrast, pattern orientation, symmetry (reviewed in Ref.
1032 [49]), and shape [50]; for a review of the recognition system in honeybees, see Ref.
1033 [51]. Social wasps can distinguish nestmates from non-nestmates based on facial
1034 markings [52]. Jumping spiders can recognize potential mates by their courtship
1035 behavior [53] and discriminate between types of prey prior to attack [54e56]. For
1036 example, *Portia fimbriata* distinguishes its favored prey, other salticids, by visual
1037 cues that include subtle details such as eye arrangement [57,58]. In another example,
1038 *Phidippus regius* uses local features to identify objects [59]. In this section, we will
1039 consider how in humans and other animals, gaze direction might inform us about
1040 object recognition and how this process depends on stimulus properties, surrounding
1041 context, and prior expectations.

1042

1043 2.1. Gaze direction and object recognition in humans

1044

1045 Humans must recognize objects in their environment for routine tasks such as
1046 identifying food or recognizing faces. Although the appearance of objects varies with
1047 viewing perspective, occlusions, variation across exemplars, and changes in lighting,
1048 we are able to recognize objects very quickly and effectively (reviewed in Ref. [60]).
1049 In the psychology literature, object recognition requires that a subject discriminates
1050 available stimulus features and matches them with representations of target objects

1051 from memory [61]. The efficiency with which an organism can detect and respond to
1052 particular stimuli is often attributed to bottom-up ‘saliency filters’ that enhance
1053 detection of particular features [23]. For example, humans shift their gaze rapidly to
1054 stimuli that appear abruptly [62], contrast with the background [63], or differ from
1055 other nearby stimuli [23,64]. While the saliency model predicts that eye movements
1056 will be directed to individual stimulus features, in real-world contexts we often target
1057 discrete objects rather than features alone [60].

1058 Contextual cues facilitate object recognition (reviewed in Ref. [65]). For
1059 example, humans are better able to correctly identify objects when the corresponding
1060 scene is appropriate [66] or when there is a collection of related objects [67], whereas
1061 if objects are presented in inappropriate scenes or object relationships are otherwise
1062 violated, recognition is impeded [68]. This shows that observers’ expectations can
1063 influence how they perceive an object. In addition, object recognition can be
1064 facilitated by information from other sensory modalities (reviewed in Ref. [69]). For
1065 instance, humans are better able to identify a partially masked image of a dog when it
1066 is paired with a barking sound [70]. To perceive objects, constituent features need to
1067 be detected and subsequently integrated in the brain of the observer, processes that
1068 may occur at different levels of visual processing. To disentangle feature-based and
1069 object-based recognition in experiments, control stimuli should have the same
1070 constituent parts but lose their distinct amalgamated meaning (e.g., Ref. [71]).

1071 Paradigms developed for studying object recognition in infants are
1072 particularly well adapted for non-verbal animals. One such approach is the
1073 simultaneous presentation of images to measure preference and visual bias in
1074 attention [72e74]. These ‘looking time’ experiments are particularly useful for
1075 exploring visual bias, habituation, and expectancy violation [75]. Similarly,
1076 anticipatory looking methods take advantage of the fact that infants and other
1077 vertebrates look to locations where they expect an event is about to occur (e.g., Ref.
1078 [76]). Infants gaze at familiar and novel stimuli for different durations, suggesting
1079 that familiar objects are recognized [77]. Furthermore, infants spend more time
1080 looking at scenes that violate their expectations [78e80]. Gaze direction and
1081 preferential looking methodologies can reveal infants’ understanding across a variety
1082 of cognitive domains, including reasoning about objects, numbers, and the social
1083 behavior of others [81e83].

1084

1085 2.2. Gaze direction and object recognition in invertebrates

1086

1087 Which stimulus features attract attention? Bottom-up control of attention has
1088 been the most commonly studied perceptual process in invertebrates, although it is
1089 arguably the least cognitive aspect in that it may be driven primarily by
1090 characteristics of the receptors rather than by higher-order processing. Here we
1091 highlight several illustrative examples. Mantids are generalist predators that track
1092 prey by moving their heads. By monitoring their gaze direction in response to
1093 computerized visual stimuli, researchers have surmised that mantids do not store
1094 representations of prey, but rather respond more simply to a suite of stimulus features
1095 including size, contrast, relative speed, and direction [84e90]. Using an
1096 ophthalmoscope, Land [91] found that jumping spiders would rotate and scan their
1097 retinas across visual stimuli that bore angles resembling those of legsda potential
1098 method for identifying prey or conspecifics quickly and efficiently based on such
1099 element configurations. In other work, a predatory generalist jumping spider species
1100 did not significantly differ in its visual exploration of non-natural versus biologically
1101 relevant stimuli, whereas predatory specialists were found to spend more time
1102 focusing on smaller regions of interest [92]. Similarly, the mosquito-specialist
1103 jumping spider *Evarcha culicivora* uses a local processing approach, analyzing the
1104 orientation of each element of prey objects for identification, rather than relying on a
1105 global or holistic approach [93]. In contrast, *Polistes fuscatus* paper wasps were found
1106 to recognize individuals from images of conspecifics with particular facial markings
1107 [52] and respond more quickly to coherent faces than scrambled or incomplete
1108 images, suggesting a higher cognitive processing for conspecific faces rather than
1109 simple pattern recognition. Honeybees demonstrate the ability to categorize objects
1110 by learning elemental configurations and assemblies of these features, which transfers
1111 to novel stimuli [reviewed in Refs. [94,95]]. Future work in arthropods should
1112 capitalize on the rich diversity in visual systems, visual environments, and life
1113 histories in comparative studies.

1114 What objects do invertebrates expect to see? Top-down visual processing occurs
1115 in at least some invertebrate species. As in human infants, looking time protocols can
1116 be used to understand whether an animal's expectations about what it will see are

1117 supported or violated. Examples come from work on *Portia*, jumping spiders that
1118 specialize in eating other spiders. Paralleling work with infants [96], a spider is
1119 shown a stimulus, which is then hidden behind a barrier. Either the same stimulus or a
1120 new stimulus is then revealed. *Portia* hesitate before attacking a revealed object if
1121 inherent features (such as color) are changed from the original stimulus, but do not
1122 hesitate if the prey orientation is changed, suggesting that *Portia* is able to
1123 differentiate prey identity independently from orientation (i.e., changes in prey
1124 orientation do not induce an expectancy violation, while changes in its inherent
1125 properties do) [97]. Again, similar to infants [83], spiders spent more time looking at
1126 a display of prey if the number of prey was modified, but not if their arrangement was
1127 modified, suggesting that *Portia* is able to cognitively represent exact numbers of prey
1128 in at least a few discrete categories [98]. These looking-time techniques could likely
1129 be adapted to additional species.

1130 How do cross-modal stimuli influence object recognition? Contextual cues such
1131 as a stimulus in another modality can alter attention to objects in the environment. For
1132 example, jumping spiders interpret images of conspecifics presenting ambiguous
1133 morphological features as displaying either threat or courtship, depending on the
1134 presence of pheromone cues [99]. Another jumping spider species freezes in response
1135 to the sound of a predatory wasp [100], but sweep their retinas back and forth,
1136 possibly looking for the source of danger [101]. With the addition of precise gaze
1137 tracking, one could test how additional senses influence visual attention and object
1138 recognition: do animals presented with cross-modal cues attend to different features
1139 than animals without those cues?

1140

1141 **3. GAZE DIRECTION AND VISUAL SEARCH**

1142

1143 Visual search is the process by which an observer looks for a target object
1144 against a background array of other, potentially distracting, items [68,102]. The most
1145 commonly studied form of visual search in non-human animals involves foraging, a
1146 convenient analog to examples of visual search in the human literature (although new
1147 human search task designs are similar to foraging behavior; see Ref. [103]). Detecting
1148 camouflaged targets requires attentive search because such targets are rarely detected
1149 passively [104], and predator performance is known to improve with experience

1150 during search tasks for cryptic prey [105]. Commonly encountered food items are
1151 consumed at proportionally higher rates than random sampling would indicate
1152 [106e108]. This is compelling evidence that previous encounters with particular
1153 objects can enhance searchers' efficiency in locating conceived target objects. Precise
1154 measurements of gaze direction during visual search tasks offer unique insights. For
1155 example, gaze shifts during foraging can indicate if a cryptic target is detected and
1156 ignored, or undetected altogether. In this section, we will investigate how gaze
1157 direction might inform us about the visual search process and how search
1158 performance in humans and other animals might depend on both context and prior
1159 knowledge.

1160

1161 3.1. Gaze direction and visual search in humans

1162

1163 Many activities in our daily lives are comprised of search tasks, whether that
1164 is looking for a car in a parking lot or picking out a face in a crowd [68]. In the
1165 human psychology literature, visual search is usually tested experimentally by
1166 instructing subjects to find a target among distractors under a variety of conditions,
1167 and their performance (usually search time) measured (although emphasis has also
1168 been placed on real-world tasks, see Refs. [68,104]). A long line of different
1169 experiments have shown that performance in these tasks can range from parallel
1170 search, in which all items are attended to simultaneously, to serial search, in which a
1171 single item or a small number of items are attended to sequentially, with search rate
1172 on many tasks falling somewhere between these extremes [68]. Townsend [109]
1173 pointed out that slow search rates may reflect limited capacity parallel search rather
1174 than serial search.

1175 When humans engage in visual search, attention can be directed by a
1176 combination of stimulus-based bottom-up and goal-directed top-down mechanisms.
1177 Bottom-up guidance is demonstrated by rapid shifts of gaze to salient stimuli that
1178 'pop out' from the background [110]. However, when people view meaningful
1179 scenes, they are less likely to be distracted by 'low level' salient stimuli [13], and
1180 these stimuli can be ignored altogether in particularly complex scenes when they are
1181 too numerous to drive shifts in attention [111]. Suppression of attentional capture to
1182 distractors depends on target-nontarget relationships rather than similarity alone

1183 [112]. In most complex search tasks, bottom-up guidance interacts with topdown
1184 guidance, which directs attention toward stimuli with visual properties known to
1185 belong to the target [113].

1186 Visual search may be facilitated by additional top-down processes including
1187 those that consider contextual cues [114]. Attention is sometimes deployed to
1188 locations that were significant for a subject in the past [115] (see Ref. [116] for a
1189 review of the role of memory in visual search). A form of ‘repetition priming’ causes
1190 subjects to be attuned to features of the last object to which they were exposed,
1191 improving search performance [117]. Awh et al. [118] argued that these different
1192 findings could be attributed to three different factors in attentional control, which they
1193 labeled physical salience, current goals, and selection history. Their third factor is
1194 especially broad and includes searches that become more efficient when targets have
1195 been associated with reward. Wolfe and Horowitz [119] proposed a longer list, with
1196 five factors that included scene structure. Cross-modal cues can also influence visual
1197 search. For example, attention may be directed to objects in a scene that are paired
1198 with related odors [120] or sounds [121,122].

1199 Measuring search time and tracking eye movements have become particularly
1200 powerful tools for determining how humans allocate attention as they look for
1201 objects. Many models have been proposed to describe mechanisms that facilitate
1202 visual search that we have not space to discuss. For a more comprehensive review
1203 and historical context of research on visual search in humans, see Nakayama and
1204 Martini [110]. We also recommend that readers consult [102,123,124] and chapter 6
1205 in Ref. [68].

1206

1207 3.2. Gaze direction and visual search in invertebrates

1208

1209 How do distracting environments affect visual search performance?
1210 Invertebrates engaged in visual search tasks often encounter many ‘distractors’, such
1211 as prey that are unlikely to be captured [125] or flowers offering fewer rewards [126].
1212 Attentional processes are often required during visual search in distracting
1213 environments, especially when target and distractor objects are visually similar. Some
1214 invertebrate species, such as dragonflies, can effectively track the movements of a
1215 target individual in a swarm of flies without being distracted by the movements of

1216 other flies [125,127], and hoverflies selectively target moving objects that contrast
1217 highly with the background [128].

1218 While the search and pursuit for such targets typically relies on ‘low-level’
1219 sensory processes such as feature-detecting neurons [129,130], ‘high-level’ cognitive
1220 processes have been implicated in many pollinator species. Honeybees take longer to
1221 complete a visual task with more distractors [131], but can learn to ignore distractions
1222 with specific stimulus properties such as a particular color [132]. Even closely related
1223 species can differ in this regard, as bumblebees were significantly less affected by
1224 distracting objects than were honeybees (parallel versus serial-like search; see Ref.
1225 [133]). Such differences are assumed to link tightly to the individual ecology of each
1226 organism. As in humans, target saliency undoubtedly also influences search time. For
1227 example, larger flower targets are easier for bees to find among distractor flowers
1228 than are smaller targets, and bees use cues such as color and illumination to assist
1229 with their search [134]. Bees also exhibit speed-accuracy trade-offs during visual
1230 search, in which more time allocated to a search task improves performance, with
1231 individuals exhibiting stable differences in their strategies [135].

1232 Most of what we know about visual search in invertebrates derives from
1233 research with bees and other pollinators, largely because flower inspection offers a
1234 convenient measure of gaze direction, but it is unclear if and how these findings
1235 extend to other invertebrates. To what extent other invertebrates use parallel or serial
1236 search would be interesting for future work. Currently, our laboratory is investigating
1237 how jumping spiders search for objects in scenes with different numbers and types of
1238 distractors.

1239 How do observer goals influence visual search? Subjective states such as
1240 hunger and the need for shelter drive visual search behavior in animals [136,137].
1241 These physiological and behavioral states influence the neuromodulation of vision to
1242 a surprisingly considerable extent (see Ref. [138] and references therein). Many
1243 animals that engage in visual search must divide their attention to remain vigilant for
1244 predators [139]. For an example in vertebrates, foraging birds often engage in
1245 scanning behaviors in which they periodically lift their heads to look for predators
1246 (but see Ref. [140]). Some prey species have lateralized eyes and preferentially use
1247 one side for certain visual search tasks, such as scanning for predators or prey (e.g.,
1248 Ref. [141]). Desert locusts searching for resources periodically pause while

1249 locomoting, which enables them to scan their surroundings with head and body turns.
1250 The duration of the pause along with their associated head and body movements can
1251 be used to determine if the locust is engaged in local search or relocation [142]. If
1252 more studies monitor gaze direction during visual search, we could develop a better
1253 understanding of how variable search patterns indicate animals' motivation.

1254 Similar to humans, exposure to one type of target can 'prime' invertebrates'
1255 attention as they search for other visually similar targets. Priming describes a process
1256 in which exposure to a particular stimulus alters stored mental representations of an
1257 object, which in turn influences future perception [117] and prepares an organism to
1258 search for a target. Priming can influence the development of a search image or
1259 search template, which allows an organism to look selectively for specific features or
1260 locations of a target object [143], and to readily ignore non-matching stimuli (but see
1261 Ref. [144]). A mechanistic example can be found in dragonflies, where neurons
1262 selectively tuned for detecting small targets are more sensitive to movement
1263 occurring ahead of the target, thereby indicating attention is modulated by the
1264 location in which priming takes place [145]. Gamberale-Stille et al. [146] recently
1265 found that two generalist butterfly species improve their search efficiency for a host
1266 plant after sequential priming. This is important because generalist species might be
1267 disadvantaged at visual search compared to specialists because there are more
1268 potential targets that are relevant to them. Many generalist pollinators such as bees
1269 use search images to look for flowers [147]. While such studies have been
1270 successfully conducted with vertebrate predators such as birds [148e150], far less
1271 work has been done with invertebrate predators. One notable exception is with the
1272 jumping spider *Evarcha culicivora*, a species that feeds preferentially on blood-fed
1273 mosquitoes. Cross and Jackson [151] demonstrated that these spiders evoke a search
1274 image for prey during visual search after exposure to olfactory cues. Currently, our
1275 laboratory is investigating if jumping spiders that are primed with supplementary
1276 visual cues are able to locate target objects faster in a cluttered scene.

1277 How do cross-modal cues affect visual search? Most animals are exposed to
1278 cues from a variety of sensory modalities, each of which can provide additional
1279 information about their surroundings and thus help to direct visual search. In *Evarcha*
1280 *culicivora* jumping spiders, as mentioned previously, priming with the scent of blood
1281 or the color red enhances the speed with which they can find partially obscured target

1282 lures [152]. Flowers often present signals from multiple modalities which enables
1283 pollinators to effectively locate them within complex scenes. For example,
1284 bumblebees are able to find an inconspicuous target flower faster when it is paired
1285 with scent [153]. Floral scents alone can trigger bees to return to specific locations
1286 where the associated reward was found [154]. *Drosophila* require visual feedback to
1287 accurately localize an invisible odor source [155] and remain oriented in an odor
1288 plume while flying [156]. Some invertebrates must also search for freely moving
1289 target hosts. For example, when flying mosquitoes detect CO₂, they steer toward
1290 certain visual stimuli that would otherwise be ignored [157,158]. Parasitoid wasps use
1291 visual cues and vibratory cues synergistically to help locate target hosts, and therefore
1292 their target capture performance is enhanced with other cues that help localize or
1293 constrain visual search to a smaller area [159]. Future work should investigate if and
1294 how visual search performance changes after exposure to cues from mates, predators,
1295 or prey in various sensory modalities.

1296

1297 **4. GAZE DIRECTION AND LEARNING**

1298

1299 Many invertebrates are capable of learning and remembering visual information,
1300 whether it be learning about newly blossoming flowers over the course of a summer
1301 or the distinctive characteristics of conspecifics. Here, we pay particular attention to
1302 the use of gaze direction in both basic associative learning [160e162] and operant
1303 tasks [163e165]. We also examine social learning: invertebrates can learn from
1304 conspecifics in the contexts of task solving [166], mate selection [167], foraging
1305 decisions [168], and predator avoidance [170].

1306

1307 4.1. Gaze direction and learning in humans

1308

1309 While our visual worlds are dynamic, many aspects are predictable, making
1310 the ability to learn about them beneficial. In humans, memory is known to play an
1311 important role in guiding visual attention [171]. For example, visual attention is often
1312 deployed more to recently attended objects [172]. Conversely, some objects that are
1313 frequently present in a scene are no longer fixated upon, a process called visual
1314 habituation [173]. Although specific details of a scene are usually not stored and

1315 changes to objects often go unnoticed [174], memory of some visual information
1316 from scenes can persist over time [172]. Selective attention can also be modified
1317 during learning in such a way that allows the learner to better optimize their ability to
1318 discriminate categories of objects [175]. While conducting visual tasks, humans learn
1319 to form associations, and these associations can fine-tune gaze direction. For
1320 example, humans are more likely to allocate their overt attention to cues that are
1321 predictive of associative outcomes when compared to unpaired cues [176]. In natural
1322 contexts, such overt attention shifts are thought to result from a reward-based learning
1323 context in which favorable outcomes guide attentionda process that can even occur
1324 unconsciously [177].

1325 Humans also learn by watching and copying how others complete tasks, a
1326 process called social or observational learning (reviewed in Ref. [178]). As Menzel et
1327 al. [179] suggested, in observational learning an obvious reinforcing stimulus is
1328 absent, so observers should have an internal representation of how the behavior might
1329 benefit themselves. Humans are exceptional social learners and are particularly
1330 attentive to faces. Emotion, gaze direction, and facial expressions influence the
1331 memory of faces [180e182]. Humans also often follow the gazes of others [183]. In
1332 some experiments, the demonstrator uses their gaze direction to draw the attention of
1333 the observer to an object (object enhancement) or a location (local enhancement)
1334 [184]. Attention to gaze direction begins early in life: infants reliably follow others'
1335 gaze direction, attending preferentially to the object at which a model gazes [185].
1336 Gaze following has also been described for non-human vertebrates, including other
1337 mammals, birds, and reptiles (e.g., Refs. [183,186,187]).

1338

1339 4.2. Gaze direction and learning in invertebrates.

1340

1341 To which features do animals attend during an associative learning task?
1342 Many invertebrates can use visual cues during associative learning. Here we highlight
1343 where gaze tracking has been implemented successfully in three taxa that are known
1344 to be adept at learning.

1345 Stomatopods possess one of the most specialized visual systems of any
1346 animal group [41]. They can learn to recognize predators and conspecifics, identify
1347 their own burrows, and discriminate among colors (reviewed in Ref. [188]). Chen

1348 [189] showed that two stomatopod species performed differently in associative
1349 learning tasks that relied on learning geometric shapes, a difference possibly related
1350 to different predatory strategies. However, none of the previous experiments
1351 attempted to acquire detailed gaze direction measurements. Recently, Daly et al.
1352 [190] developed a technique that allows for well-controlled measurements of gaze
1353 direction. They found that stomatopod eyes can move independently of each other
1354 and simultaneously engage in different tasks. They were also able to demonstrate how
1355 the animals achieve gaze stabilization even with the additional challenge of three
1356 rotational degrees of freedom: torsion, pitch, and yaw [191,192]. This system is
1357 suitable for including gaze direction in studies of learning and discrimination.

1358 Another invertebrate group that uses visual and tactile cues during
1359 associative learning and operant conditioning, or learning from the consequences of
1360 their behavior, are the cephalopods [193]. For example, cuttlefish learn to
1361 discriminate among prey images and treat sketches and altered images of reduced size
1362 as equivalents when performing a task [194] (Fig. 2). They can also associate food
1363 with a flashing light [160] and have episodic-like memory to keep track of ‘what,
1364 when, and where’ they have eaten [169]. Octopuses are capable of operant
1365 conditioning [195] and use visual information to track and control the location of
1366 their arms when completing tasks [165]. A few techniques have been developed to
1367 track gaze direction in cephalopods. Recordings of cuttlefish with high-speed cameras
1368 showed that these animals can adopt different saccadic movements depending on the
1369 behavioral situation [196]. Levy and Hochner [197] tracked the inclination of
1370 octopuses’ eyes during locomotion and showed that the interaction between the arms
1371 and surrounding visual cues gives feedback regarding head orientation. A very
1372 promising tool for non-invasive eyetracking in *Octopus bimaculoides* has been
1373 recently developed using an IR camera and pose tracking (DeepLabCut; see Ref.
1374 [246]) [43]. These new techniques for monitoring head and eye position and the
1375 dexterity of cephalopod movements will improve our understanding of how gaze
1376 direction changes with experience with a task or when observing others, analogous to
1377 human research.

1378 Jumping spiders also use visual cues in associative learning [45]. These
1379 spiders learn to avoid distasteful prey [198], associate colors with food [161] or nests
1380 [199], and associate images with an aversive shock and vibration [162,200].

1381 Eyetracking during learning trials would help us ascertain how learning influences the
1382 allocation of attention to different visual cues. Of particular interest is how innate
1383 preferences for particular forms and movements [93,201,202] are modified by
1384 learning, and how spiders' attention to different parts of an object shift with
1385 experiences that work in opposition to innate tendencies. Eyetracking can also
1386 provide a more finely calibrated tool to measure the effort that spiders expend to
1387 make sense of confusing images. For example, de Agro et al. [59] showed that
1388 *Phidippus regius* spiders can learn to associate abstract images with a reward or
1389 punishment, but then fail to discriminate fragments of the same stimuli. If spiders that
1390 have learned about whole images examine image fragments in a different way than do
1391 naive spiders, we would have evidence that they recognize something is familiar. For
1392 a review of these and related issues in invertebrate learning, see Abramson and Wells
1393 [203].

1394 What do animals attend to during social learning? Observational learning
1395 has been described in a number of invertebrate taxa. In *Octopus vulgaris*, individuals
1396 can learn to perform reward-choosing tasks by watching other individuals [166,204].
1397 Social invertebrates like bees and wasps also engage in social learning [205]. For
1398 example, bumblebees can learn how to recognize rewarding flower colors after
1399 watching other individuals forage in an observational arena [168]. Dawson et al.
1400 [206] showed that flower preference can be driven by second-order conditioning. The
1401 observer first associates the conspecific with the presence of food, and then watches
1402 the conspecific forage on a particular color of flower. The observer then associates
1403 flower color with food even without direct experience with the flower. In another
1404 study, bumblebees even demonstrated cognitive flexibility during an observational
1405 learning task in which observers did not simply copy the behavior of the
1406 demonstrator, rather they improved upon it [207]. With precise measurement of gaze
1407 direction, it would be possible to test whether animals watching a conspecific attend
1408 particularly to the conspecific's interaction with the object of interest, as in the
1409 phenomenon of object enhancement described in the psychological literature, and
1410 whether experienced social learners are more likely to direct their gaze to the most
1411 informative part of the scene.

1412 Non-social invertebrates can also perform observational learning.
1413 *Drosophila melanogaster* females watch other conspecifics copulate and use this

1414 learned information to select their own mates [167]. Wood crickets exhibit social
1415 learning in predator avoidance [170]. Despite the small number of reported cases
1416 compared to social invertebrates, these examples emphasize how the implementation
1417 of gaze direction measures could be helpful when investigating the cues to which
1418 observers attend, and how these cues differ between social and non-social animals
1419 given the substantial difference in their lifestyles.

1420

1421 **5. GAZE DIRECTION AND NAVIGATION**

1422

1423 Navigation, a complex task that is essential for many invertebrates,
1424 requires learning both visual landmarks and proprioceptive cues. We will examine
1425 how tracking gaze direction can be informative for studying navigation tasks such as
1426 homing (reviewed in Ref. [208]) and route planning [209,210]. In some invertebrate
1427 taxa, navigational abilities have been well studied, especially social insects (e.g.,
1428 Refs. [205,211]); cephalopods (e.g., Refs. [212,247]); and to a lesser extent, spiders
1429 (reviewed in. Ref. [45]).

1430

1431 **5.1. Gaze direction and navigation in humans**

1432

1433 The ability to shift our gaze is essential for daily tasks in our lives such as
1434 moving around obstacles and traveling to and from particular locations. While
1435 walking, humans precede changes in direction with head and eye movements [213]
1436 and learn to fixate on objects with which they may potentially collide (reviewed in
1437 Ref. [214]). However, while navigating cluttered environments, humans do not
1438 always fixate on all obstructing objects but rely on peripheral vision as well [215].
1439 Using an assay that employed virtual reality, Andersen et al. [216] found that humans
1440 tasked with navigation fixate on visual landmarks. To select these landmarks, humans
1441 use cues including visual and structural salience, which are characteristics of
1442 landmarks that are visually conspicuous or immediately related to navigation,
1443 respectively. The intention to learn a route leads to more gaze shifts to landmarks at
1444 structurally salient locations along a route, while gaze to more visually salient
1445 landmarks occurs regardless of learning intention [217].

1446

5.2. Gaze direction and navigation in invertebrates

How can gaze direction improve our understanding of navigation in invertebrates? Many species must travel long distances to nest sites after foraging, and the ability to learn the arrangement of landmarks around their nests plays an important role in navigation and homing [179]. Octopus and cuttlefish use landmarks to orient and guide them through mazes [218,219]. Jumping spiders orient toward familiar beacons when returning to nest sites [199]. Navigating ants, bees, wasps, and many other invertebrates orient their gaze to match their view of a scene to memory of familiar scenes [208]. As Tinbergen [220] famously described, sometimes insects perform learning flights or walks close to their nest to learn a visual representation of the surrounding environment to guide their return after foraging.

Considering the diversity of the environments in which animals must navigate, it is not surprising that there are different strategies to optimize information filtering and processing [18,221]. The measurement of gaze direction can give us a better understanding about what specifically animals learn from these flights and walks and how they acquire this information [222], and a number of studies have done just that. Usually learning flights or walks start with the animals leaving the nest and rotating around to obtain a frontal view of the nest entrance. After that, they move along arcs, sometimes pivoting around the nest, while performing saccadic movements [223]. By tracking the gaze and body position of wasps, Stürzl et al. [39] showed that learning flights occur in a coneshaped formation allowing the animals to control the gain of horizontal distance in a fairly constant rate as they gain height above ground. When returning to the nest, wasps perform a predictable flight maneuver when they encounter the previously memorized view of their nest. In bumblebees, changes in gaze direction also occur with short and fast head turns, reducing image rotation to short time intervals. Boeddeker et al. [224] found that these saccadic movement patterns are very similar to those known for vertebrates including humans. For ants, learning walks are also a common homing strategy; however, Fleischmann et al. [225] found the pivoting and saccadic movements can be species-specific, with ants that live in a visually richer environment exhibiting a larger repertory of behaviors during the learning walk. Baddeley et al. [226], by analyzing panoramic images generated from the perspective of the ant, found that

1480 ants use scene familiarity during navigation even in the absence of distinct landmarks.
1481 Using anesthetic injection into the mushroom body, an area associated with learning
1482 and memory in arthropods, Kamhi et al. [227] showed that, in ants, this region is
1483 necessary for retrieving visual memories. Ants treated with anesthesia showed no
1484 preference in their gaze direction to environmental cues compared to ants with
1485 functional mushroom bodies, which oriented their gaze preferentially to visual
1486 landmarks.

1487 How can gaze direction improve our understanding of route planning in
1488 invertebrates? Analyzing gaze direction might help us understand how animals plan
1489 routes. Jumping spiders from the subfamily *Spartaeinae* specialize in preying on
1490 other spiders, and approach their prey via complex routes, including reversed-route
1491 detours when the prey is out of sight [55,209,210,228]. For example, *Portia africana*
1492 decides when to use a detour or not based on the goal objects it sees before making a
1493 plan, selecting a shorter route when presented with a prey rather than a non-prey goal
1494 [229]. By monitoring cephalothorax orientation and thus gaze direction, Taristano and
1495 Andrew [221] showed that *Portia labiata* first scan all possible routes leading away
1496 from the target, but then narrow their attention to a complete route that leads back
1497 towards themselves. Spiders do not always try to solve a complex detour all at once,
1498 but rather appear to establish secondary objectives along the route, thereby solving
1499 the detour in small blocks. *Spartaiene* spiders are exceptionally good at navigating
1500 detours, whereas nonspartaeine salticid species that typically prey on insects vary in
1501 this ability. For example, for *Marpissa marina* visual cues seem important when
1502 planning a route, whereas *Trite planiceps* typically prefer shorter routes [230], and
1503 *Phidippus audax* fail to complete reversed route detours at all [231]. With careful
1504 measurements of gaze direction across species, we might be better able to understand
1505 the proximate sources of interspecific variation in detouring success [221].

1506

1507 6. CONCLUSION AND FUTURE DIRECTIONS

1508

1509 Our goal here has been to make the case that the rich psychological
1510 literature on human gaze direction deserves more attention, and its methods more
1511 direct application, by students of invertebrate behavior and cognition. Gaze direction
1512 measurements are rich in information, and they can be used to form inferences about

1513 cognitive functions that are either not apparent in other behaviors or are difficult to
1514 discern in the measurements of neural activity currently possible. Because eye
1515 movements and the underlying neural control of gaze direction of invertebrates
1516 evolved separately from that of vertebrates [17], identifying parallel and divergent
1517 aspects of the rules underlying the control of gaze direction will be informative for
1518 future research. While we mostly focused on similarities between findings in humans
1519 and invertebrates, we also expect fundamental differences. Within invertebrates, the
1520 potential for comparative studies is rich, given the sheer diversity of species and
1521 visual systems, the availability of closely related species that are in very different
1522 visual habitats, the availability of distantly related species facing similar visual
1523 challenges, and our growing understanding of underlying neural structures that
1524 influence both cognitive processes and visual processing (e.g., Refs. [232,233]).

1525 Precise measures of gaze have started to provide surprising insights into
1526 animal behavior that would be otherwise difficult to come by. Eyetracking reveals
1527 that peahens direct their gaze only to the lower third of a peacock's display, while the
1528 extravagant feathers that are not being fixated upon might instead serve to capture the
1529 attention of females from afar [33]. Male *Habronattus pyrithrix* jumping spiders
1530 present an elaborate courtship display that includes 'knee pops' and flicks of the
1531 upraised tarsi. Eyetracking shows that females direct their gaze at the pops.
1532 Moreover, tarsal flicks do not usually attract the gaze, but rather stimulate the anterior
1533 lateral eyes to direct the principal eyes to the center of the display (unpubl. data). It
1534 would be valuable to study whether females differ in how they attend to male
1535 displays, as such variation can have consequences for sexual selection (see Ref. [234]
1536 for a review).

1537 However, numerous operational challenges remain. First, the visual system
1538 of the study organisms must be well understood. For example, even across vertebrate
1539 species, there is enormous variation in the presence and shape of retinal areas with
1540 higher spatial acuity (e.g., Refs. [16,235]). At a minimum, one must understand the
1541 field of view of the eyes. Within compound eyes, the positions of different ommatidia
1542 makes them suitable for different behavioral tasks [236,237]. Second, devising ways
1543 to measure gaze direction can be difficult. Accurate measures of head direction may
1544 require high-speed cameras positioned at different angles (e.g., Refs. [39,238]), and,
1545 as we previously described, species with eyes that move independently from their

1546 bodies offer particular problems. Cross-disciplinary collaborations, as our lab had
1547 with optics designers [48,239], are often necessary.

1548 A promising potential technique for future work will be to combine
1549 measure of gaze direction with virtual reality (VR). VR systems have been designed
1550 for jumping spiders [240], *Drosophila* [241], and honeybees [242] (Fig. 3). To our
1551 knowledge, no invertebrate studies have explicitly tracked dynamic changes in gaze
1552 direction while an animal is engaged in VR. We believe tracking the gaze direction of
1553 freely moving animals in VR systems such as FreemoVR [243] will be a powerful
1554 technique. A closed-loop system that updates with animal choices might offer great
1555 insight into how animals use their vision to explore scenes and to complete behavioral
1556 tasks.

1557 Given the dearth of studies investigating bottom-up versus topdown
1558 mechanisms underlying visual processes in invertebrates, we hope to encourage more
1559 research in this area. Recent studies have further supported the view that bees in
1560 particular demonstrate exceptional plasticity in visual processing [244], and how this
1561 occurs in the brain is an area of active research [245]. Embracing a comparative
1562 approach will provide novel insights for visual cognition research in humans and
1563 invertebrates alike.

1564

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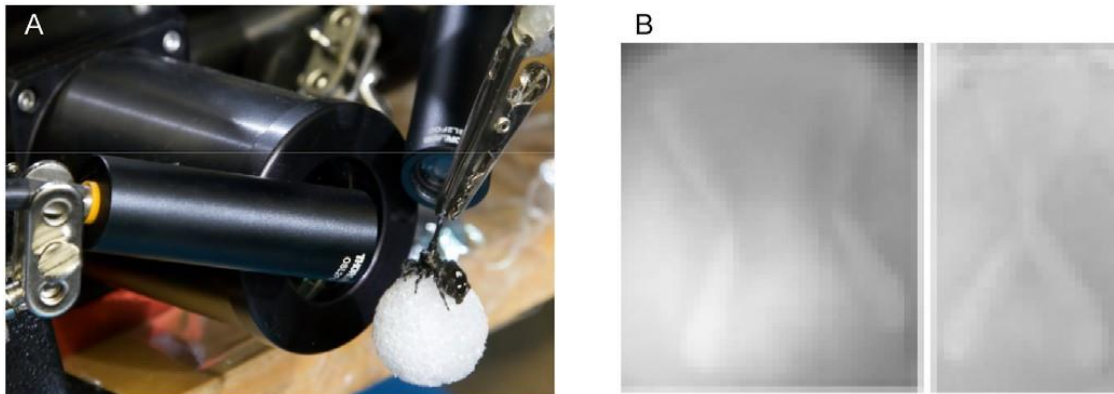
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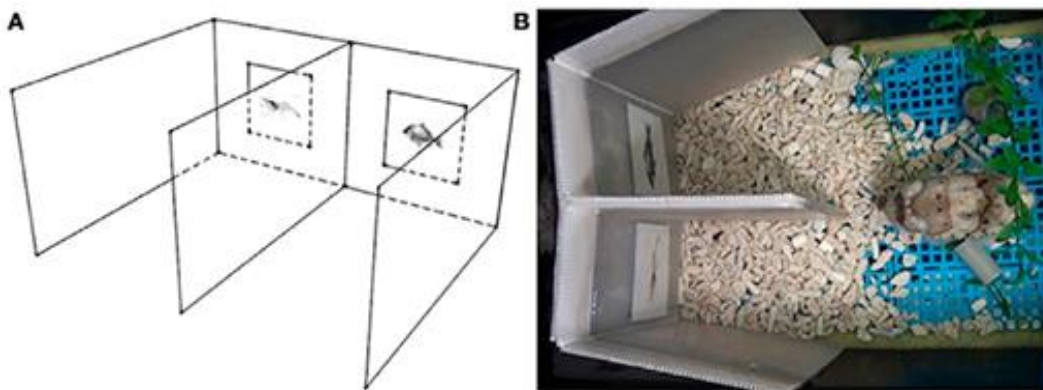
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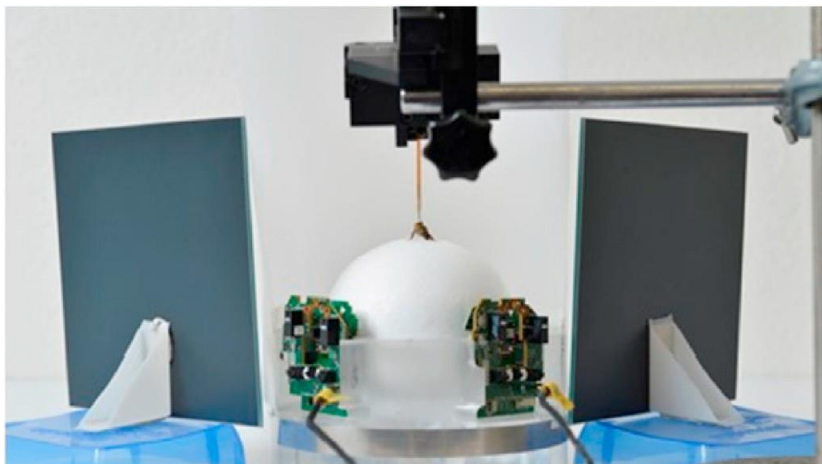
2260 **FIGURES**



2261
2262 Fig. 1. (A) A tethered jumping spider looking at an image in the eyetracker. The retinas are illuminated by IR light, which
2263 penetrates the carapace. (B) Reflections of the boomerang-shaped retinas as captured by an IR camera. On the left, the retinas are
2264 in relaxed position; on the right, the spider is examining a stimulus and has directed both retinas to it
2265
2266



2267
2268 Fig. 2. A schematic (A) and photograph (B) of an experimental setup for examining cuttlefish choice. The cuttlefish can view
2269 both options at the decision point. Reproduced from Ref. [194].
2270



2271
2272 Fig. 3. A honeybee in a virtual reality experiment (reproduced from Ref. [242]).
2273

2274 **CHAPTER 4**

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2276

2277 **DANGEROUS ATTRACTION: RISKS AND BENEFITS OF PHEROMONE-**
2278 **INDUCED BEHAVIORAL STATE CHANGES**

2279

2280 **ATRAÇÃO PERIGOSA: RISCOS E BENEFÍCIOS DAS MUDANÇAS NO**
2281 **ESTADO COMPORTAMENTAL INDUZIDAS POR FEROMÔNIOS.**

2282

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2301 **ABSTRACT**

2302 Exposure to ecologically relevant sensory cues can alter an animal’s behavioral state.
2303 Previous work across taxa has shown that exposure to female pheromones can lead
2304 males to increase mate-searching behaviors. However, this behavioral state change
2305 might involve risks through reduced general attention. Here we investigate the risks
2306 and benefits of pheromone-induced state changes in the jumping spider *Phidippus*
2307 *audax* (Araneae: Salticidae). We hypothesized that males exposed to female
2308 pheromones will increase courtship behaviors but will be less responsive to predatory
2309 cues. To investigate courtship behaviors, we tested whether pheromone-exposed
2310 males are faster to detect, and more likely to vigorously court, a female image relative
2311 to control spiders. We found that pheromone-exposed males have a lower latency to
2312 court and presenting more attempts to court once the female is detected. To
2313 investigate the effect of pheromones on responsiveness to predatory cues, we tested if
2314 spiders were less responsive to a predatory wasp buzz sound. We found that, contrary
2315 to predictions, pheromone-exposed spiders were more likely to freeze in response to
2316 predator sounds. We also predicted that pheromone-exposed spiders that were
2317 inspecting a conspecific image would be less likely to redirect their gaze to a looming
2318 stimulus that might indicate danger. Using a specialized eyetracker, we found that all
2319 spiders, regardless of treatment group, looked at the looming stimulus. Thus, our data
2320 suggest that pheromone exposure increases courtship effort and potentially mating
2321 success without reducing attention to predator cues in either the visual or auditory
2322 modality, and in fact, pheromone exposure increases the responsiveness of spiders to
2323 predator cues.

2324 Key – words: Selective attention; Mate-searching; Arousal; Predation risk; Decision
2325 making

2326 **RESUMO**

2327 A exposição a sinais sensoriais ecologicamente relevantes pode alterar o estado
2328 comportamental de um animal. Trabalhos anteriores demonstraram que a exposição A
2329 feromônios de fêmeas pode levar os machos a aumentar os comportamentos de

2330 procura por parceiras sexuais. Entretanto, esta mudança de estado comportamental
2331 pode envolver riscos através da redução da atenção geral. Aqui investigamos os riscos
2332 e benefícios das mudanças de estado induzidas por feromônios na aranha *Phidippus*
2333 *audax* (Araneae: Salticidae). Hipotetizamos que os machos expostos às feromônios de
2334 fêmeas aumentariam os comportamentos de cortejo, mas serão menos sensíveis aos
2335 sinais predatórios. Para investigar os comportamentos de cortejo, testamos se os
2336 machos expostos a feromônios são mais rápidos para detectar, e mais propensos a
2337 cortejar vigorosamente, uma imagem de fêmea do que machos do grupo controle.
2338 Descobrimos que os machos expostos a feromônios têm uma latência menor para
2339 cortejar e apresentam mais tentativas de cortejar uma vez que a fêmea é detectada.
2340 Para investigar o efeito dos feromônios na resposta a sinais predatórios, testamos se
2341 as aranhas eram menos sensíveis a um som de zumbido de vespa. Descobrimos que,
2342 ao contrário das previsões, os machos expostos a feromônios eram mais propensos a
2343 apresentar *freezing* em resposta a sons predadores. Também previmos que os machos
2344 expostos a feromônios que estavam inspecionando uma imagem específica seriam
2345 menos propensas a redirecionar seu olhar para um estímulo que poderia indicar
2346 perigo. Usando um eyetracker especializado, descobrimos que todas os machos,
2347 independentemente do grupo de tratamento, olhavam para o estímulo que se
2348 aproximava. Assim, nossos dados sugerem que a exposição a feromônios aumenta o
2349 esforço de cortejo e o sucesso potencial do acasalamento sem reduzir a atenção a
2350 sinais predadores na modalidade visual ou auditiva e, de fato, a exposição a
2351 feromônios aumenta a capacidade de resposta das aranhas a estímulos predatórios.

2352 Palavras-chave: Atenção seletiva; Busca por parceiros sexuais; Excitação; Risco de
2353 predação; Tomada de decisão

2354 INTRODUCTION

2355 Trade-offs between different activities are widely known in animal behavior
2356 (Danchin et al 2008). A classical one is mating vs getting preyed upon (Cooper 1999),
2357 in which males engaging in mate-searching behaviors can become more conspicuous
2358 and more likely to be attacked (Magnhagen 1991). As a consequence, the mortality
2359 during mate searching may be up to 80% in some species (Vollrath 1980; Andrade

2360 2003; Kasumovic et al. 2007; Scott et al. 2019). Trade-offs are, however, less
2361 investigated when it comes to the role that attention plays in general and particularly
2362 in mate searching (Hebets 2005; Shettleworth 2010; Bagheri et al. 2020). On the
2363 benefits side, males exposed to female pheromones recognize faster images with
2364 female features (Tedore and Johnsen 2013). It also can increase males' speed to
2365 explore the environment and consequently find the female faster (Ram et al. 2007).
2366 Finally, male spiders searching for mates can change the strategy after contacting
2367 female pheromone and follow other male's dragline as a shortcut to encounter the
2368 female (Scott et al. 2019). Though exposition to female cues seem to help securing
2369 mating, there may be costs to it: when performing mate-searching behaviors, the
2370 remaining attention resources to unrelated tasks could be limited. In situations where
2371 the information received exceeds an animal's processing ability, it might cause some
2372 relevant information to remain unprocessed, leading to several critical risks (Dukas
2373 and Kamil 2000; Dukas 2004). Simultaneous behavioral tests have shown that the
2374 probability of encountering/detecting a given stimulus could be increased while the
2375 probability to respond or detect a secondary stimulus is decreased (Dukas 2004). For
2376 example, blue jays were less effective to detect peripheral potential predator cues
2377 when doing a visual task (Dukas and Kamil 2000). Trained blue jays focused on
2378 cryptic prey even in situations when non-cryptic food was available (Dukas and
2379 Kamil 2001). Bumblebees multitasking and learning a pollen-color association
2380 presented more errors when choosing a reward flower simultaneously with collecting
2381 nectar (Muth et al. 2017). Female crickets can recognize and filter between several
2382 male calls even when there is background sound, but they take more time to
2383 recognize the calls (Nityananda 2016). Considering that mate-searching behaviors are
2384 being triggered by the pheromone exposure, pheromone might cause a change on the
2385 focus of the animals to certain environmental cues, which should reduce general
2386 attention (Dukas 2004). Ultimately, such selective attention may lead animals not to
2387 pay attention to important cues, such as those of predators. This subject is not always
2388 easy to study in invertebrates because attention may be hard to define operationally,
2389 requiring special machines to score gaze direction, for example.

2390 Jumping spiders in general are good models for cognitive studies in general
2391 (Cross and Jackson 2013; Jakob and Long, 2016; Aguilar-Arguello and Nelson,

2392 2021). They can perform several complex tasks and use different kinds of sensory
2393 cues such as auditory, visual, and chemical (see Aguilar-Arguello and Nelson, 2021).
2394 Just like other spiders, we can use females' silk to increase male arousal, presumably
2395 via pheromones (Gaskett 2007). In the jumping spider *Phidippus audax*, we observed
2396 that males increase exploratory behaviors after exposure to chemicals from female
2397 silk (Winsor A, unpublished data). Because of a presumable increased attention, we
2398 asked if the changes caused by exposure to female pheromones promoted mating
2399 benefits. We tested if males exposed to female pheromones would start courtship
2400 faster and attempt to court more. We were also interested in the costs of such
2401 exposure. We tested if males exposed to female pheromones would be less attentive
2402 to potential predator cues. We investigated this question by simulating auditory and
2403 visual potential predator cues, the latter with a special device that allows scoring
2404 where the retinas of the principal eyes of a jumping spider are focusing on.

2405 **MATERIAL AND METHODS**

2406 **Experimental subjects**

2407 For all experiments we used adult male *Phidippus audax* (Hentz 1845) jumping
2408 spiders. We collected them using sweep nets and by hand with vials from fields and
2409 structures in Hampshire County, MA, USA. For housing, we kept the spiders in
2410 individual plastic boxes (18×13×10 cm), with a wood stick, a hollow black tube and
2411 plastic foliage for habitat enrichment (Cardducci and Jakob 2000). We kept the
2412 temperature at 25°C with a 16 :8 h light: dark cycle. We fed the spiders twice a week
2413 with crickets (*Acheta domesticus*) and provided them with water *ad libitum*.

2414 **Procedure to prepare the pheromone treatment**

2415 To expose males to female pheromones, we used the protocol detailed below
2416 for the first two experiments. We first separated adult female spiders in plastic cages
2417 (18×13×10 cm) and fed them a cricket. One day after feeding, we transferred the
2418 female spiders into different individual circular plastic boxes (15 cm diameter x 15
2419 cm high) lined with filter paper substrate for 24 hours before starting a trial. To avoid

2420 inadvertent pheromone exposure, we kept the plastic boxes in a different room from
2421 the male spiders for at least 24 hours with temperature at 25°C and a 16 : 8 h light :
2422 dark cycle. The female spiders were used only once for each experiment and
2423 randomly assignment to a male spider treatment. To create a control group, we used
2424 the same procedure but did not place a female in the pheromone chamber. For the
2425 experiment we assigned the male spiders to be in the treatment or control group by
2426 giving them an individual cage containing a filter paper impregnated with
2427 pheromones via silk dragline deposits or a control filter paper. After 30 minutes in
2428 contact with the substrate, the males were ready to be used in a single experimental
2429 trial.

2430 For experiment III, which was conducted with the eyetracker, we used a
2431 different protocol. Following Jakob et al (2018) and Bruce et al. (2021) we tethered
2432 spiders using a plastic dental disposable micro brush (Easyinsmile, Passaic, NJ, USA)
2433 attached to the cephalothorax using a 1:1 mixture of gum rosin (Acros Organics,
2434 Fairlawn, NJ, USA) and beeswax (Stackich Inc., Troy, MI, USA). After having the
2435 spider tethered, we gave one polystyrene ball to support their legs. The spider could
2436 move the ball freely and deposit its dragline during a 24-hour period. After the
2437 procedure with the female to obtain the pheromone ball, we repeated the same
2438 process to tether the males to a wax hat and they were subsequently aligned in the
2439 eyetracker. Therefore, the male stood on a pheromone-infused polystyrene ball
2440 impregnated with a female silk throughout the test.

2441 **Courtship advantages**

2442 The experiment goal was to test if pheromone-induced males have courtship
2443 advantages over the control spider. To test those potential advantages, we
2444 investigated if males start courtship faster and for longer durations. For this
2445 experiment, we used a rectangular arena made of foam core (15 cm X 9.5 cm X 11
2446 cm height) with an Apple iPod (generation 5; Apple Inc., Cupertino, CA) on the
2447 ending wall. To test that we used 40 male spiders divided equally in the treatment and
2448 the control groups. We conducted all trials in a room under full spectrum lights
2449 (Philips 20W 24 in T12 Daylight). We transferred the spider to the arena, and it was

2450 positioned on the opposite side of the iPod and left to acclimate for 3 min inside a
2451 transparent cup before we started trials. Each trial consisted of a video of a female
2452 image (Figure 1) with global movements around the screen for 1min. A global
2453 movement is such that the entire image moves in synchrony, so that body parts do not
2454 move relative to other body parts. We then scored the latency for males to start
2455 courtship and the duration of it. We considered a male started courting when it raised
2456 legs I and displayed latero-lateral movements. Courtship stopped when the male
2457 moved the body to another direction or stopped courting.

2458 **Response to a predator sound**

2459 The goal of this experiment was to test if pheromone-exposed spiders would
2460 present different reactions to a predator sound when moving around an arena. To test
2461 that, we used 40 male spiders divided in two groups: the treatment and the control.
2462 We used individual circular arenas (15 cm diameter x 15 height), and a mini speaker
2463 (Sony - SRS-X11) 20 cm above the arena on a separated substrate to avoid seismic
2464 transmission through the substrate. We used a recording of a wasp sound (e.g.
2465 Shamble et al. 2016). Each trial consisted of playing the wasp sound for 2 seconds
2466 after a 1 min period of acclimatization. The wasp sound was played 5 times with 1
2467 min intervals. We scored if the spiders froze or not when the sound was played and
2468 how many times during the whole trial (see Shamble et al. 2016). We investigated not
2469 only the first response to the sound, but also the potential difference in habituation,
2470 that is, we scored the response in all 4 successive trials.

2471 **Distraction when eye scanning**

2472 *Use of the specialized eyetracker* -For this experiment we used a customized spider-
2473 specific eyetracker (Canavesi et al., 2011; Jakob et al., 2018) to visualize the position
2474 of the spider's principal eye retinas as they watch stimuli. The eyetracker is a
2475 modified type of ophthalmoscope, that was inspired by the work of Land (Land,
2476 1969a,b; Land and Nilsson, 2012). Spiders can watch videos or images that are
2477 projected through the eyetracker while the position of their retinas are recorded with
2478 an infrared camera. More details of the procedure can be found in Jakob et al. (2018).

2479 We observed the stimulus presentation and the retinal position simultaneously in a
2480 separate window in real time on a computer monitor and recorded it. For scoring the
2481 experiments, the videos of the retinal movement were superimposed on and aligned
2482 with the stimulus videos. Thus, we knew exactly where the retinas were scanning
2483 according to the video length.

2484 *Distractor experiment* - This experiment's goal was to test if spiders become less
2485 attentive to potential predator visual cues when scanning a conspecific image if they
2486 are exposed to pheromones. To investigate that, we used 27 male spiders divided in a
2487 pheromone exposed (N = 13) and a control groups (N = 14). In contrast to the
2488 previous experiment, we adapted the pheromone-induced protocol for the eyetracker
2489 experiment. Male spiders were tethered using a similar tethering protocol explained
2490 for getting the female pheromone for experiment III. After tethering the male spiders,
2491 we gave a polystyrene ball impregnated with pheromone or a control ball with no
2492 pheromone for them to stand on. After that, we applied the calibration routine
2493 described in Jakob et al. (2018), making sure the spiders could visualize all the fields
2494 of view we were using in the trials. We then presented a grey scale female image
2495 stimulus created using the software Processing (v2.2) (Figure 1) at the center of the
2496 spider's visual field. From there we watched the spider's principal eyes retinas on the
2497 computer screen. We waited until the retinas started to actively scan the female image
2498 with back-and-forth and twisting movements, then we keystroke to trigger the
2499 presentation of the looming distractor stimulus in an area of the screen that was
2500 visible only to the anterior lateral eyes (ALEs). The looming stimulus increases in
2501 size for 1 second and then were motionless (similar protocol from Bruce et al. 2021).
2502 We scored if the retinas moved away from the female image in the direction of the
2503 distractor stimulus or not.

2504 **Statistical analyses**

2505 *Courtship advantages* - We used the software JAMOVI 1.1.9 for the statistical
2506 analysis. For latency to start courtship we first applied a Shapiro-Wilk normality test,
2507 and because our data was non-parametric we applied a Mann-Whitney test. For the

2508 number of attempts to court we applied a chi-square considering animals that courted
2509 or did not court.

2510 *Response to a predator sound* - For the 1st trial with the buzz sound, we ran a 2x2 chi-
2511 square test to test for differences in the response, considering freeze (1) or ignore (0)
2512 as response variables. We also ran a 2x2 chi-square comparing the responses from
2513 the 1st trial and the 5th trial to verify if the animals are responding different and
2514 comparing it with the GLMM.

2515 We tested habituation to the sound with a GLMM test. We applied a
2516 generalized linear mixed model (GLMM, package lme4) with a binomial structure
2517 (logit-link). All the response variables were either 1 or 0 (if the responses occurred or
2518 not, respectively). Trials were included as fixed effects and individual ID as a random
2519 effect to account for repeated measures.

2520 *Distraction when eye scanning* - No statistical procedure was applied since all
2521 animals from treatment group (N = 20) and from control group (N = 20) all looked to
2522 the distractor (100%).

2523 **RESULTS**

2524 **Courtship advantages**

2525 In the analysis of latency to start courtship after detecting the female image,
2526 we found that animals from the treatment group started a courtship in a median of
2527 7.5s after detection (max = 24 s; min = 2 s). Animals from the control group started a
2528 courtship in a median of 17s (max = 51 s; min = 5 s). We found that males that were
2529 exposed to female pheromone, treatment group, had a lower latency to start a
2530 courtship after detecting a female image (U = 95.0; p = 0.005) (Figure 3A).

2531 After detection, animals in the treatment group (N = 20) attempted to court a
2532 female image on the screen 11 times, whereas individuals in the control group (n=20)
2533 engaged in courtship only 4 times ($x^2 = 6.40$; $df = 1$; $p = 0.011$) (Figure 3B).

2534 **Response to a predator sound**

2535 We considered the first trial to compare freeze response between the groups,
2536 considering that it would be the first encounter with the predator. Animals from the
2537 treatment group (N = 20) responded in 14 out of 20 opportunities whereas animals in
2538 the control group responded only in 6 out of 20 opportunities ($x^2 = 6.4$; $df = 1$;
2539 $p=0.011$) (Figure 2 - trial 1).

2540 For the habituation tests, we found a general tendency to respond more in the
2541 1st trial from both groups (N = 40 with 20 responses) compared to the 5th trial (N = 40
2542 with 6 responses) ($x^2 = 11.16$; $df = 1$; $p = 0.008$), but we did not find an habituation
2543 pattern comparing the difference between trials in general (Std = -0.774; z value = -
2544 1.021; $p = 0.307$). However, when considering trials-treatment-response, we did find
2545 a difference for the treatment group, with spiders responding more in general (std = -
2546 0.840; z value = -2.616; $p = 0.0089$) (Figure 2).

2547 **Distraction when eye scanning**

2548 All animals from both the control (n=14) and treatment (n=13) groups looked
2549 away when the looming oval stimulus was presented, showing no difference in
2550 attention between groups to detect peripheral cues.

2551 **DISCUSSION**

2552 We found that males of the jumping spider *Phidippus audax* previously
2553 exposed to female chemicals responded more to predator sounds, court more and with
2554 lower latency and did not respond less to distractors than spiders in the control group.

2555 Courting more and faster after being exposed to female cues shows that cues
2556 of the opposite sex not only trigger sexual behaviors, but also enhance them. This is
2557 probably an advantage in terms of reproductive success (Elias et al. 2010) but it often
2558 comes at a cost (Andrade 2003). According to previous studies, searching for mates
2559 and defending against predators are often conflicting activities, since mate searching
2560 requires exposure, leading to an increase in predation rate (Magnhagen 1991;
2561 Andrade 2003). Indeed, males exposed to female cues have been reported to respond
2562 less to predators' cues than males not aroused by female cues (Ram et al. 2007). Our
2563 results contradict this idea, since we found that males exposed to female chemicals
2564 actually respond more to predators. Therefore, selective attention does not seem to
2565 explain our data, but rather we may be facing a case of an atypical overall increase in
2566 arousal. A possible explanation is that sexual cannibalism is a common practice in
2567 jumping spiders (Cross et al. 2008; Taylor & McGraw 2013; Lietzenmayer et al.
2568 2013). Therefore, being sexually aroused may also require not being preyed upon by
2569 the female, which may result in increased vigilance as a whole. This may be within
2570 the pool of strategies animals use to make mating decisions when they are under
2571 potential predation risk (reviewed in Lima and Dill, 1990). Moreover, in our
2572 particular case, wasps in nature may eavesdrop female signals to males on their
2573 draglines (Fei et al. 2023), which would justify increased vigilance in males.

2574 Another unexpected and interesting result is that males always looked at the
2575 looming distractor irrespective of being or not exposed to female chemicals. We were
2576 expecting that the pheromone-induced state would cause reduction in the general
2577 attention level since the increasing in mate-searching behaviors would be taking most
2578 of the processing resources (Dukas 2004). Animals with small brains are expected to
2579 have even more declared consequences of limited attention, due to the potential
2580 reduced processing power (Menzel and Giurfa 1999; Chittka and Niven 2009).
2581 However, we did not find any reduction in the attention level at least for the tasks we
2582 tested, showing that jumping spiders' attention in this case seems not to be limited to
2583 their brain size or cognition (e.g. Japyassu and Laland 2017; Cross et al. 2020). It also
2584 corroborates the idea that exposure to female pheromone could be increasing the
2585 attention level as a whole, and not limiting it as would be expected according to the
2586 traditional idea of limited attention and brain processing (Dukas 2004).

2587 Why would salticid differ from most taxa? These spiders have a pair of
2588 principal eyes with high spatial resolution, which we have monitored in our
2589 experiments. The fact that it is looking at stimuli in nature (or the distractor in our
2590 experiment) does not mean it is not aware of movement of the female it was focusing
2591 on (Jakob et al. 2018). This is because the lateral eyes are motion detectors, and these
2592 are active irrespective of where the principal eyes are looking at (Zurek and Nelson
2593 2012; Morehouse et al. 2017; Jakob et al. 2018). This important difference about gaze
2594 direction when comparing jumping spiders with other animals may explain our results
2595 (Winsor et al. 2021). The costs of looking a distractor are probably lower for jumping
2596 spiders than for animals with two eyes (Morehouse et al. 2017). Should the female
2597 move, the lateral eyes will detect such movement even if the principal eyes are
2598 looking at a distractor.

2599 Jumping spiders may be an exception in classical examples of selective
2600 attention due to specificities of their life history (sexual cannibalism) and sensory
2601 capabilities (multiple eyes with distinct functions). We should also mention that, in
2602 jumping spiders, the neural connection between the optic neuropils allows rapid
2603 integration of the information and consequently a quick response, differently from
2604 other groups of spiders that have fewer connection between those neuropils (see
2605 examples in Long 2016, 2021). The relative importance of these three factors could
2606 be tested in arachnids with no sexual cannibalism, with those having a single pair of
2607 eyes and/or with spiders with a different brain organization, with less complex
2608 communication between the neuropils.

2609

2610

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FIGURES

A.

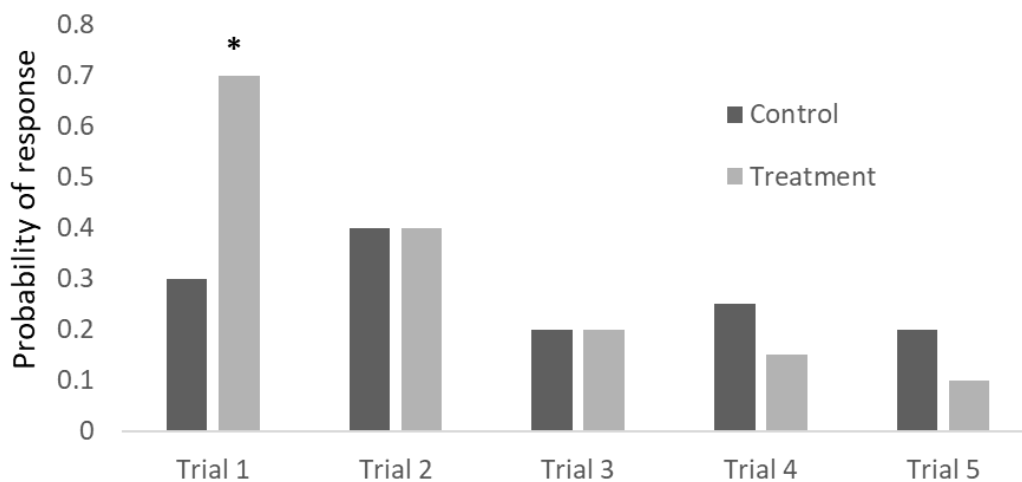


B.



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Figure 1. Female of the jumping spider *Phidippus audax* (Araneae, Salticidae) image used in the tests (A). (B) shows an elliptical distractor on the upper right and the boomerang shaped retinas of a male focusing on the female.

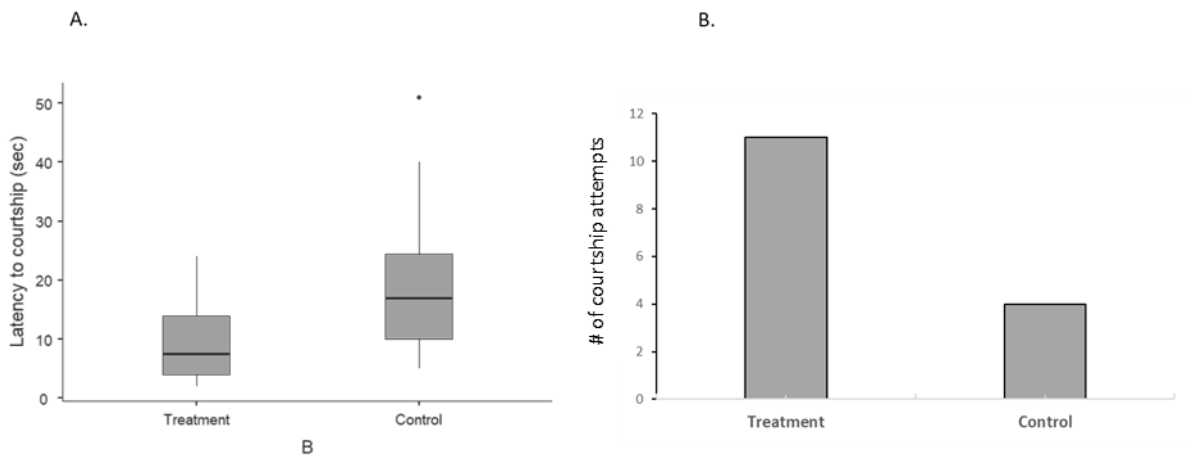


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Figure 2. Percentage of number of males of the jumping spider *Phidippus audax* (Araneae, Salticidae) freezing after a wasp sound played in five successive trials with

2751 1 min intervals. Males were exposed to female pheromones before the trials only in
2752 the treatment group.

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2754

2755 Figure 3. Latency to start displaying courtship behaviors (A) and number of males
2756 that initiated courtship behaviors (B) in males of the jumping spider *Phidippus audax*
2757 (Araneae, Salticidae) watching a conspecific female on a screen. Males were exposed
2758 to female pheromones only in the treatment control. In “A”, vertical lines correspond
2759 to maximum and minimum, and the dot indicate an outlier.

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Discussão Geral e Conclusões

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2766 De forma geral, investigamos aqui a aprendizagem e a atenção utilizando
2767 perguntas e abordagens menos convencionais. Enquanto grande parte dos estudos em
2768 aprendizado e atenção focam nos benefícios desses para os animais, aqui tentamos
2769 investigar possíveis custos e até mesmo possíveis malefícios associados a esses
2770 processos. Utilizando opiliões, realizamos testes de aprendizado considerados menos
2771 complexos, habituação e aprendizagem associativa, uma vez que esses animais foram
2772 pouquíssimos estudados em relação a aprendizado. Acredito, entretanto, que fomos
2773 capazes de estabelecer protocolos e demonstrar que esse grupo pode sim ser um
2774 modelo para estudos de aprendizado e memória. Inclusive o Capítulo 2 traz um novo
2775 método e uma ferramenta para que o estudo de condicionamento aversivo possa ser
2776 realizado em outros opiliões. Já para investigarmos situações mais complexas e até
2777 mesmo levantar uma discussão mais teórica sobre cognição e atenção, fizemos uso
2778 das papa-moscas (Salticidae) e suas habilidades visuais. O capítulo 3, foi um
2779 excelente exercício de comparação entre a literatura de cognição em humanos e
2780 invertebrados. Traçamos paralelos que nos permitiram sugerir que a direção do olhar
2781 pode ser uma importante proxy para estudos de cognição em invertebrados visuais, e
2782 que talvez com o eyetracker, por exemplo, possamos ter tantos avanços como os que
2783 ocorreram com uso dessa ferramenta em humanos.

2784 Falando dos capítulos de uma maneira mais geral, o capítulo 1 evidenciou a
2785 universalidade da habituação, mesmo em situações em que o estímulo é
2786 potencialmente muito perigoso. O capítulo 2 mostrou que opiliões podem aprender e
2787 armazenar informação, mas não por muito tempo, ao menos para estímulos aversivos.
2788 No capítulo 3 levantamos muitas possibilidades de estudo, mas deixando claro as
2789 dificuldades para se estudar o direcionamento do olhar devido à complexidade de
2790 certos sistemas. No capítulo 4, contrariando nossa hipótese, revelamos que a atenção
2791 seletiva em um estímulo sexual pode aumentar a atenção também em outros
2792 contextos.

2793 Especificamente no que diz respeito aos resultados, penso que avançamos em
2794 estudos mecanístico de aracnídeos, como tentamos mostrar na discussão de cada
2795 capítulo. Mas aqui queria ressaltar a complexidade dos assuntos abordados. Nos três

2796 capítulos experimentais, obtivemos resultados que não esperávamos. Pensávamos, no
2797 capítulo 1, que não deveria haver habituação para um estímulo que pode matar. No
2798 capítulo 2, a teoria sugeria que, dependendo do treinamento dado, a formação de
2799 memória deveria ser diferente, mas isso também não foi observado. Já no 4,
2800 hipotetizamos que a atenção na realização de uma tarefa tiraria atenção das demais,
2801 mas notamos um aumento da atenção também em outras tarefas. As explicações
2802 potenciais específicas estão nos respectivos capítulos, mas queria ressaltar, para um
2803 eventual leitor da graduação ou no início de sua pós-graduação, que não corroborar
2804 hipóteses não necessariamente é algo ruim. Ao contrário, por vezes nos permitem
2805 fazer descobertas ainda mais interessantes por abrir portas que não esperávamos. A
2806 probabilidade de um paradigma ser quebrado quando o resultado difere do esperado
2807 possivelmente é maior do que quando se obtém o que seria esperado pela teoria.
2808 Talvez os próprios autores não sejam as pessoas que irão fazer as tais grandes
2809 descobertas, mas podem contribuir humildemente abrindo a porta do desconhecido
2810 para que, no futuro, um leitor explore mais a fundo tal assunto e passe a construir
2811 conhecimento a partir de um início diferente.

2812 Outro ponto interessante é a complexidade dos sistemas e como nosso olhar
2813 pode ser limitado. Talvez não tenhamos corroborado nossas hipóteses porque elas
2814 foram frutos de nossa incapacidade de entender ou de acessar a complexidade de um
2815 campo de conhecimento. A simplificação por vezes é uma necessidade da ciência
2816 para fazer avanços, mas pode levar a explicações imprecisas de casos específicos, por
2817 exemplo. A falta de conhecimento da fisiologia do animal, de dados de história
2818 natural também podem ser fatores relevantes. Tomemos por exemplo o capítulo 1.
2819 Partimos de premissas que acreditamos serem corretas, mas, assim como em muitos
2820 trabalhos, não podemos ter 100% de certeza. Estamos pressupondo, talvez
2821 simplisticamente, que o opilião entende o estímulo oferecido como algo similar a uma
2822 ave. Fizemos provavelmente a melhor aproximação que podíamos levando em conta
2823 nosso conhecimento atual. Mas talvez, no futuro, descubramos que opiliões
2824 reconhecem aves visualmente e pelo odor conjuntamente, e que a sensibilidade ao
2825 contato é tal que ele diferencia nosso estímulo de uma bicada de ave que tentamos
2826 simular. E por essa razão a habituação ocorreu, o estímulo não era considerado
2827 mortal. Isto é, nosso método não é um problema em 2023, mas talvez seja em 2050. A
2828 complexidade dos sistemas é revelada conforme os estudamos, e nisso reside

2829 justamente uma das belezas da ciência. Sempre adicionamos ao conhecimento
2830 anterior, os avanços são graduais e não podemos tirar um conhecimento de seu
2831 contexto temporal (vide discussão sobre isso em Willemart, no prelo).
2832

2833

2834

Resumo

2835

2836 Esta tese apresenta o resultado de estudos sobre aprendizado e atenção em
2837 dois grupos de aracnídeos, aranhas e opiliões. Ela é fruto de trabalhos desenvolvidos
2838 tanto no Brasil quanto em estágio no exterior durante a pandemia de COVID 19.
2839 Apresento uma introdução geral, os capítulos: 1. Habituation to a predatory stimulus
2840 in a harvester (Arachnida, Opiliones); 2. Aversive conditioning and memory in the
2841 harvester *Mischonyx squalidus* (Arachnida: Opiliones); 3. What gaze direction can
2842 tell us about cognitive processes in invertebrates; 4. Dangerous attraction: risks and
2843 benefits of pheromone-induced behavioral state changes; e uma conclusão geral com
2844 dificuldades encontradas.

2845 No capítulo 1, estudamos uma das formas mais elementares de aprendizado, a
2846 habituação. Testamos se um animal deveria deixar de reagir a um estímulo que,
2847 embora não estivesse causando danos, fosse um estímulo potencialmente letal. Para
2848 isso, estimulamos indivíduos do opilião *Mischonyx squalidus* com um estímulo
2849 predatório por repetidas vezes, com intervalos controlados, em um mesmo dia e em
2850 dias diferentes. Medimos a ocorrência e magnitude do comportamento defensivo
2851 destes animais de pinçar com as pernas IV. Os animais habituaram-se ao estímulo,
2852 contrariando o que esperávamos, e a probabilidade e magnitude das respostas
2853 diminuíram. A resposta foi menor nos segundo e terceiro dias e observamos outros
2854 comportamentos defensivos frente ao estímulo predatório. Discutimos os dados em
2855 função dos papéis da ambiguidade, relevância da sucessão de estímulos e a relevância
2856 dos vários comportamentos defensivos de uma espécie.

2857 No capítulo 2, investigamos memória de curto (STM, *short term memory*) e
2858 longo (LTM, *long term memory*) prazos, geradas por diferentes protocolos de
2859 aprendizado associativo. Testamos se a maneira pela qual um estímulo foi aprendido
2860 influenciaria no aprendizado e no tempo de retenção deste aprendizado.
2861 Desenvolvemos um aparato para realizar os testes que fizemos no opilião *Mischonyx*
2862 *squalidus*, utilizando um estímulo aversivo. Cada indivíduo passou por três choques
2863 de 3s consecutivos, pareando-se um químico e o estímulo aversivo (choque) com
2864 intervalos de um (STM) ou trinta (LTM) minutos. Então o animal foi colocado em

2865 arena onde podia escolher entre um local com o químico previamente associado ao
2866 choque ou o lado controle. O teste nesta arena foi feito imediatamente após o
2867 aprendizado ou 24h depois. Com os dois protocolos, houve formação de memória no
2868 mesmo dia, mas não no dia seguinte, demonstrando que a maneira como o animal
2869 aprendeu não teve influência e que o aprendizado não foi retido até o dia seguinte.

2870 No capítulo 3, tentamos demonstrar que por meio de medidas precisas do
2871 direcionamento do olhar, podemos entender para onde a atenção está focada, o que
2872 pode beneficiar estudos de cognição em geral e em particular de invertebrados,
2873 animais menos exploramos neste quesito. Para tanto revisamos a literatura sobre o
2874 assunto e evidenciamos que paradigmas desenvolvidos em vertebrados também se
2875 aplicam a invertebrados, mostrando ainda como técnicas específicas podem ser úteis.

2876 Já no capítulo 4, testamos, utilizando uma aranha papa-moscas (Salticidae), se
2877 a exposição a feromônios de fêmeas faz com que machos fiquem mais focados em
2878 encontrar fêmeas, sejam mais eficientes em conseguir acasalar e passam a prestar
2879 menos atenção em outros estímulos no ambiente. Para tanto utilizamos um aparelho
2880 exclusivo, o *Eyetracker*, para estudar o direcionamento do olhar e a atenção. Este
2881 aparelho permite que consigamos acessar para onde uma aranha visual de menos de 1
2882 cm está olhando, e assim para onde está voltada sua atenção. Verificamos que o
2883 macho, quando exposto aos feromônios da fêmea, começa a cortejá-la mais rápido e
2884 por mais tentativas do que animais do grupo controle. No entanto, contrariando nossa
2885 hipótese, os machos também apresentam mais reações defensivas ao ouvirem áudios
2886 de vespas do que o grupo controle e que ambos os grupos direcionam seu olhar
2887 igualmente para um estímulo projetado ao lado da imagem de uma fêmea. Logo,
2888 concluímos que parece haver um aumento na atenção de maneira geral, não apenas
2889 para reprodução, contrariando a ideia clássica de atenção seletiva. Explicamos o
2890 resultado por meio de risco de canibalismo sexual e aparato visual destas aranhas.

2891

2892

2893

Abstract

2894

2895 This thesis presents the results of studies on learning and attention in two
2896 groups of arachnids, spiders and harvesters. It is the result of work done both in
2897 Brazil and in an internship at the University of Massachusetts (USA) during the
2898 COVID 19 pandemic. I present a general introduction, the chapters: 1. Habituation to
2899 a predatory stimulus in a harvester (Arachnida, Opiliones); 2. Aversive conditioning
2900 and memory in the harvester *Mischonyx squalidus* (Arachnida, Opiliones); 3. What
2901 gaze direction can tell us about cognitive processes in invertebrates; 4. Dangerous
2902 attraction: risks and benefits of pheromone-induced behavioral state changes; and a
2903 general conclusion with difficulties encountered.

2904

2905 In chapter 1, we studied one of the most elementary forms of learning,
2906 habituation. We tested whether an animal should stop reacting to a stimulus that,
2907 although it was not causing harm, was a potentially lethal stimulus. To do this, we
2908 stimulated individuals of the harvester *Mischonyx squalidus* with a predatory stimulus
2909 repeatedly, at controlled intervals, on the same day and on different days. We
2910 measured the occurrence and magnitude of these animals' defensive behavior of
2911 pinching with legs IV. The animals habituated to the stimulus, contrary to what we
2912 expected, and the probability and magnitude of responses decreased. The response
2913 was lower on the second and third days, and we observed other defensive behaviors
2914 in reaction to a predatory stimulus. We discuss the data considering the roles of
2915 ambiguity, relevance of stimulus succession, and the relevance of various defensive
2916 behaviors in a species.

2916

2917 In Chapter 2, we investigate short term memory (STM) and long-term
2918 memory (LTM) generated by different associative learning protocols. We tested
2919 whether the way by which a stimulus was learned would influence learning and
2920 retention time of what had been learned. We developed an apparatus to perform the
2921 tests we did on the harvester *Mischonyx squalidus*, using an aversive stimulus. Each
2922 individual underwent three consecutive 3s shocks, pairing a chemical and the aversive
2923 stimulus (shock) at one (STM) or thirty (LTM) minute intervals. Then the animal was
2924 placed in an arena where it could choose between a location with the chemical

2924 previously associated with the shock or the control side. Testing in this arena was
2925 done either immediately after learning or 24h later. With both protocols, there was
2926 memory formation on the same day, but not the next day, demonstrating that the way
2927 the animal learned had no influence and that the learned behavior was not retained
2928 until the next day.

2929 In chapter 3, we try to demonstrate that through accurate measures of gaze
2930 direction, we can understand where attention is focused, which may benefit cognition
2931 studies in general and in particular in invertebrates, animals less explored in this
2932 aspect. To this end, we reviewed the literature on the subject and showed that
2933 paradigms developed in vertebrates can also be applied to invertebrates, also showing
2934 how specific techniques can be useful.

2935 In chapter 4 we test, using the jumping spider *Phidippus audax* (Salticidae), if
2936 exposure to female pheromones makes males more focused on finding females, more
2937 efficient in achieving mating and pay less attention to other stimuli in the
2938 environment. For this we use a unique device, the Eyetracker, to study gaze direction
2939 and attention. This device allows us to access the gaze direction of the spider, and
2940 thus where its attention is focused. We found that the males exposed to female
2941 pheromones attempt to court more often and begins to court her faster than animals in
2942 the control group. However, contrary to our hypothesis, males also displayed
2943 defensive behaviors more often when listening to wasp audios than the control group,
2944 and both groups direct their gaze equally to a stimulus projected next to the image of
2945 a female. Thus, we conclude that there seems to be an increase in attention in general,
2946 not only for reproduction, contrary to the classical idea of selective attention. We
2947 explain the results by the risk of sexual cannibalism and the visual apparatus of these
2948 spiders.

2949

Anexos e Apêndices

- 2951 Anexo A – Trabalho publicado no Journal of Biochemical and Biophysical
 2952 Research Communications em 2021 – Referente ao Chapter 4.

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What gaze direction can tell us about cognitive processes in invertebrates

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Most visually guided animals shift their gaze using body movements, eye movements, or both to gather information selectively from their environments. Psychological studies of eye movements have advanced our understanding of perceptual and cognitive processes that mediate visual attention in humans and other vertebrates. However, much less is known about how these processes operate in other organisms, particularly invertebrates. We here make the case that studies of invertebrate cognition can benefit by adding precise measures of gaze direction. To accomplish this, we briefly review the human visual attention literature and outline four research themes and several experimental paradigms that could be extended to invertebrates. We briefly review selected studies where the measurement of gaze direction in invertebrates has provided new insights, and we suggest future areas of exploration.

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1. Introduction

How do animals acquire, evaluate, and process visual information? How do they decide which parts of the rich visual environment to attend to? Animal behaviorists typically study these questions by presenting animals with a variety of different stimuli and observing their responses. This fruitful approach has led to an understanding of male traits that are preferred by females (e.g., Ref. [1]), how animals detect and assess threats (e.g., Ref. [2]), and how animals detect prey (e.g., Ref. [3]) or avoid aposematic prey (e.g., Ref. [4]), among many examples. This experimental approach has been particularly productive since the advent of software that enables researchers to manipulate videos and create animations to present stimuli (reviews in Refs. [5-7]).

Here we advocate for increased use of a complementary approach: the precise measurement of gaze direction. Eye movements have long been used to study cognitive processing in humans (see Ref. [8], reviewed in Ref. [9]). Humans direct their gaze and as such selectively gather visual information: only a small amount of information that is present in the environment is processed by the high-resolution foveal area of the eye. Thus, a person's gaze is a direct measurement of overt selective attention that tells us something about cognitive processes underlying visual attention. The development of increasingly accurate and easy-to-use eyetrackers has generated thousands of papers addressing a range of questions on human visual processing and cognition, including how humans recognize objects (e.g., Ref. [10]); explore visual scenes (e.g., Ref. [11]); complete active tasks (e.g., Ref. [12]); and adjust their gaze based on their goals, expectations, and prior knowledge (reviewed in Refs. [13,14]).

Of course, gaze control is not restricted to humans: nearly all visually guided animals control their gaze using coordinated body or eye movements [15]. While much elegant comparative work has been done on the study of eye movements (reviewed in Refs. [16,17]), most of the research questions have not been framed

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in the context of cognition. Rather, many papers have focused on how animals use eye movements to solve particular functional problems, such as stabilizing their vision or pursuing moving objects (reviewed in Ref. [17]). Our goal in this paper is not to revisit that body of literature, but rather to highlight some of the questions and experimental approaches commonly used in psychology and ask whether they can be applied to other animals. We focus on invertebrates and particularly on jumping spiders, our own research organisms.

1.1. Common themes in visual attention research

The research on visual attention, particularly in humans, is vast. However, there are some recurring ideas threading through the literature. First, the process of visual attention must be inherently selective [18]. It is not possible to process all incoming information, necessitating that relevant information be prioritized over extraneous information [19]. Neural tissue required for computation is energetically expensive both in its development [20] and use [21], which is thought to constrain selective attention.

Second, visual attention can be influenced by bottom-up processes, in which stimulus properties drive attentional shifts [19], or by top-down processes, in which attentional shifts result from goals of the observer [22]. Top-down processing is characteristic of higher-order processing as it relies on working memory to determine which information is stored and attended to [23]. A large body of evidence in humans and a smaller body in non-human animals suggests that both stimulus-driven and goal-directed processes direct visual attention (see Ref. [24] for humans, [25] for insects). Top-down and bottom-up processing can interact: topdown attentional states can regulate attention to bottom-up cues [26], and bottom-up and top-down guidance can even work in opposition to each other [27].

Here we focus on four areas where we think an expanded study of gaze direction in invertebrates might prove profitable: object recognition (section 2), visual search (section 3), learning (section 4), and navigation (section 5). For a detailed discussion on why these processes are thought to be cognitive, see Shettleworth [28]. Before covering these areas,

we first consider the methods by which gaze direction can be measured.

1.2. The measurement of gaze direction and eye movement across species

Techniques for measuring gaze direction are, as one might expect, most advanced for humans. Humans have foveated visual systems where the central area of the retina has substantially higher acuity than the periphery. Eye movements typically follow a pattern of saccades and fixations: rapid shifts in gaze direction followed by periods of stasis as people scan their environment and direct their foveas to areas of interest [17]. It is these fixation points on a visual scene that are often the behavioral variable of interest for cognitive research. A number of methods for tracking human gaze direction have been developed (reviewed in Ref. [29]), including those based on corneal reflection or on the measure of electrical currents on skin surrounding the eyes. Whereas many eyetrackers require a person to be sitting in place, lightweight head-mounted systems [30] have allowed researchers to track eye movements in infants and in study participants that are moving freely about (see Ref. [29]).

Researchers have adapted human eyetracker technology to monitor gaze direction in a small number of non-human vertebrate species. Vertebrates tend to scan with saccade-and-fixation patterns, similar to those described in humans, which makes the application of previously developed technologies relatively feasible [15]. Birds have flexible necks and light heads, which enable them to rotate their heads at similar speeds to human saccades [17]. Eyetrackers have been successfully implemented in non-human primates (e.g., Ref. [31]), birds (e.g., Refs. [32e37]), and rodents (e.g., Ref. [38]).

By contrast, most invertebrate species, including insects, do not have movable eyes and can only shift their gaze by shifting their body or head orientation. Precise measures of head orientation (e.g., using high-speed cameras [39]), coupled with an understanding of the visual field of view, can generate insight into how animals with immovable eyes explore their visual environment. Some

crustaceans have stalked eyes that can move independently of the body. By monitoring eye position, it is possible to reconstruct the panoramic visual field of fiddler crabs [40]. The independently moving eyes of stomatopods, marine crustaceans also known as mantis shrimp, can also be measured (see Ref. [41] for a review). For example, Marshall et al. [42] videoed stomatopod eyes, tracked their eye positions frame-by-frame, and fitted a three-dimensional outline of each eye to infer gaze angle of the fovea, revealing several distinct eye movement strategies. Cephalopods such as squid and octopuses have moveable eyes resembling those of vertebrates [17], and only recently have techniques to measure eye movement in these species been developed and deployed [43]. Other invertebrate species have eyes that move only internally. Our lab works on jumping spiders (Family Salticidae), which are appealing subjects for visual attention research as they are highly visual in many aspects of their lives, possess high-acuity vision [44], demonstrate an ability to learn (reviewed in Refs. [45,46]), and exhibit complex decision-making behaviors [46]. The jumping spider visual system is modular, with a pair of principal eyes that are responsible for high spatial acuity and color vision, and three pairs of secondary eyes that are particularly attuned to detecting and processing movement. The retinas of the principal eyes are situated at the back of long tubes within the cephalothorax, with an immovable lens on the exterior of the spider. The most forwardfacing pair of secondary eyes direct the principal eye retinas to locate and track objects [47]. In our lab, we have conducted experiments in which we show spiders video stimuli while we monitor gaze direction with a specially designed eyetracker that captures infrared (IR) light reflected from the retinas [47,48] (Fig.1).

2. Gaze direction and object recognition

Recognition is the process by which animals perceive and classify objects in appropriate categories, such as food, predators, and conspecifics. The behavior of many invertebrate taxa can be driven by images of objects. There are a number of well-studied examples. Bees use many

visual features to detect and recognize flowers, including color contrast, pattern orientation, symmetry

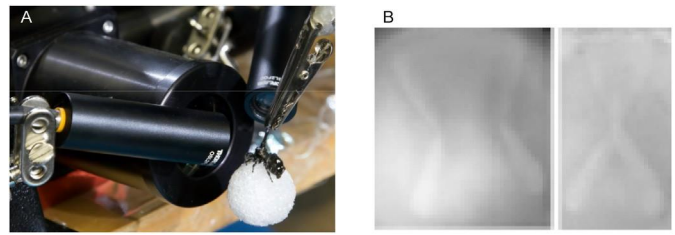


Fig. 1. (A) A tethered jumping spider looking at an image in the eyetracker. The retinas are illuminated by IR light, which penetrates the carapace. (B) Reflections of the boomerang-shaped retinas as captured by an IR camera. On the left, the retinas are in relaxed position; on the right, the spider is examining a stimulus and has directed both retinas to it.

(reviewed in Ref. [49]), and shape [50]; for a review of the recognition system in honeybees, see Ref. [51]. Social wasps can distinguish nestmates from non-nestmates based on facial markings [52]. Jumping spiders can recognize potential mates by their courtship behavior [53] and discriminate between types of prey prior to attack [54e56]. For example, *Portia fimbriata* distinguishes its favored prey, other salticids, by visual cues that include subtle details such as eye arrangement [57,58]. In another example, *Phidippus regius* uses local features to identify objects [59]. In this section, we will consider how in humans and other animals, gaze direction might inform us about object recognition and how this process depends on stimulus properties, surrounding context, and prior expectations.

2.1. Gaze direction and object recognition in humans

Humans must recognize objects in their environment for routine tasks such as identifying food or recognizing faces. Although the appearance of objects varies with viewing perspective, occlusions, variation across exemplars, and changes in lighting, we are able to recognize objects very quickly and effectively (reviewed in Ref. [60]). In the psychology literature, object recognition requires that a subject discriminates available stimulus features and matches them with representations of target objects from memory [61]. The efficiency with which an organism can detect and respond to particular stimuli is often attributed to bottom-up ‘salience filters’ that enhance detection

of particular features [23]. For example, humans shift their gaze rapidly to stimuli that appear abruptly [62], contrast with the background [63], or differ from other nearby stimuli [23,64]. While the saliency model predicts that eye movements will be directed to individual stimulus features, in real-world contexts we often target discrete objects rather than features alone [60].

Contextual cues facilitate object recognition (reviewed in Ref. [65]). For example, humans are better able to correctly identify objects when the corresponding scene is appropriate [66] or when there is a collection of related objects [67], whereas if objects are presented in inappropriate scenes or object relationships are otherwise violated, recognition is impeded [68]. This shows that observers' expectations can influence how they perceive an object. In addition, object recognition can be facilitated by information from other sensory modalities (reviewed in Ref. [69]). For instance, humans are better able to identify a partially masked image of a dog when it is paired with a barking sound [70]. To perceive objects, constituent features need to be detected and subsequently integrated in the brain of the observer, processes that may occur at different levels of visual processing. To disentangle feature-based and object-based recognition in experiments, control stimuli should have the same constituent parts but lose their distinct amalgamated meaning (e.g., Ref. [71]). Paradigms developed for studying object recognition in infants are particularly well adapted for non-verbal animals. One such approach is the simultaneous presentation of images to measure preference and visual bias in attention [72e74]. These 'looking time' experiments are particularly useful for exploring visual bias, habituation, and expectancy violation [75]. Similarly, anticipatory looking methods take advantage of the fact that infants and other vertebrates look to locations where they expect an event is about to occur (e.g., Ref. [76]). Infants gaze at familiar and novel stimuli for different durations, suggesting that familiar objects are recognized [77]. Furthermore, infants spend more time looking at scenes that violate their expectations [78e80]. Gaze direction and preferential looking methodologies can reveal infants' understanding across a variety of cognitive

domains, including reasoning about objects, numbers, and the social behavior of others [81e83].

2.2. Gaze direction and object recognition in invertebrates

Which stimulus features attract attention? Bottom-up control of attention has been the most commonly studied perceptual process in invertebrates, although it is arguably the least cognitive aspect in that it may be driven primarily by characteristics of the receptors rather than by higher-order processing. Here we highlight several illustrative examples. Mantids are generalist predators that track prey by moving their heads. By monitoring their gaze direction in response to computerized visual stimuli, researchers have surmised that mantids do not store representations of prey, but rather respond more simply to a suite of stimulus features including size, contrast, relative speed, and direction [84e90]. Using an ophthalmoscope, Land [91] found that jumping spiders would rotate and scan their retinas across visual stimuli that bore angles resembling those of legs as a potential method for identifying prey or conspecifics quickly and efficiently based on such element configurations. In other work, a predatory generalist jumping spider species did not significantly differ in its visual exploration of non-natural versus biologically relevant stimuli, whereas predatory specialists were found to spend more time focusing on smaller regions of interest [92]. Similarly, the mosquito-specialist jumping spider *Evarcha culicivora* uses a local processing approach, analyzing the orientation of each element of prey objects for identification, rather than relying on a global or holistic approach [93]. In contrast, *Polistes fuscatus* paper wasps were found to recognize individuals from images of conspecifics with particular facial markings [52] and respond more quickly to coherent faces than scrambled or incomplete images, suggesting a higher cognitive processing for conspecific faces rather than simple pattern recognition. Honeybees demonstrate the ability to categorize objects by learning elemental configurations and assemblies of these features, which transfers to novel stimuli [reviewed in Refs. [94,95]]. Future work in arthropods should capitalize on the rich diversity in visual systems,

visual environments, and life histories in comparative studies.

What objects do invertebrates expect to see? Top-down visual processing occurs in at least some invertebrate species. As in human infants, looking time protocols can be used to understand whether an animal's expectations about what it will see are supported or violated. Examples come from work on *Portia*, jumping spiders that specialize in eating other spiders. Paralleling work with infants [96], a spider is shown a stimulus, which is then hidden behind a barrier. Either the same stimulus or a new stimulus is then revealed. *Portia* hesitate before attacking a revealed object if inherent features (such as color) are changed from the original stimulus, but do not hesitate if the prey orientation is changed, suggesting that *Portia* is able to differentiate prey identity independently from orientation (i.e., changes in prey orientation do not induce an expectancy violation, while changes in its inherent properties do) [97]. Again, similar to infants [83], spiders spent more time looking at a display of prey if the number of prey was modified, but not if their arrangement was modified, suggesting that *Portia* is able to cognitively represent exact numbers of prey in at least a few discrete categories [98]. These looking-time techniques could likely be adapted to additional species.

How do cross-modal stimuli influence object recognition? Contextual cues such as a stimulus in another modality can alter attention to objects in the environment. For example, jumping spiders interpret images of conspecifics presenting ambiguous morphological features as displaying either threat or courtship, depending on the presence of pheromone cues [99]. Another jumping spider species freezes in response to the sound of a predatory wasp [100], but sweep their retinas back and forth, possibly looking for the source of danger [101]. With the addition of precise gaze tracking, one could test how additional senses influence visual attention and object recognition: do animals presented with cross-modal cues attend to different features than animals without those cues?

3. Gaze direction and visual search

Visual search is the process by which an observer looks for a target object against a background array of other, potentially distracting, items [68,102]. The most commonly studied form of visual search in non-human animals involves foraging, a convenient analog to examples of visual search in the human literature (although new human search task designs are similar to foraging behavior; see Ref. [103]). Detecting camouflaged targets requires attentive search because such targets are rarely detected passively [104], and predator performance is known to improve with experience during search tasks for cryptic prey [105]. Commonly encountered food items are consumed at proportionally higher rates than random sampling would indicate [106e108]. This is compelling evidence that previous encounters with particular objects can enhance searchers' efficiency in locating concealed target objects. Precise measurements of gaze direction during visual search tasks offer unique insights. For example, gaze shifts during foraging can indicate if a cryptic target is detected and ignored, or undetected altogether. In this section, we will investigate how gaze direction might inform us about the visual search process and how search performance in humans and other animals might depend on both context and prior knowledge.

3.1. Gaze direction and visual search in humans

Many activities in our daily lives are comprised of search tasks, whether that is looking for a car in a parking lot or picking out a face in a crowd [68]. In the human psychology literature, visual search is usually tested experimentally by instructing subjects to find a target among distractors under a variety of conditions, and their performance (usually search time) measured (although emphasis has also been placed on real-world tasks, see Refs. [68,104]). A long line of different experiments have shown that performance in these tasks can range from parallel search, in which all items are attended to simultaneously, to serial search, in which a single item or a small number of items are attended to sequentially, with search rate on many tasks falling somewhere between these extremes [68]. Townsend [109] pointed out that slow search rates may reflect

limited capacity parallel search rather than serial search.

When humans engage in visual search, attention can be directed by a combination of stimulus-based bottom-up and goal-directed top-down mechanisms. Bottom-up guidance is demonstrated by rapid shifts of gaze to salient stimuli that ‘pop out’ from the background [110]. However, when people view meaningful scenes, they are less likely to be distracted by ‘low level’ salient stimuli [13], and these stimuli can be ignored altogether in particularly complex scenes when they are too numerous to drive shifts in attention [111]. Suppression of attentional capture to distractors depends on target-nontarget relationships rather than similarity alone [112]. In most complex search tasks, bottom-up guidance interacts with topdown guidance, which directs attention toward stimuli with visual properties known to belong to the target [113].

Visual search may be facilitated by additional top-down processes including those that consider contextual cues [114]. Attention is sometimes deployed to locations that were significant for a subject in the past [115] (see Ref. [116] for a review of the role of memory in visual search). A form of ‘repetition priming’ causes subjects to be attuned to features of the last object to which they were exposed, improving search performance [117]. Awh et al. [118] argued that these different findings could be attributed to three different factors in attentional control, which they labeled physical salience, current goals, and selection history. Their third factor is especially broad and includes searches that become more efficient when targets have been associated with reward. Wolfe and Horowitz [119] proposed a longer list, with five factors that included scene structure. Cross-modal

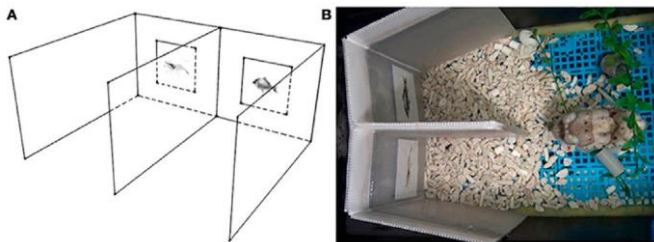


Fig. 2. A schematic (A) and photograph (B) of an experimental setup for examining cuttlefish choice. The cuttlefish can view both options at the decision point. Reproduced from Ref. [194].

cues can also influence visual search. For example, attention may be directed to objects in a scene that are paired with related odors [120] or sounds [121,122].

Measuring search time and tracking eye movements have become particularly powerful tools for determining how humans allocate attention as they look for objects. Many models have been proposed to describe mechanisms that facilitate visual search that we have not space to discuss. For a more comprehensive review and historical context of research on visual search in humans, see Nakayama and Martini [110]. We also recommend that readers consult [102,123,124] and chapter 6 in Ref. [68].

3.2. Gaze direction and visual search in invertebrates

How do distracting environments affect visual search performance? Invertebrates engaged in visual search tasks often encounter many ‘distractors’, such as prey that are unlikely to be captured [125] or flowers offering fewer rewards [126]. Attentional processes are often required during visual search in distracting environments, especially when target and distractor objects are visually similar. Some invertebrate species, such as dragonflies, can effectively track the movements of a target individual in a swarm of flies without being distracted by the movements of other flies [125,127], and hoverflies selectively target moving objects that contrast highly with the background [128].

While the search and pursuit for such targets typically relies on ‘low-level’ sensory processes such as feature-detecting neurons [129,130], ‘high-level’ cognitive processes have been implicated in many pollinator species. Honeybees take longer to complete a visual task with more distractors [131], but can learn to ignore distractions with specific stimulus properties such as a particular color

[132]. Even closely related species can differ in this regard, as bumblebees were significantly less affected by distracting objects than were honeybees (parallel versus serial-like search; see Ref. [133]). Such differences are assumed to link tightly to the

individual ecology of each organism. As in humans, target saliency undoubtedly also influences search time. For example, larger flower targets are easier for bees to find among distractor flowers than are smaller targets, and bees use cues such as color and illumination to assist with their search [134]. Bees also exhibit speed-accuracy trade-offs during visual search, in which more time allocated to a search task improves performance, with individuals exhibiting stable differences in their strategies [135].

Most of what we know about visual search in invertebrates derives from research with bees and other pollinators, largely because flower inspection offers a convenient measure of gaze direction, but it is unclear if and how these findings extend to other invertebrates. To what extent other invertebrates use parallel or serial search would be interesting for future work. Currently, our laboratory is investigating how jumping spiders search for objects in scenes with different numbers and types of distractors.

How do observer goals influence visual search? Subjective states such as hunger and the need for shelter drive visual search behavior in animals [136,137]. These physiological and behavioral states influence the neuromodulation of vision to a surprisingly considerable extent (see Ref. [138] and references therein). Many animals that engage in visual search must divide their attention to remain vigilant for predators [139]. For an example in vertebrates, foraging birds often engage in scanning behaviors in which they periodically lift their heads to look for predators (but see Ref. [140]). Some prey species have lateralized eyes and preferentially use one side for certain visual search tasks, such as scanning for predators or prey (e.g., Ref. [141]). Desert locusts searching for resources periodically pause while locomoting, which enables them to scan their surroundings with head and body turns. The duration of the pause along with their associated head and body movements can be used to determine if the locust is engaged in local search or relocation [142]. If more studies monitor gaze direction during visual search, we could develop a better understanding of how variable search patterns indicate animals' motivation.

Similar to humans, exposure to one type of target can 'prime' invertebrates' attention as they search for other visually similar targets. Priming describes a process in which exposure to a particular stimulus alters stored mental representations of an object, which in turn influences future perception [117] and prepares an organism to search for a target. Priming can influence the development of a search image or search template, which allows an organism to look selectively for specific features or locations of a target object [143], and to readily ignore non-matching stimuli (but see Ref. [144]). A mechanistic example can be found in dragonflies, where neurons selectively tuned for detecting small targets are more sensitive to movement occurring ahead of the target, thereby indicating attention is modulated by the location in which priming takes place [145]. Gamberale-Stille et al. [146] recently found that two generalist butterfly species improve their search efficiency for a host plant after sequential priming. This is important because generalist species might be disadvantaged at visual search compared to specialists because there are more potential targets that are relevant to them. Many generalist pollinators such as bees use search images to look for flowers [147]. While such studies have been successfully conducted with vertebrate predators such as birds [148e150], far less work has been done with invertebrate predators. One notable exception is with the jumping spider *Evarcha culicivora*, a species that feeds preferentially on blood-fed mosquitoes. Cross and Jackson [151] demonstrated that these spiders evoke a search image for prey during visual search after exposure to olfactory cues. Currently, our laboratory is investigating if jumping spiders that are primed with supplementary visual cues are able to locate target objects faster in a cluttered scene.

How do cross-modal cues affect visual search? Most animals are exposed to cues from a variety of sensory modalities, each of which can provide additional information about their surroundings and thus help to direct visual search. In *Evarcha culicivora* jumping spiders, as mentioned previously, priming with the scent of blood or the color red enhances the speed with which they can find partially obscured target lures [152]. Flowers often present signals from multiple modalities

which enables pollinators to effectively locate them within complex scenes. For example, bumblebees are able to find an inconspicuous target flower faster when it is paired with scent [153]. Floral scents alone can trigger bees to return to specific locations where the associated reward was found [154]. *Drosophila* require visual feedback to accurately localize an invisible odor source [155] and remain oriented in an odor plume while flying [156]. Some invertebrates must also search for freely moving target hosts. For example, when flying mosquitoes detect CO₂, they steer toward certain visual stimuli that would otherwise be ignored [157,158]. Parasitoid wasps use visual cues and vibratory cues synergistically to help locate target hosts, and therefore their target capture performance is enhanced with other cues that help localize or constrain visual search to a smaller area [159]. Future work should investigate if and how visual search performance changes after exposure to cues from mates, predators, or prey in various sensory modalities.

4. Gaze direction and learning

Many invertebrates are capable of learning and remembering visual information, whether it be learning about newly blossoming flowers over the course of a summer or the distinctive characteristics of conspecifics. Here, we pay particular attention to the use of gaze direction in both basic associative learning [160e162] and operant tasks [163e165]. We also examine social learning: invertebrates can learn from conspecifics in the contexts of task solving [166], mate selection [167], foraging decisions [168], and predator avoidance [170].

4.1. Gaze direction and learning in humans

While our visual worlds are dynamic, many aspects are predictable, making the ability to learn about them beneficial. In humans, memory is known to play an important role in guiding visual attention [171]. For example, visual attention is often deployed more to recently attended objects [172]. Conversely, some objects that are frequently present in a scene are no longer fixated upon, a process called visual habituation [173]. Although specific details of a scene are usually not stored and changes

to objects often go unnoticed [174], memory of some visual information from scenes can persist over time [172]. Selective attention can also be modified during learning in such a way that allows the learner to better optimize their ability to discriminate categories of objects [175]. While conducting visual tasks, humans learn to form associations, and these associations can fine-tune gaze direction. For example, humans are more likely to allocate their overt attention to cues that are predictive of associative outcomes when compared to unpaired cues [176]. In natural contexts, such overt attention shifts are thought to result from a reward-based learning context in which favorable outcomes guide attentiona process that can even occur unconsciously [177]. Humans also learn by watching and copying how others complete tasks, a process called social or observational learning (reviewed in Ref. [178]). As Menzel et al. [179] suggested, in observational learning an obvious reinforcing stimulus is absent, so observers should have an internal representation of how the behavior might benefit themselves. Humans are exceptional social learners and are particularly attentive to faces. Emotion, gaze direction, and facial expressions influence the memory of faces [180e182]. Humans also often follow the gazes of others [183]. In some experiments, the demonstrator uses their gaze direction to draw the attention of the observer to an object (object enhancement) or a location (local enhancement) [184]. Attention to gaze direction begins early in life: infants reliably follow others' gaze direction, attending preferentially to the object at which a model gazes [185]. Gaze following has also been described for non-human vertebrates, including other mammals, birds, and reptiles (e.g., Refs. [183,186,187]).

4.2. Gaze direction and learning in invertebrates

To which features do animals attend during an associative learning task? Many invertebrates can use visual cues during associative learning. Here we highlight where gaze tracking has been implemented successfully in three taxa that are known to be adept at learning.

Stomatopods possess one of the most specialized visual systems of any animal group [41]. They can learn to recognize predators and conspecifics, identify their own burrows, and discriminate among colors (reviewed in Ref. [188]). Chen [189] showed that two stomatopod species performed differently in associative learning tasks that relied on learning geometric shapes, a difference possibly related to different predatory strategies. However, none of the previous experiments attempted to acquire detailed gaze direction measurements. Recently, Daly et al. [190] developed a technique that allows for well-controlled measurements of gaze direction. They found that stomatopod eyes can move independently of each other and simultaneously engage in different tasks. They were also able to demonstrate how the animals achieve gaze stabilization even with the additional challenge of three rotational degrees of freedom: torsion, pitch, and yaw [191,192]. This system is suitable for including gaze direction in studies of learning and discrimination.

Another invertebrate group that uses visual and tactile cues during associative learning and operant conditioning, or learning from the consequences of their behavior, are the cephalopods [193]. For example, cuttlefish learn to discriminate among prey images and treat sketches and altered images of reduced size as equivalents when performing a task [194] (Fig. 2). They can also associate food with a flashing light [160] and have episodic-like memory to keep track of 'what, when, and where' they have eaten [169]. Octopuses are capable of operant conditioning [195] and use visual information to track and control the location of their arms when completing tasks [165]. A few techniques have been developed to track gaze direction in cephalopods. Recordings of cuttlefish with high-speed cameras showed that these animals can adopt different saccadic movements depending on the behavioral situation [196]. Levy and Hochner [197] tracked the inclination of octopuses' eyes during locomotion and showed that the interaction between the arms and surrounding visual cues gives feedback regarding head orientation. A very promising tool for non-invasive eyetracking in *Octopus bimaculoides* has been recently developed using an IR camera and pose tracking

(DeepLabCut; see Ref. [246]) [43]. These new techniques for monitoring head and eye position and the dexterity of cephalopod movements will improve our understanding of how gaze direction changes with experience with a task or when observing others, analogous to human research.

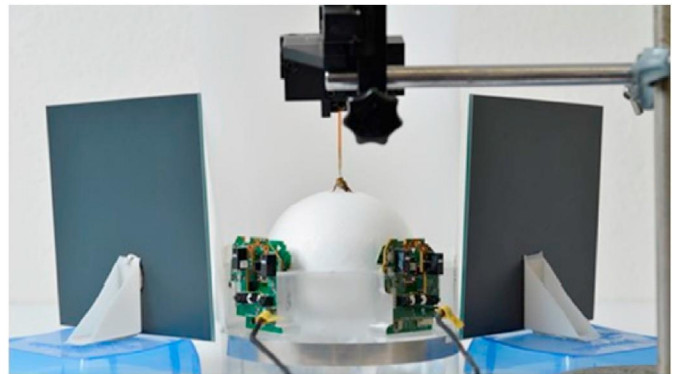


Fig. 3. A honeybee in a virtual reality experiment (reproduced from Ref. [242]).

Jumping spiders also use visual cues in associative learning [45]. These spiders learn to avoid distasteful prey [198], associate colors with food [161] or nests [199], and associate images with an aversive shock and vibration [162,200]. Eyetracking during learning trials would help us ascertain how learning influences the allocation of attention to different visual cues. Of particular interest is how innate preferences for particular forms and movements [93,201,202] are modified by learning, and how spiders' attention to different parts of an object shift with experiences that work in opposition to innate tendencies. Eyetracking can also provide a more finely calibrated tool to measure the effort that spiders expend to make sense of confusing images. For example, de Agro et al. [59] showed that *Phidippus regius* spiders can learn to associate abstract images with a reward or punishment, but then fail to discriminate fragments of the same stimuli. If spiders that have learned about whole images examine image fragments in a different way than do naive spiders, we would have evidence that they recognize something is familiar. For a review of these and related issues in invertebrate learning, see Abramson and Wells [203].

What do animals attend to during social learning? Observational learning has been described in a number of invertebrate taxa. In *Octopus vulgaris*, individuals can learn to perform reward-choosing

tasks by watching other individuals [166,204]. Social invertebrates like bees and wasps also engage in social learning [205]. For example, bumblebees can learn how to recognize rewarding flower colors after watching other individuals forage in an observational arena [168]. Dawson et al. [206] showed that flower preference can be driven by second-order conditioning. The observer first associates the conspecific with the presence of food, and then watches the conspecific forage on a particular color of flower. The observer then associates flower color with food even without direct experience with the flower. In another study, bumblebees even demonstrated cognitive flexibility during an observational learning task in which observers did not simply copy the behavior of the demonstrator, rather they improved upon it [207].

With precise measurement of gaze direction, it would be possible to test whether animals watching a conspecific attend particularly to the conspecific's interaction with the object of interest, as in the phenomenon of object enhancement described in the psychological literature, and whether experienced social learners are more likely to direct their gaze to the most informative part of the scene. Non-social invertebrates can also perform observational learning. *Drosophila melanogaster* females watch other conspecifics copulate and use this learned information to select their own mates [167]. Wood crickets exhibit social learning in predator avoidance [170]. Despite the small number of reported cases compared to social invertebrates, these examples emphasize how the imple-

mentation of gaze direction measures could be helpful when investigating the cues to which observers attend, and how these cues differ between social and non-social animals given the substantial difference in their lifestyles.

5. Gaze direction and navigation

Navigation, a complex task that is essential for many invertebrates, requires learning both visual landmarks and proprioceptive cues. We will examine how tracking gaze direction can be informative for studying navigation tasks such as homing (reviewed in Ref. [208]) and route planning [209,210]. In some invertebrate taxa, navigational abilities have been well studied, especially social insects (e.g., Refs. [205,211]); cephalopods (e.g., Refs. [212,247]); and to a lesser extent, spiders (reviewed in Ref. [45]).

5.1. Gaze direction and navigation in humans

The ability to shift our gaze is essential for daily tasks in our lives such as moving around obstacles and traveling to and from particular locations. While walking, humans precede changes in direction with head and eye movements [213] and learn to fixate on objects with which they may potentially collide (reviewed in Ref. [214]). However, while navigating cluttered environments, humans do not always fixate on all obstructing objects but rely on peripheral vision as well [215]. Using an assay that employed virtual reality, Andersen et al. [216] found that humans tasked with navigation fixate on visual landmarks. To select these landmarks, humans use cues including visual and structural salience, which are characteristics of landmarks that are visually conspicuous or immediately related to navigation, respectively. The intention to learn a route leads to more gaze shifts to landmarks at structurally salient locations along a route, while gaze to more visually salient landmarks occurs regardless of learning intention [217].

5.2. Gaze direction and navigation in invertebrates

How can gaze direction improve our understanding of navigation in invertebrates? Many species must travel long distances to nest sites after foraging, and the ability to learn the arrangement of landmarks around their nests plays an important role in navigation and homing [179]. Octopus and cuttlefish use landmarks to orient and guide them through mazes [218,219]. Jumping spiders orient toward familiar beacons when returning to nest sites [199]. Navigating ants, bees, wasps, and many other invertebrates orient their gaze to match their view of a scene to memory of familiar scenes [208]. As Tinbergen [220] famously described, sometimes insects perform learning flights or walks close to their nest to learn a visual representation of the surrounding environment to guide their return after foraging.

Considering the diversity of the environments in which animals must navigate, it is not surprising that there are different strategies to optimize information filtering and processing [18,221]. The measurement of gaze direction can give us a better understanding about what specifically animals learn from these flights and walks and how they acquire this information [222], and a number of studies have done just that. Usually learning flights or walks start with the animals leaving the nest and rotating around to obtain a frontal view of the nest entrance. After that, they move along arcs, sometimes pivoting around the nest, while performing saccadic movements [223]. By tracking the gaze and body position of wasps, Stürzl et al. [39] showed that learning flights occur in a coneshaped formation allowing the animals to control the gain of horizontal distance in a fairly constant rate as they gain height above ground. When returning to the nest, wasps perform a predictable flight maneuver when they encounter the previously memorized view of their nest. In bumblebees, changes in gaze direction also occur with short and fast head turns, reducing image rotation to short time intervals. Boeddeker et al. [224] found that these saccadic movement patterns are very similar to those known for vertebrates including humans. For ants, learning walks are also a common homing strategy; however, Fleischmann et al. [225] found the pivoting and saccadic movements can be species-specific, with ants that live in a visually richer environment exhibiting a larger repertory of behaviors during the learning walk. Baddeley et al. [226], by analyzing panoramic images generated from the perspective of the ant, found that ants use scene familiarity during navigation even in the absence of distinct landmarks. Using anesthetic injection into the mushroom

body, an area associated with learning and memory in arthropods, Kamhi et al. [227] showed that, in ants, this region is necessary for retrieving visual memories. Ants treated with anesthesia showed no preference in their gaze direction to environmental cues compared to ants with functional mushroom bodies, which oriented their gaze preferentially to visual landmarks.

How can gaze direction improve our understanding of route planning in invertebrates? Analyzing gaze direction might help us understand how animals plan routes. Jumping spiders from the subfamily Spartheinae specialize in preying on other spiders, and approach their prey via complex routes, including reversed-route detours when the prey is out of sight [55,209,210,228]. For example, *Portia africana* decides when to use a detour or not based on the goal objects it sees before making a plan, selecting a shorter route when presented with a prey rather than a non-prey goal [229]. By monitoring cephalothorax orientation and thus gaze direction, Taristano and Andrew [221] showed that *Portia labiata* first scan all possible routes leading away from the target, but then narrow their attention to a complete route that leads back towards themselves. Spiders do not always try to solve a complex detour all at once, but rather appear to establish secondary objectives along the route, thereby solving the detour in small blocks. Spartheine spiders are exceptionally good at navigating detours, whereas nonspartheine salticid species that typically prey on insects vary in this ability. For example, for *Marpissa marina* visual cues seem important when planning a route, whereas *Trite planiceps* typically prefer shorter routes [230], and *Phidippus audax* fail to complete reversedroute detours at all [231]. With careful measurements of gaze direction across species, we might be better able to understand the proximate sources of

interspecific variation in detouring success [221].

6. Conclusion and future directions

Our goal here has been to make the case that the rich psychological literature on human gaze direction deserves more attention, and its methods more direct application, by students of invertebrate behavior and cognition. Gaze direction measurements are rich in information, and they can be used to form inferences about cognitive functions that are either not apparent in other behaviors or are difficult to discern in the measurements of neural activity currently possible. Because eye movements and the underlying neural control of gaze direction of invertebrates evolved separately from that of vertebrates [17], identifying parallel and divergent aspects of the rules underlying the control of gaze direction will be informative for future research. While we mostly focused on similarities between findings in humans and invertebrates, we also expect fundamental differences. Within invertebrates, the potential for comparative studies is rich, given the sheer diversity of species and visual systems, the availability of closely related species that are in very different visual habitats, the availability of distantly related species facing similar visual challenges, and our growing understanding of underlying neural structures that influence both cognitive processes and visual processing (e.g., Refs. [232,233]).

Precise measures of gaze have started to provide surprising insights into animal behavior that would be otherwise difficult to come by. Eyetracking reveals that peahens direct their gaze only to the lower third of a peacock's display, while the extravagant feathers that are not being fixated upon might instead serve to capture the attention of females from afar [33]. Male *Habronattus pyrrithrix* jumping spiders present an elaborate courtship

display that includes ‘knee pops’ and flicks of the upraised tarsi. Eyetracking shows that females direct their gaze at the pops. Moreover, tarsal flicks do not usually attract the gaze, but rather stimulate the anterior lateral eyes to direct the principal eyes to the center of the display (unpubl. data). It would be valuable to study whether females differ in how they attend to male displays, as such variation can have consequences for sexual selection (see Ref. [234] for a review). However, numerous operational challenges remain. First, the visual system of the study organisms must be well understood. For example, even across vertebrate species, there is enormous variation in the presence and shape of retinal areas with higher spatial acuity (e.g., Refs. [16,235]). At a minimum, one must understand the field of view of the eyes. Within compound eyes, the positions of different ommatidia makes them suitable for different behavioral tasks [236,237]. Second, devising ways to measure gaze direction can be difficult. Accurate measures of head direction may require high-speed cameras positioned at different angles (e.g., Refs. [39,238]), and, as we previously described, species with eyes that move independently from their bodies offer particular problems. Cross-

disciplinary collaborations, as our lab had with optics designers [48,239], are often necessary.

A promising potential technique for future work will be to combine measure of gaze direction with virtual reality (VR). VR systems have been designed for jumping spiders [240], *Drosophila* [241], and honeybees [242] (Fig. 3). To our knowledge, no invertebrate studies have explicitly tracked dynamic changes in gaze direction while an animal is engaged in VR. We believe tracking the gaze direction of freely moving animals in VR systems such as FreemoVR [243] will be a powerful technique. A closed-loop system that updates with animal choices might offer great insight into how animals use their vision to explore scenes and to complete behavioral tasks.

Given the dearth of studies investigating bottom-up versus topdown mechanisms underlying visual processes in invertebrates, we hope to encourage more research in this area. Recent studies have further supported the view that bees in particular demonstrate exceptional plasticity in visual processing [244], and how this occurs in the brain is an area of active research [245]. Embracing a comparative approach will provide novel insights for visual cognition research in humans and invertebrates alike.

Declaration of competing interest

The authors declare no conflicts of interest.

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Biografia

Dificuldades encontradas e trabalhos de pesquisa paralelos à tese

Durante o ano de 2020 e começo de 2021 estive em um doutorado sanduiche nos Estados Unidos, na University of Massachusetts. Em meio à pandemia, a condição para continuar a ter acesso ao laboratório durante a pandemia era de que apenas uma pessoa o fizesse e eu fui esta pessoa. Sobrecarregado com tarefas que seriam divididas em 4-5 pessoas, tive que alterar prazos de desenvolvimento da escrita e elaboração de manuscritos já finalizados no Brasil. Fiquei extremamente sobrecarregado de tarefas relacionadas não só ao meu projeto, mas também com cuidado geral e funcionamento do laboratório. Entre as tantas tarefas realizadas durante o projeto tive que, sozinho, criar, alimentar, cuidar, acasalar, separar, e desenvolver uma linhagem de aranhas para um experimento, tarefa que eu nunca havia realizado. Mantive 600 aranhas no laboratório por meses, sozinho. Também tive que montar arenas, criar estímulos para experimentos de comportamento, fazer pilotos, rodar os experimentos, aprender técnicas de histologia em 6 meses (sendo que tais técnicas levaram cerca de 5 anos para serem aperfeiçoadas pelos responsáveis), aprender a utilizar um microscópio confocal, tudo isso ainda cuidando e limpando cerca das aranhas. Também atravessei inúmeros problemas técnicos no laboratório e estes dificultaram muito a rotina de trabalho. Além do mais, foram quase 5 meses finais do projeto sem nenhuma remuneração vinda da agência de fomento brasileira, visto que o prazo da bolsa tinha se encerrado. Ao retornar ao Brasil, trabalhei em loja para me sustentar, com centenas de horas de vídeos a serem analisados, por volta de 500 fotos de cortes histológicos, 2 programas de edição de cortes histológicos para serem aprendidos, além do Doutorado para ser escrito. Tenho tentado conciliar os empregos de professor em duas escolas com a confecção da tese e infelizmente não consegui incluir tudo o que tenho neste documento. Ainda tenho dados coletados para os seguintes trabalhos (Tabela 1) que deveriam compor a tese e trabalhos paralelos que desenvolvi nos últimos anos (Tabela 2). Nos próximos anos pretendo finalizar estes trabalhos.

Tabela 1. Trabalhos desenvolvidos como parte do Doutorado que não consegui incluir na Tese.

ASSUNTO E CONTEXTO	DETALHAMENTO DO QUE JÁ FOI FEITO
Comportamentos inatos vs aprendidos. Opiliões possuem clara preferência por escuro. Seria possível reverter esta preferência por meio de estímulos aversivos? Trabalho que conduzi no Brasil.	Dados 100% coletados e analisados
Efeito da complexidade do ambiente no desenvolvimento do cérebro e comportamento. Criamos aranhas em diferentes ambientes e testamos como reagem a determinados estímulos e como ficou a morfologia interna de seus cérebros. Trabalho que conduzi nos EUA, com a colaboração de Skye Long e Alex Winsor, sob supervisão de Elizabeth Jakob.	Dados 100% coletados, dependendo das análises

Tabela 2. Trabalhos desenvolvidos paralelos ao Doutorado que não foram planejados para compor a Tese.

ASSUNTO E CONTEXTO	DETALHAMENTO DO QUE JÁ FOI FEITO
Orientação e homing em um opilião ripário. Parceria informal com meus colegas de laboratório Norton FS Silva (autor principal), Gabriel P Murayama e meu orientador Rodrigo H Willemart. Foi a continuação de um trabalho iniciado no mestrado do Norton.	Dados 100% coletados e analisados
Galinhas, animais diurnos, poderiam predação escorpiões, animais noturnos? É um capítulo da tese do Gabriel P Murayama, o autor principal do trabalho	Dados 100% coletados
Manutenção e criação de aranhas Salticidae do ovo ao adulto. Trabalho sobre métodos visto que é comum não ser bem sucedido nesta tarefa e	Dados 100% coletados e

obtive sucesso. O conhecimento destes métodos pode ser de grande ajuda para responder inúmeras perguntas em comportamento animal.
