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1 **Polarization sensitivity as a visual contrast enhancer in the Emperor dragonfly larva,**
2 *Anax imperator* (Leach, 1815)

3
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15
16 **Running title**

17 Dragonfly larval polarization sensitivity

18
19 **Keywords**

20 Polarization vision, optomotor response, turbidity, Rayleigh scattering

21
22 **Summary Statement**

23
24 Behavioural evidence that polarization sensitivity in the Emperor dragonfly larva, *Anax*
25 *imperator*, reduces the contrast-degrading effect of scattered light under naturalistic
26 horizontally polarized underwater lighting conditions.

27
28 **Abstract**

29
30 Polarization sensitivity (PS) is a common feature of invertebrate visual systems. In insects, PS
31 is well known for its use in several different visually guided behaviours, particularly
32 navigation and habitat search. Adult dragonflies use the polarization of light to find water but
33 a role for PS in aquatic dragonfly larvae, a stage that inhabits a very different photic
34 environment to the adults, has not been investigated. The optomotor response of the larvae of
35 the Emperor dragonfly, *Anax imperator*, was used to determine whether these larvae use PS

36 to enhance visual contrast underwater. Two different light scattering conditions were used to
37 surround the larval animals: a naturalistic horizontally polarized light field and non-
38 naturalistic weakly polarized light field. In both cases these scattering light fields obscured
39 moving intensity stimuli that provoke an optokinetic response in the larvae. Animals were
40 shown to track the movement of a square-wave grating more closely when it was viewed
41 through the horizontally polarized light field, equivalent to a similar increase in tracking
42 ability observed in response to an 8% increase in the intensity contrast of the stimuli. Our
43 results suggest that larval PS enhances the intensity contrast of a visual scene under partially
44 polarized lighting conditions that occur naturally in freshwater environments.

45

46 **Introduction**

47

48 Amongst insects, polarization sensitivity (PS) plays an important role in navigation where it is
49 mediated by the highly specialised visual photoreceptors located in the dorsal rim area (DRA)
50 of the compound eye, these photoreceptors being used to detect polarized patterns in skylight
51 (Labhart and Meyer, 1999; Homberg et al., 2011). Some species also use polarization signals
52 for mate recognition (Sweeney et al., 2003) or to aid the detection of food sources (Kelber et
53 al., 2001; Foster et al., 2014). The ventral short-wave sensitive photoreceptors of many water-
54 seeking insects can be polarization sensitive and are used to detect and approach horizontally
55 polarized light reflected from water bodies, a behaviour termed positive polarotaxis (Schwind,
56 1991; Schwind, 1995; Lerner et al., 2008; Kriska et al., 2009).

57

58 Adult dragonflies (Odonata: Aeshnidae) have a polarization sensitive DRA (Meyer and
59 Labhart, 1993) as well as ventrally directed PS that is mediated by photoreceptors in the
60 ventral part of the compound eyes (Laughlin, 1976; Laughlin and McGinness, 1978).

61 Electrophysiological studies have shown that these regions are both maximally sensitive to
62 short wavelengths: the UV in *Hemicordulia tau*, and the “blue” region of the spectrum in
63 *Hemianax papuensis* (Laughlin, 1976). Positive polarotaxis has been demonstrated
64 behaviourally in odonates indicating that the polarization of light is an important visual cue
65 for locating suitable freshwater sites, which are extensively used for mating (Kriska et al.,
66 2009) and oviposition (Horváth et al., 1998; Horváth et al., 2007; Kriska et al., 2009).

67 Compound eye mediated PS in terrestrial adult odonates may be limited to navigational and
68 water-seeking tasks, although it is possible it is also used in other contexts. Aeshnid dragonfly
69 larvae are also highly dependent on vision and, like adults, possess large compound eyes

70 (Corbet, 2004). Despite this, little research attention has been paid to the visual adaptations of
71 dragonfly larvae, particularly in the context of their natural underwater environment.

72

73 The photic environment of aquatic dragonfly larvae differs considerably from that
74 experienced by the adult animals. Larvae inhabit slow moving streams or ponds where there
75 is often high levels of light scattering and spectral attenuation due to turbidity and the
76 presence of dissolved organic matter that absorbs strongly at short wavelengths (Lythgoe,
77 1979; Davies-Colley and Vant, 1987; Markager and Vincent, 2000). Light underwater can
78 also become partially polarized depending on its interaction with suspended particles smaller
79 than the wavelength of light and the direction of entry from the aerial hemisphere via Snell's
80 window (Horváth and Varjú, 1995). The degree of polarization has been measured in
81 freshwater at *ca.* 35% at midday and up to 67% at crepuscular periods when the sun is near
82 the aerial horizon (Novales Flamarique and Hawryshyn, 1997). The predominant angle of
83 polarization of light underwater is predictable, and when the sun is close to its zenith, at solar
84 midday, or the sky is overcast, the angle of polarization is predominately horizontal (i.e.
85 parallel to the water surface) (Novales Flamarique and Hawryshyn, 1997). On clear days,
86 polarization angle changes depending on the position of the sun, with a maximum deviation
87 from the horizontal, in directions perpendicular to the direction of the sun, of approximately
88 48.5° occurring at sunset or sunrise when the sun is at the terrestrial horizon (Hawryshyn,
89 1992; Waterman, 2006).

90

91 Scattering of light that occurs underwater between a viewer and an object, often called veiling
92 light, degrades the visual contrast between an object and its background. A proportion of this
93 scattered light is polarized at one predominant angle, due to Rayleigh scattering from sub-
94 wavelength particles present in the water. Thus, the intensity contrast of the scene can be
95 increased by selectively filtering the polarized component of the scattered light (Lythgoe and
96 Hemmings, 1967; Schechner and Karpel, 2005). Visual PS has been shown in several aquatic
97 animals. It has been suggested that PS may have evolved due to the advantages that can be
98 gained by processing out naturally occurring underwater linearly polarized light, improving
99 visual contrast. Such processing could, for instance, significantly enhance the visual contrast
100 of prey and predators seen against their background. A range of different behavioural
101 experiments have been carried out on diverse marine aquatic animals including octopus
102 (Shashar and Cronin, 1996), cuttlefish (Shashar et al., 2000; Temple et al., 2012; Cartron et
103 al., 2013), squid (Shashar et al., 1998; Pignatelli et al., 2011), and stomatopods (Marshall et
104 al., 1999; How et al., 2014), and although each study set out with a different aim, all

105 demonstrated the potential for PS to enhance object detection underwater. Such ability also
106 has clear adaptive potential for freshwater aquatic animals, particularly to visual predators
107 such as dragonfly larvae that often need to detect and assess possible prey against partially
108 polarized background spacelight.

109

110 The aim of this study was to test the effect of the polarization of the aquatic light environment
111 on the visually-mediated tracking behaviour of the hawker Emperor dragonfly larva, *Anax*
112 *imperator*, in response to moving square-wave gratings seen by subject animals through a
113 polarized veiling light field. To infer a biologically relevant relationship between contrast
114 detection and PS, the degree of polarization in our experiments was kept to levels known to
115 occur underwater. We show that animals were more responsive to the stimuli when they were
116 viewed through a naturalistic horizontally polarized light field with a percentage polarization
117 in the range 14.5 – 21.3% rather than through a non-naturalistic weakly polarized light field
118 of between 5.5 – 7.2% percentage polarization and a vertical angle of polarization. We
119 demonstrate that this increase in response is equivalent to that observed when the intensity
120 contrast of the square-wave grating is increased by 8%. Findings are discussed in relation to
121 the ecology, behaviour and development of *A. imperator*.

122

123 **Results**

124

125 **Experiment 1**

126

127 Experiment 1 tested the optomotor response of larvae to moving square-wave gratings, of
128 four different fundamental spatial frequencies, viewed either through veiling light that was
129 naturalistically horizontally polarized, or that was non-naturalistically weakly vertically
130 polarized. We aimed to test the hypothesis that, if polarization sensitive, larvae use the
131 polarization of light to enhance their ability to perceive intensity stimuli in a naturally
132 polarized aquatic environment. Gain, the ratio of the angular rotation rate of the larva's head
133 relative to the rotation rate of the grating was used as a measure of response. In total, 18
134 (instar f-3, n = 7; f-2, n = 5; f, n = 6) of the total 20 animals responded to a moving $16.35 \pm$
135 0.05% intensity contrast grating above the threshold level of 0.1 gain (see Material and
136 Methods section for details of the gain threshold), averaged across all 8 paired trials per
137 animal. Response, either saccadic or smooth tracking (Fig. 1A and B) was measured as the
138 average across 8 trials per animal. Saccadic tracking was less common, only occurring in 12
139 of 99 trials (i.e. 12%) in which a response was observed. Fitting linear mixed models revealed

140 a number of significant fixed factors (Table 1). The animals' responses to the two different
141 polarized light fields (LF) were found to depend on the spatial frequency (SF) of the grating
142 (Linear Mixed Model (LMM), $df = 3$, $\text{Chi}^2 = 13.3$, $p = 0.004$; Fig. 1C). In both light fields,
143 gain was low for both low and high spatial frequencies, SF1 and SF4 (Fig. 1C), and higher in
144 response to intermediate spatial frequency, SF2 (Fig. 1C). Responses to SF3 varied with light
145 field and there was a significantly greater response when animals viewed SF3 through the
146 strongly horizontally polarized light field (mean gain = 0.37, 95% CIs = 0.25 to 0.53)
147 compared with the weakly vertically polarized light field (mean gain = 0.13, 95% CIs = 0.06
148 to 0.21) (Tukey's test, $p < 0.001$; Fig. 1C). Both trial order (ORDER) and drum direction
149 (DIR) independently affected the responses of animals to the moving grating. However, the
150 order of trials was pseudorandomised to account for these order effects and both fixed effects
151 were controlled for in the analysis. No significant difference in response was observed
152 between different larval instars (LMM, $df = 2$, $\text{Chi}^2 = 2.03$, $p = 0.363$).

153

154 **Experiment 2**

155

156 Experiment 2 was designed to test whether the change in response observed in Experiment 1
157 between the naturalistic and non-naturalistic light fields could be replicated by altering the
158 intensity contrast of moving gratings. This explored the hypothesis that stronger optomotor
159 responses in the naturalistic light field would match increased responses to an enhanced
160 perceived intensity contrast of the grating. Larvae were tested with the same four moving
161 square-wave gratings as Experiment 1, with three different intensity contrasts (16.3%, 20.3%
162 and 24.3%) that were seen through the non-naturalistic, weakly vertically polarized veiling
163 light. All 15 animals (instar f-2, $n = 10$; f-1, $n = 3$; f, $n = 2$) responded above the threshold of
164 0.1 gain averaged across all 12 trials and all data were therefore included in further analyses.
165 Animals' responses were again influenced by a number of factors (Table 2). The responses
166 depended on both the spatial frequency of the grating (SF) and grating contrast
167 (CONTRAST), indicated by a significant interaction between these two factors (LMM, $df =$
168 6 , $\text{Chi}^2 = 16.1$, $p = 0.013$; Fig. 2). Inspection of Fig. 2 shows that changing the contrast of the
169 grating stripes affected the responses of animals to the SF3 grating. This was similar to the
170 observed change in response to the different polarizations of surrounding light fields when
171 SF3 was tested in Experiment 1. A significant increase in gain was observed at SF3 when the
172 contrast was increased from 16.3% (the grating contrast used in Experiment 1) to 24.3%, an
173 8.0% increase in the absolute contrast (Tukey's test, $p = < 0.01$; Fig. 2). Average gain was not
174 significantly different between contrasts of 16.3% and 20.3% (Tukey's test, $p = 0.207$), nor

175 between contrasts of 20.3% and 24.3% (Tukey's test, $p = 0.418$). Responses were not
176 significantly different between contrasts at all other spatial frequencies. The order of drum
177 rotation (ORDER), direction of rotation (DIR), and animal instar (INSTAR) did not
178 significantly affect the responses of animals to the moving grating (Table 2).

179

180 **Discussion**

181

182 This study is the first to demonstrate polarization sensitivity (PS) in a larval odonate. The
183 most parsimonious interpretation of our results is that the PS of *Anax imperator* larvae
184 functions to improve visual contrast by selectively filtering polarized light scattered by the
185 underwater light environment. Whilst previous experiments (Shashar et al., 1998; Shashar et
186 al., 2000) have suggested that PS and opponent processing could improve visual contrast for
187 any object whose polarization differs from the background, or by cutting out intervening
188 polarized scattered light (Lythgoe and Hemmings, 1967; Schechner and Karpel, 2005), this
189 study presents behavioural evidence for the latter mechanism in an aquatic insect.

190 Importantly, the methodologies used tested the contrast enhancement capability of larvae
191 under naturalistic levels of degree of polarization.

192

193 Dragonfly larvae exhibited optomotor responses to the moving square-wave gratings by
194 movement of the head and, in some cases, the body, in the direction of drum rotation. These
195 mirror similar innate optomotor responses to moving gratings that have been demonstrated in
196 a range of different species (Collewijn, 1970; David, 1979; Maaswinkel and Li, 2003). These
197 responses provide a mechanism to reduce the motion of the visual image on the retina (retinal
198 slip) when the visual scene is displaced relative to the gaze of the animal. In practice, this
199 enables animals experiencing retinal slip during periods of motion to stabilise their position
200 relative to the environment, for example during flight (Srinivasan and Zhang, 2004) or in
201 moving water (Maaswinkel and Li, 2003). Such wide field motion detection is highly
202 important for aeshnid dragonfly larvae, to maintain body position in moving water during
203 periods of active hunting.

204

205 Whether an animal responds to an optomotor stimulus depends principally on an individual's
206 contrast sensitivity function (CSF), a function of both spatial frequency and contrast. The CSF
207 has been characterized for many different taxa, including humans (De Valois et al., 1974),
208 goldfish (Northmore and Dvorak, 1979) and blowflies (Dvorak et al., 1980), and has a
209 characteristic inverted-U shape. The inverse of the CSF describes the contrast sensitivity

210 threshold (CST): the minimum contrast required by the visual system to detect a certain
211 spatial frequency. Therefore, generally speaking, a higher contrast is needed to detect or
212 respond to higher or lower spatial frequencies than to mid-range spatial frequencies. The
213 optomotor responses (gain) of the dragonfly larvae to all four different spatial frequencies
214 (SF1 = 0.03, SF2 = 0.06, SF3 = 0.01 and SF4 = 0.12 cycles/°) were consistent with such a
215 CST. Larvae exhibited their highest level of response when tested with mid spatial
216 frequencies (SF2 and SF3) and lagged behind the rotation of the grating to a greater degree at
217 upper and lower spatial frequencies (SF1 and SF4).

218

219 Only the responses of larvae to SF3 gratings were affected by the polarization of the veiling
220 light field and by changes in the intensity contrast of the square-wave gratings. No such
221 changes in response were observed for the other three spatial frequencies tested and we
222 propose the following explanation to describe this relationship. We suggest that, in both
223 experiments, the animal's CST curve can explain the responses of larvae to the different
224 spatial frequencies and contrasts. This interpretation is shown diagrammatically in Fig. 3.
225 The perceived contrast of the grating must exceed the CST for subjects to detect and respond
226 to its rotation. Therefore, in Experiment 2, even the highest intensity contrast tested (24.3%)
227 did not exceed the CST at SF1 or SF4, leading to weak or absent responses. Similarly all
228 contrasts tested (16.3%, 20.3% and 24.3%) were above the CST at SF2. We propose that, at
229 SF3, only the highest contrast (24.3%) was sufficient to exceed the CST (Fig. 3). Conversely,
230 at the lower intensity contrasts of 16.3% and 20.3% that were closer to, or below, the CST,
231 only weaker and absent responses respectively were seen as responses to the moving grating.
232

233 A similar change in response was also observed only at SF3 in Experiment 1, when animals
234 viewed the grating through differently polarized light fields. Specifically, at SF3, animals
235 only exhibited a strong optomotor response when viewing the grating through the naturalistic,
236 more strongly horizontally polarized light field. We suggest that these data are consistent with
237 the explanation that the larval PS reduces the visual interference of the scatter in the veiling
238 light field, elevating the perception of the visual contrast above the CST. This increase in
239 perceived contrast is greater in the light field, mimicking that found in nature (more strongly
240 horizontally polarized), than the non-naturalistic light field (low percentage polarization and
241 vertically polarized) suggesting that larval PS may be well adapted to reduce the partially
242 polarized scatter found naturally occurring in the freshwater environment. A mechanism
243 based on PS to reduce the contrast-degrading effect of veiling light or haze, would be adaptive

244 both for broad field visual functions (e.g. optomotor associated motion stabilization) and for
245 small field visual behaviours such as prey tracking and capture.

246

247 Mechanisms underlying PS in insects have been well studied, particularly in species that
248 utilise polarized light for navigation (Homberg et al., 2011). These mechanisms include the
249 alignment of dichroic visual pigment chromophores within the photoreceptor microvilli and
250 the orthogonal arrangement of adjacent photoreceptor microvilli (Labhart and Meyer, 1999;
251 Roberts et al., 2011). In the ventral region of the adult dragonfly eye (*Hemicordulia tau*),
252 polarization sensitive cells also have microvilli oriented in two perpendicular directions,
253 horizontally and vertically, relative to the body axis (Laughlin, 1976; Laughlin and
254 McGinness, 1978). This suggests a putative two-channel polarization system, capable of
255 analysing the angle and degree of polarization, albeit with predictable neutral points and
256 confusion states that would only be overcome with additional channels (Bernard and Wehner,
257 1977).

258

259 In the larval visual system of *A. imperator*, polarized light could be used to enhance the
260 perceived contrast of the visual scene by one of a number of independent mechanisms. For
261 example, using an opponent two-channel polarization detector could de-haze an image
262 (Bernard and Wehner, 1977; Tyo et al., 1996). Even more simply, a single channel detector
263 with a vertically oriented axis would decrease the absorption of horizontally polarized light
264 (Roberts et al., 2011; Roberts et al., 2014) with an example of this mechanism previously
265 being found in certain regions of the fiddler crab eye, where it is thought to remove the glare
266 from mud flats (Alkaladi et al., 2013). It has also been suggested that similar mechanism
267 exists in the ventral part of the eyes of pond skaters, *Gerris lacustris* (Schneider and Langer,
268 1969), serving to filter glare from the surface of the water. However, the structural basis of PS
269 larval *Anax imperator* is still to be determined.

270

271 Behavioural experiments have suggested that the visual systems of various aquatic animals
272 including trout (Novales Flamarique and Browman, 2001), squid (Shashar et al., 1998),
273 cuttlefish (Shashar et al., 2000; Pignatelli et al., 2011; Temple et al., 2012; Cartron et al.,
274 2013) and crayfish (Tuthill and Johnsen, 2006) are able to analyse the polarization of light as
275 a way to increase the detection of objects underwater. In many of these experiments, however,
276 animals were tested under non-naturalistic lighting conditions, for example, using point-
277 source illumination or percentages of polarization that far exceed those found in nature. In the
278 methodology developed in this experiment, the light field experienced by the subject was

279 created to be as naturalistic as possible by using downwelling light and percentage
280 polarization levels within the range of that found in the habitat of the dragonfly larva. It
281 should be noted in the future that such methods provide a tractable way to demonstrate
282 environmentally relevant behavioural responses.

283

284 As a final consideration, the PS of the adult dragonfly visual system has been demonstrated
285 both by using electrophysiology and by multiple-choice behavioural experiments (Laughlin,
286 1976; Horváth et al., 1998; Horváth et al., 2007; Kriska et al., 2009). Whilst adult dragonflies
287 likely use the horizontally polarized light reflected from water surfaces to detect suitable
288 habitats for oviposition (females) and mating (males), and possibly also for flight
289 stabilization, these behaviours are specific to the terrestrial/aerial stage. Our results
290 demonstrate that larvae also have PS, but for behaviours specific to the aquatic life stage:
291 contrast enhancement of a visual scene in a partially polarized, turbid environment. These
292 findings suggest that there is considerable developmental plasticity in the PS of the dragonfly
293 compound eye, with PS being used for markedly different visual tasks in adults and larvae.

294

295 **Materials and Methods**

296

297 **Experimental set up**

298

299 Four larval instars (f, f-1, f-2 and f-3; where f is the final instar before metamorphosis, f-1 is
300 one instar before final etc.) of the Emperor dragonfly *Anax imperator*, obtained from Blades
301 Biological Ltd. (Essex, UK), were used for behavioural experiments. Individuals were housed
302 in separate compartments, to avoid cannibalism, within a large aquarium filled with clear de-
303 chlorinated tap water maintained at 15°C. White fluorescent room lighting provided a 12 h:12
304 h daily light:dark cycle. Animals were fed *ad libitum* with live annelid worms, *Dendrobaena*
305 sp. (Wormsdirect, Essex, UK) but were tested 3-5 days after a feeding bout.

306

307 For behavioural experiments, a subject dragonfly larva was housed in a small, clear,
308 cylindrical, 10 cm diameter transparent PerspexTM (polymethylmethacrylate) tank filled with
309 clear de-chlorinated tap water and a 1.5 cm layer of aquarium sand (Fig. 4A). A black, opaque
310 plastic collar prevented the subject from viewing the scene below. Black tape covered the top
311 5cm of the inner tank creating a 2.5 cm high clear window through which the animal could
312 view the outside environment. This tank was held within a larger PerspexTM cylindrical tank
313 (25 cm diameter) filled with very dilute milk solution (0.042 g/l skimmed milk powder, 0.1%

314 fat, Sainsbury's Ltd.) in de-chlorinated tap water. Both tanks were held stationary within a
315 large (30 cm diameter) clear Perspex™ cylindrical drum, that could be rotated in a clockwise
316 (CW) or counter-clockwise (CCW) direction (as viewed from above) at 12 and 11 °/second,
317 respectively. Animals were tested in a dark room where illumination was provided only by a
318 circular fluorescent bulb (Circline 22W cool white deluxe Sylvania). The top of the bulb was
319 painted with matte black spray paint and placed directly above the milky water tank to
320 prevent light from the bulb illuminating the grating directly (Fig. 4A). Animals were tested
321 using a square-wave grating, made by printing vertical monochrome stripes on paper that was
322 placed on the outside of the rotatable outer drum, and which was viewed by subjects through
323 the milky water tank. Subjects were filmed from above using a HD digital video camera
324 (Panasonic HC-X900) recording in 1080p/50 mode (1920 x 1080 pixels), at 50 fps.

325

326 **Degree of polarization measurements**

327

328 All spectral measurements were made using a spectrophotometer (USB2000, Ocean Optics)
329 coupled to fibre optic (Ocean Optics UV-vis, 200 µm diameter) and a collimating lens (Ocean
330 Optics 74-UV) which focussed light from a small (approx. 5 mm diameter) area on the
331 surface to be measured into the fibre optic. To avoid bending the fibre into the apparatus, light
332 from the square-wave grating was reflected from a front-surface polished aluminium mirror
333 angled at 45° positioned inside the clear water tank, which was filled with distilled water.

334 The intensity of small areas of the grating, as seen through the milky water tank, was thus
335 measured, and the Michelson contrast calculated between the grating stripes. To characterize
336 the polarization of light, a rotatable linear polarizer was fixed to the lens at the end of the
337 optic fibre. Spectral measurements were made through the milky water tank of the light and
338 dark stripes of the grating were obtained, and the percentage polarization of the grating stripes
339 was calculated, for both horizontally and vertically polarized light fields, using to the equation

340

$$341 \quad \text{Percentage Polarization} = \left(\frac{I_{max} - I_{min}}{I_{max} + I_{min}} \right) \times 100, \quad (1)$$

342

343

344 where I_{max} and I_{min} are the radiant intensities of the light when the transmission axis of the
345 linear analyser polarizer is rotated until the maximum and minimum number of counts are
346 recorded, respectively. This use of this equation assumes there was no ellipticity in the
347 polarization of the light field.

348

349 **Illumination**

350

351 The polarization of the light field that surrounded the animal was controlled by the
352 transmission axis orientation of linear Polaroid™ filters placed directly above the milky water
353 tank, beneath the light source. Milk was used as it created a turbid, contrast-degrading
354 environment with a high proportion of Rayleigh scattering, due to the presence of sub-
355 wavelength particles. Sector-shaped pieces of linear polarizer (Rosco 730011, London, UK)
356 were sandwiched between two circular pieces of 3 mm thick Perspex™. The transmission
357 axes of the filter segments were oriented either radially or tangentially to create two polarizer
358 discs with differently oriented transmission axes (Fig. 4B). The polarization of the incoming
359 light affected both the degree and polarization angle of the light, scattered by the milky water
360 (See Supplemental Figure 1). When the disc with tangentially arranged polarizer segments
361 was placed above the milky water tank, the light field surrounding the inside clear water tank
362 housing the animal was strongly horizontally polarized. When the disc with radially oriented
363 polarizer segments was used, the light field was weakly vertically polarized.

364

365 Square-wave gratings with four fundamental spatial frequencies (SF1 0.03 (± 0.01), SF2 0.06
366 (± 0.02), SF3 0.010 (± 0.03), and SF4 0.12 (± 0.04) cycles/° measured from the centre of the
367 experimental chamber) were printed on paper and were used to test the optomotor response.
368 The error quoted is the maximum deviation in spatial frequency with visualization distance
369 within the arena about the mean. The grey levels of the printed dark and light stripes were
370 varied until their radiances were as near equal as possible when viewed through the two light
371 fields (See Supplemental Figure 2). Consequently, the difference in the intensity contrast of
372 the gratings, averaged over the wavelength range 400 to 700 nm, between the two light fields
373 was not significant ($n = 3$, average difference = 0.09%, $sd = 0.42\%$). Light in the UV region
374 of the spectrum was not used in these experiments as odonate larvae lack a dedicated UV-
375 sensitive visual pigment (Futahashi et al., 2015). In both light fields the lighter stripe had a
376 lower percentage polarization than the darker stripe, likely due to the brighter paper reflecting
377 more unpolarized light towards the central tank thus lowering the value. The percentage
378 polarization of the light and dark stripes in the vertically polarized light field was 5.5 and
379 7.2%, respectively. Values were higher under the horizontally polarized conditions at 14.5
380 and 21.3%.

381

382 The intensity contrast of the grating was measured in the horizontally or vertically polarized
383 light fields, with and without linear polarizing analysers in the light path, and the resulting

384 change in contrast, compared with measurements in the absence of a linear analyser, was
385 quantified. When the grating was viewed through the horizontally polarized light field, the
386 addition of a vertically oriented linear polarizing analyser increased the contrast by 4.2% (Fig.
387 4C). In the vertically polarized light field with a low percentage polarization, there was an
388 increase in contrast of 1.0% when vertically polarized light was excluded with the analyser
389 (Fig. 4D). The measured contrast of the gratings was reduced by 2.9% and 1.0% when the
390 transmission axis of the linear polarizer was aligned with the predominant angle of
391 polarization in the horizontally and vertically polarized light fields, respectively (Fig. 4C, D).
392 In summary, filtering the respective predominant angle of polarization in each light field
393 caused an increase in intensity contrast of the grating but this increase was greater in the
394 horizontally polarized light field due to its higher percentage polarization.

395

396 For each set of behavioural experiments, individual larvae were transferred from their home
397 aquarium to the inner chamber of the apparatus and allowed to acclimatise to the new
398 environment for 30 minutes. After this, once the subject animal had been stationary for at
399 least 5 seconds in the clear water tank, a square-wave grating was rotated in either the CW or
400 CCW direction for 30 seconds. Preliminary trials indicated that when a square-wave grating
401 was rotated in the opposite direction to that which the animal was oriented then erratic
402 swimming behaviours were likely to be elicited. For this reason, the grating was always
403 rotated in the direction that the animal was facing or in a randomized direction if there was no
404 clearly directed starting orientation. A minimum 4 minute interval was allowed between each
405 trial. The order of trial presentation was pseudorandomised using a Latin square design to
406 minimize the effect of presentational order.

407

408 **Experiment 1**

409

410 Each animal (instar f-3, n = 7; f-2, n = 6; f, n = 7) was tested with all four grating spatial
411 frequencies in both the natural horizontally and weakly vertically polarized light fields with a
412 grating intensity contrast of $16.35\% \pm 0.05\%$.

413

414 **Experiment 2**

415

416 Each animal (instar f-2, n = 10; f-1, n = 3; f, n = 2) was tested with all four spatial frequencies
417 in a weakly vertically polarized light field. The intensity contrast of the gratings tested were
418 16.30%, 20.30% and 24.30%

419

420 **Video analysis**

421

422 The video recording of each trial was split into individual frames using Avidemux open
423 source, non-linear video editing software and, for every 30th frame, a rostro-caudal line was
424 drawn equidistant from both eyes along the head of the animal and the absolute head angle
425 measured using ImageJ (Abràmoff et al., 2004). The head angle of the animal was plotted for
426 each 30 second trial and, where applicable, used to extract a 6 second region during which the
427 animal exhibited an optomotor response, indicated by a change in angle of at least 2° per 30
428 frame interval (or 0.6 seconds). When saccades were present, only periods of smooth tracking
429 between the rapid movements in the opposite direction were used for measurements of the
430 optomotor response. For saccadic tracking, gain was calculated for each separated non-
431 saccadic period by regression, and a mean value obtained from these. In cases where there
432 was no obvious optomotor response data between 3 and 9 seconds from start of drum rotation
433 were used. For non-saccadic tracking a regression line was fitted to the angular change data
434 and the head angular velocity calculated. Gain, a commonly used measure of the optomotor
435 response that compares the ratio of the rotational angular velocity of the animal compared
436 with the grating, was calculated according to the equation

437

438

439

$$Gain = \frac{\text{animal head angular velocity}}{\text{drum angular velocity}} . \quad (2)$$

440

441

442 **Statistical analysis**

443

444 Quality checks were performed on gain data prior to statistical analysis such that
445 unresponsive animals with gain values less than 0.1, averaged across all trials, were removed
446 from the analysis. Linear mixed models were fitted to the data (gain) in R version 3.0.2 (R
447 Core Team, 2013) using the package *lme4* (Bates et al., 2014) and the function *lmer*. Data
448 from Experiments 1 and 2 were log and square root transformed, respectively, so that they
449 were normally distributed about their means before statistical analysis. Data were back-
450 transformed before presentation in figures. Fixed effects used in Experiment 1 were the
451 polarization of the light field, spatial frequency of the grating, direction of drum rotation,
452 order of trial presentation, and animal instar. In Experiment 2, the contrast of the grating
453 replaced the polarization of the light field as a fixed effect. The significance of each effect on

454 the fit of the model was compared using an analysis of variance (ANOVA) with a probability
455 significance threshold of 0.05 and the Akaike Information Criterion (AIC) used to identify the
456 better fitting model. As this experiment had a repeated measures design, animal identity was
457 included as a random factor.

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462

463 **Author contributions**

464

465 C.R.S carried out behavioural experiments, analysed the data and wrote the paper. N.W.R and
466 J.C.P interpreted data and edited the paper.

467

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471

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593

594 **Figure Captions**

595

596 **Fig. 1. Smooth and saccadic responses to moving square-wave gratings and averaged**
597 **responses to gratings seen through the naturalistic horizontally or weakly vertically**
598 **polarized light field.** A: In a relatively small number of trials (see text) animals responded to
599 the movement of the grating (indicated by the dashed line) with periods of smooth tracking
600 followed by rapid, saccadic, movement of the head in the opposite direction (A; black arrows
601 indicating start of saccade and grey arrows indicating start of smooth tracking). B: The
602 majority of animals tracked the drum smoothly but, typically, lagged behind the movement of
603 the drum, indicated by gain values < 1.0 in all cases. C: the responses (gain) of larvae to four
604 spatial frequencies (SF1 to SF4; 0.032, 0.063, 0.095, 0.121 cycles per degree respectively) in
605 horizontally polarized (black solid lines) and vertically polarized (red dashed lines) light
606 fields. There was a significant difference in response to grating SF3 between the two light
607 fields. Error bars represent ± 1 standard deviation.

608

609 **Fig. 2. Averaged responses to different grating contrasts.** Responses (gain) of larvae to
610 gratings having four different spatial frequencies (SF1 – SF4; 0.032, 0.063, 0.095, 0.121
611 cycles per degree, respectively) and three different intensity contrasts, 16.3% (red dotted
612 lines), 20.3% (blue dashed lines) and 24.3% (black solid lines), seen through a vertically
613 polarized light field. Error bars represent ± 1 standard deviation. Responses varied most to
614 grating SF3, with gain increasing with grating contrast.

615

616 **Fig. 3. Hypothetical *A. imperator* contrast sensitivity threshold (CST).** The proposed CST,
617 solid line, of the *A. imperator* larval visual system superimposed on the experimental grating
618 contrasts and spatial frequencies tested in Experiment 2; 16.3% contrast (solid circles), 20.3%
619 (crosses) and 24.3% contrast (open circles) are plotted for all four spatial frequencies. We
620 suggest that the responses of larvae were determined by the perceived contrast of the grating,
621 and whether this contrast fell above or below the CST. This proposed CST curve explains the
622 low or lack of response to SF1 and SF4 as both contrasts fall below the CST. At SF2, both
623 contrasts fall above the CST but at SF3, only the higher contrast, 24.4% (black) exceeds the
624 CST thus at this spatial frequency we see a difference in response to different intensity grating
625 contrasts. We propose that the difference in response between polarized light fields is
626 explained in the same way, by means of a difference in perceived contrast.

627 **Fig. 4. Experimental apparatus used to test the optomotor response and the changes in**
628 **contrast of the square-wave gratings when polarization filtering was applied. A:**
629 Experimental set up to test the optomotor response of dragonfly larvae to a moving square-
630 wave grating of vertical stripes. The subject animal was contained in the stationary central
631 cylinder of clear water, which was surrounded by a concentric outer tank containing dilute
632 milk solution. Light to the latter, from above, was polarized by one of two linear polarizing
633 discs (B), consisting of radially or tangentially orientated linear polarizers, resulting in
634 vertically or horizontally polarized light (respectively) being scattered towards the subject.
635 This veiling light field reduced the contrast of the grating, which was fixed to a rotatable outer
636 drum. The animal's response was assessed by their measuring their ability to visually track
637 the rotation of the grating. B: Polarizer discs used to change the polarization of the light
638 illuminating the milky water tank, constructed of sectors of Polaroid™ filter. The arrows
639 indicate the transmission axis of the linear polarizer in each sector. Two light fields were
640 created using these discs independently: one vertically polarized (left disc), and the other
641 horizontally polarized (right disc). C and D: Change in intensity contrast of the grating
642 stripes, from measurements made without a linear polarizer, when measurements were made
643 with a linear polarizer placed in front of the fibre with transmission axis oriented vertically
644 (black lines) or horizontally (red lines), selectively filtering horizontally or vertically
645 polarized light, respectively. The data are presented for the two scattering light conditions
646 used in experiments: (C) horizontally polarized scatter, and (D) vertically polarized scatter.
647 The change in the intensity contrast was higher in the horizontally polarized light field with a
648 maximum increase in contrast of 4.2% when horizontally polarized light was filtered using a
649 vertically oriented analyser (see text)

650 **Tables**

651 Table 1. Statistics of the fitted model, for Experiment 1, showing the highest order terms
652 tested with the minimum model. Asterisks indicate significant factors and/or interactions at p
653 < 0.05.

654

Factor/interaction	DF	Chi²	P-value
LF:SF	3	13.3	0.004 *
LF:ORDER	7	24.5	0.001 *
LF:DIR	1	0.32	0.859
SF:ORDER	21	43.7	0.003 *
SF:DIR	3	24.3	0.000 *
ORDER:DIR	7	5.83	0.559
INSTAR	2	2.03	0.363

655

656 Table 2. Statistics of the fitted model, for Experiment 2, showing the highest order terms
657 tested with the minimum model. Asterisks mark significant factors and/or interactions at p <
658 0.05.

659

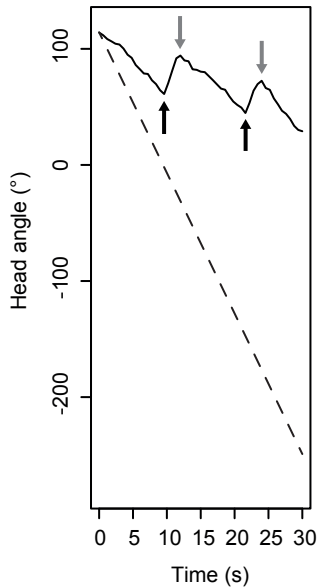
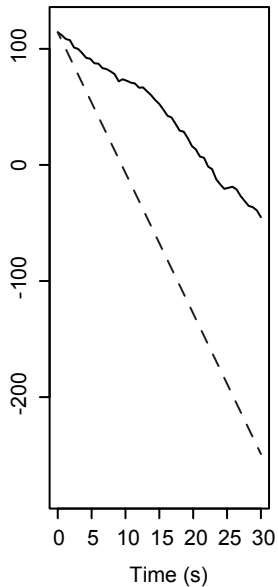
Factor/interaction	DF	Chi²	P-value
CONTRAST:SF	6	16.1	0.013 *
ORDER	11	2.81	0.993
DIR	1	0.13	0.288
INSTAR	2	0.13	0.936

660

661

662

663

A**B****C**