

Sink trap: duckweed and dye attractant reduce mosquito populations

Article

Accepted Version

Cuthbert, R. N., Coughlan, N. E., Dick, J. T. A. and Callaghan, A. ORCID: <https://orcid.org/0000-0002-2731-3352> (2020) Sink trap: duckweed and dye attractant reduce mosquito populations. *Medical and Veterinary Entomology*, 34 (1). pp. 97-104. ISSN 1365-2915 doi: <https://doi.org/10.1111/mve.12417> Available at <https://centaur.reading.ac.uk/87146/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1111/mve.12417>

Publisher: Wiley

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1 *Original Article*

2 **Sink trap: duckweed and dye attractant reduce mosquito populations**

3 *Duckweed and dye cause mosquito sink*

4

5 Ross N. Cuthbert^{*1,2}, Neil E. Coughlan¹, Jaimie T.A. Dick¹, Amanda Callaghan²

6 ¹*Institute for Global Food Security, School of Biological Sciences, Queen's University*

7 *Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, Northern Ireland, United Kingdom*

8 ²*Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading,*

9 *Harborne Building, Reading RG6 6AS, England, United Kingdom*

10

11 *Corresponding author: Ross N. Cuthbert (email, rcuthbert03@qub.ac.uk; telephone,

12 +44(0)28 4272 8230)

13

14

15

16

17

18

19

20

21 **Abstract**

22 Duckweeds, such as *Lemna minor* Linnaeus (Alismatales:Lemnaceae), are common in
23 aquatic habitats and have been suggested to reduce larval mosquito survivorship through
24 mechanical and chemical effects. Further, pond dyes are increasingly used in aquatic habitats
25 to enhance their aesthetics, but have been shown to attract mosquito oviposition. The present
26 study examines the coupled effects of *L. minor* and black pond dye on oviposition selectivity
27 of *Culex pipiens* Linnaeus (Diptera:Culicidae) mosquitoes in a series of laboratory choice
28 tests. Then, using outdoor mesocosms, the combined influence of duckweed and pond dye on
29 mosquito abundances in aquatic habitats is quantified. Mosquitoes were strongly attracted to
30 duckweed, and oviposited significantly greater numbers of egg rafts in duckweed-treated
31 water compared to untreated controls, even when the duckweed was ground. The presence of
32 pond dye interacted with the duckweed and further enhanced positive selectivity towards
33 duckweed-treated water. The presence of duckweed caused significant and sustained
34 reductions in larval mosquito numbers, whilst the relative effects of dye were not evident.
35 The use of floating aquatic plants such as duckweed, combined with dye, may help reduce
36 mosquito populations through the establishment of population sinks, characterised by high
37 rates of oviposition coupled with high levels of larval mortality.

38 **Keywords**

39 biological control; *Culex*; disease vector; floating weed; *Lemna*; lethal effects; oviposition;
40 pond; population sink

41

42

43

44 **Introduction**

45 Habitat selection processes are fundamental to the determination of population and
46 community-level dynamics, and can consequently shape landscape-level patterns of
47 biodiversity (MacArthur and Wilson, 1967; Rosenzweig, 1981; Chesson, 2000). In particular,
48 habitat choice may mitigate detrimental effects through the selective colonisation of available
49 habitat patches which minimise the fitness risk to reward ratio (Werner and Gilliam, 1984;
50 Nonacs and Dill, 1990). In aquatic systems, for example, fitness rewards may be accrued
51 from high resource levels, low competitor densities and low predator abundances, thus
52 ensuring high individual and population fitness whilst mitigating predation risk
53 (Kershenbaum et al. 2012; Albeny-Simões et al. 2014). Moreover, species with terrestrial
54 adult and aquatic larval life stages, such as mosquitoes, must differentiate between discrete
55 aquatic habitat patches in order to minimise the fitness risk to reward relationship
56 (Kershenbaum et al. 2012; Pintar et al. 2018). However, although ovipositional responses to
57 the presence of natural enemies have been well documented for a variety of insects (e.g.
58 Chesson, 1984; Eid et al. 1992a; Åbjörnsson et al. 2002; Vonesh et al. 2009; Vonesh and
59 Blaustein, 2010; Cuthbert et al. 2018b), relatively little is known about trade-offs between
60 detrimental and desirable environmental features in relation to habitat selection (McPeck,
61 2004; Pintar et al. 2018; Cuthbert et al. 2019a, b).

62 Recently, in response to substantial environmental damage and increasing levels of
63 pesticide resistance resulting from the use of synthetic insecticides (Naqqash et al. 2016),
64 there has been an increasing scientific interest in the use of plants or plant extracts for insect
65 pest control (Shaalán and Canyon, 2018; Oladipupo et al. 2019). Indeed, insecticidal
66 properties have been identified across a range of plant species within both aquatic and
67 terrestrial habitats, and many pesticides are based on plant allelochemicals (Shaalán et al.
68 2005). Accordingly, this may also represent an alternative strategy for the control of disease

69 vector insect species, such as mosquitoes (Elango et al. 2010). Yet, ovipositional responses of
70 pest species may modulate the efficacy of such plants or plant compounds in the control of
71 mosquito populations through selective avoidance behaviours; however, these effects have
72 remained largely unquantified (but see Shaalan and Canyon, 2018). Indeed, if mosquitoes
73 avoid plants which have deleterious effects on their aquatic progeny, this could impede
74 population-level control efficacies.

75 Mosquitoes are major vectors of arboviruses and a variety of parasites which have
76 caused unprecedented disease and mortality rates worldwide (Hemingway et al. 2006; Benelli
77 and Mehlhorn, 2016; World Health Organisation, 2018). In their terrestrial adult stage,
78 through the use of visual, olfactory and tactile cues (Bentley and Day, 1989), mosquitoes are
79 frequently observed to be highly responsive to the presence of aquatic predators which
80 consume their larvae, and often display an active avoidance of oviposition within predator
81 colonised habitats (Vonesh and Blaustein, 2010). However, the presence of plants or plant
82 compounds can also profoundly influence larval mosquito survival (e.g. Eid et al. 1992b).

83 The presence of floating aquatic plants can act as a physical barrier which
84 mechanically inhibits larval mosquito respiration and egg hatchability on the water surface
85 (e.g. Hobbs and Molina, 1983). Duckweed spp. (Lemnaceae) are common free-floating
86 aquatic plants which form dense monospecific mats on surface waters. Many species of
87 duckweed are widespread due to an extensive variety of dispersal mechanisms (e.g. Coughlan
88 et al. 2015b, 2017), coupled with high levels of environmental resilience that facilitate long-
89 distance movement by mobile vectors (Coughlan et al. 2015a, b, 2018). Indeed, duckweed
90 spp. have also been found to colonise container-style habitats (Cuthbert pers. obs.), where
91 vectorially important mosquitoes proliferate in peri-urban and urban areas (Townroe and
92 Callaghan, 2014). Duckweed extracts have insecticidal properties which cause high rates of
93 mortality in larval mosquito populations (Eid et al. 1992b). In addition, such plants have been

94 reported to repel female mosquitoes from ovipositing, whilst also favouring mosquito
95 predators such as copepods (Eid et al. 1992a; Yang et al. 2005; Cuthbert et al. 2018c).
96 Accordingly, to enhance mosquito control effects, the development of measures to counteract
97 such ovipositional avoidance behaviour is vital.

98 Recently, commercial pond dyes have been identified as a strong oviposition
99 attractant for mosquitoes (Ortiz-Perea and Callaghan, 2017; but see Ortiz-Perea et al. 2018).
100 Darkened containers may be more attractive to mosquitoes due to a perceived greater water
101 depth, larger load of organic matter for larvae to develop (Williams, 1962; Hoel et al. 2011),
102 or higher degree of shading than alternative habitats (Vezzani et al. 2005). Further, the use of
103 dye has been shown to have a negative effect on the survivorship of mosquitoes to the adult
104 stage (Ortiz-Perea and Callaghan, 2017). In addition, the use of pond dyes has become
105 increasingly common to improve the aesthetics of ponds and lakes (see Ortiz-Perea and
106 Callaghan, 2017). Although the application of pond dyes can reduce the growth of submerged
107 plants and algae through reductions in the penetration of visible light spectrums into water
108 (620-740 nm; Douglas et al. 2003), there is no evidence to suggest that their use will impede
109 the growth of free-floating aquatic plants on the water surface. Thus, the co-application of
110 duckweed and pond dyes may synergise mosquito control efficacy through ovipositional
111 attraction and greater net lethal effects, given that the presence of duckweed or pond dyes can
112 separately facilitate increased rates of larval mortality (Eid et al. 1992a, b; Ortiz-Perea and
113 Callaghan, 2017). Indeed, the use of black pond dye has been shown to reverse ovipositional
114 predator avoidance behaviours by mosquitoes, which has resulted in an enhanced potential
115 for effective biological control (Cuthbert et al. 2018b).

116 The present study therefore examines the effects of duckweed and black pond dye on
117 oviposition selectivity behaviour and natural colonisation of aquatic habitats by wild *Culex*
118 *pipiens* mosquitoes. First, using a series of laboratory ovipositional choice tests, the

119 responsiveness of gravid mosquitoes to the presence of duckweed and duckweed extract is
120 determined. Second, whether the presence of dye further influences observed mosquito
121 oviposition behaviour in response to duckweed is examined. Finally, in an array of outdoor
122 mesocosms, natural colonisation by mosquitoes under factorial duckweed and dye treatments
123 is quantified over time by monitoring larval mosquito abundances. Specifically, whether
124 mosquitoes will avoid ovipositing in the presence of duckweed or duckweed cues, given its
125 reported mechanical and larvicidal effects, and whether the presence of dye will further
126 modulate behavioural responses of mosquitoes to duckweed through enhanced attraction of
127 dye-treated habitats is assessed. Further, whether the presence of duckweed and dye will act
128 in synergy to reduce wild population numbers of larval mosquitoes in aquatic habitats is
129 tested.

130 **Materials and Methods**

131 *Experimental organisms*

132 All experimental organisms were obtained on the University of Reading Whiteknights
133 campus (51°26'12.8"N 0°56'31.8"W). Gravid adult female *C. pipiens* were collected using
134 modified Reiter gravid box traps (Reiter 1987; Townroe and Callaghan 2015). The trap
135 consists of separable upper and lower components. The upper component contains a motor,
136 fan and lead acid battery which creates an air vacuum to draw adult mosquitoes into a
137 collection chamber. The lower portion comprises a tray containing 3 L of bait, a hay and
138 yeast infusion, prepared in advance by fermenting 300 g of hay with 2.5 g of fast-action dried
139 yeast in sealed 80 L outdoor containers for 7 days, stirring occasionally. The bait was then
140 strained and decanted into the lower tray portions of the gravid traps. Trapping occurred
141 overnight, with fresh bait used on each sampling occasion. The following morning, adult
142 mosquitoes were transferred into 30 cm³ cages and were maintained at 25 °C (\pm 1 °C) within

143 a laboratory and under a 16 h light:8 h dark photoperiod. Each cage contained 10 % sucrose-
144 soaked cotton for sustenance. Although *C. pipiens* comprised > 99 % of the mosquitoes
145 trapped, individuals of *Culiseta annulata* (Schrank) (Diptera:Culicidae) and *Anopheles*
146 *plumbeus* Stephens (Diptera:Culicidae) were also collected and dispatched. Duckweed,
147 *Lemna minor* was collected from artificial container-style aquatic habitats by trawling a 1
148 mm mesh net along the surface waters, before being transferred to the same laboratory, where
149 it was rinsed and stored in 5 L dechlorinated tap water.

150 *Oviposition preferences*

151 During July-August 2018, in the laboratory (25 ± 1 °C, 16:8 light:dark), oviposition
152 responses of wild-caught gravid adult female *C. pipiens* to the presence of duckweed were
153 determined using choice tests. Groups of adult mosquitoes were released into 30 cm² cages
154 and given a choice of 200 mL arenas (9.5 cm dia.) in which to oviposit. Arenas were
155 positioned in random corners of each cage to avoid positional effects. In all oviposition
156 experiments, egg rafts were removed and enumerated daily from each cage, over a total of 3
157 days.

158 *Laboratory paired choice tests*

159 In paired choice tests (treatment/control), treatment cups contained either 5 g (30 adults cage⁻¹
160 ¹, $n = 6$ cages), 20 g (30 adults cage⁻¹, $n = 4$ cages), 50 g (20 adults cage⁻¹, $n = 5$ cages) of
161 intact duckweed plantlets (Figure 1; Experiment 1a–c), or 5 g of ground (30 adults cage⁻¹, n
162 = 4 cages; Figure 1; Experiment 1d) duckweed plantlets in dechlorinated tap water from an
163 aerated source, paired with a control treatment of dechlorinated tap water alone. The 5 g
164 ground duckweed treatment was also separately paired with a dyed control treatment (Dyofix
165 black liquid pond dye, 0.3g L⁻¹; 20 adults cage⁻¹, $n = 5$ cages: Figure 1; Experiment 1e).
166 Ground duckweed was prepared using a pestle and mortar until it was a paste. Oviposition

167 activity indices (OAI) were calculated for each treatment pair as per Kramer and Mulla
168 (1979):

$$169 \quad OAI = (NT - NC)/(NT + NC) \quad (1)$$

170 where OAI is a function of the number of egg rafts laid in treated water (*NT*) in relation to
171 controls (*NC*). The OAI range is from -1 to 1, where 0 corresponds to no preference, values
172 closer to 1 indicate increasing preference for duckweed treatments and values closer to -1
173 indicate increasing preference for control treatments (i.e. duckweed avoidance).

174 *Laboratory factorial choice tests*

175 In the factorial choice tests, duckweed (present/absent) and dye (present/absent) were
176 presented to wild gravid adult mosquitoes (50 adults cage⁻¹; *n* = 6 cages). Duckweed
177 treatments comprised 5 g of intact duckweed and dye treatments comprised 0.3 g L⁻¹ black
178 liquid pond dye (Dyofix), in dechlorinated tap water from a continuously aerated source
179 (Figure 1; Experiment 2a). The factorial experiment was repeated with ground duckweed
180 plantlets (Figure 1; Experiment 2b).

181 *Natural colonisation*

182 Between August and October 2014, sixteen 40 L mesocosms (48 cm dia.) were established in
183 the experimental gardens of the University of Reading Whiteknights campus (51°26'12.8"N
184 0°56'31.8"W). These mesocosms consisted of artificial containers which had been dug into
185 the ground. In a completely randomised factorial design, mesocosms were treated with
186 duckweed (present/absent) and dye (present/absent) (*n* = 4 per experimental group; Figure 1
187 Experiment 3). Duckweed treatments comprised total coverage of the pond surface by
188 duckweed, whilst dye treatments comprised 0.3g L⁻¹ black liquid pond dye (Dyofix). Each
189 pond was sampled weekly for nine weeks, using a 250 µm mesh dipping net (6 × 12 cm). The
190 net was moved in four figure-of-eight sweeps on each sampling occasion from the top to

191 bottom of mesocosms (as per Ortiz-Perea et al. 2018). Larval *C. pipiens* were identified and
192 then enumerated on a sampling tray before reintroduction into their respective source
193 mesocosms.

194 *Statistical analyses*

195 For the laboratory choice tests, generalised linear mixed models (Bates et al. 2015) assuming
196 a Poisson error distribution were used to analyse total counts of egg rafts with respect to
197 treatment groups. Where residuals were found to be overdispersed (deviation larger than
198 mean), a negative binomial error distribution was employed. In each experiment, 'cage' was
199 included as a random effect to account for the blocked design.

200 A zero-inflated generalised linear mixed model (Fournier et al. 2012) assuming a
201 negative binomial distribution was used to examine the effects of duckweed and dye
202 treatments on counts of larval mosquitoes for the outdoor natural colonisation experiment.
203 Zero-inflation was specified as a constant term across the model. Sampling period was
204 included as a covariate, with individual ponds included as a random effect to account for
205 repeated measures through time. Locally-weighted scatterplot smoothing (LOWESS; 9/10
206 smoother span) lines were also fitted to display the treatment effects on larval mosquito
207 abundances over the experimental period. All statistical analyses were undertaken in R v3.4.4
208 (R Core Development Team 2018).

209 **Results**

210 *Oviposition preferences*

211 In pairwise choice tests, significantly greater numbers of egg rafts were oviposited by *C.*
212 *pipiens* in the presence of duckweed than duckweed-free controls, irrespective of duckweed
213 density (5 g, $z = 6.66$, $p < 0.001$; 20 g, $z = 3.98$, $p < 0.001$; 50 g, $z = 5.71$, $p < 0.001$). Where 5
214 g of ground duckweed was present with an undyed control, significantly more egg rafts were

215 oviposited in the presence of ground duckweed ($z = 5.62, p < 0.001$). However, when paired
216 with a dyed control, there was no significant preference towards ground duckweed-treated
217 water ($z = 0.83, p = 0.41$). These trends were further reflected in OAI values, where
218 preference was significantly displayed towards duckweed-treated water as compared to
219 undyed control groups (Figure 2).

220 In the factorial oviposition experiment, in the presence of intact duckweed,
221 significantly greater numbers of egg rafts were oviposited with duckweed ($z = 4.47, p <$
222 0.001) and dye ($z = 3.35, p < 0.001$) overall (Figure 3a). However, there was no significant
223 ‘duckweed \times dye’ interaction effect here ($z = 1.26, p = 0.21$), although considerably more egg
224 rafts were oviposited under both treatments combined. Similarly, significantly greater
225 numbers of egg rafts were oviposited with ground duckweed ($z = 5.14, p < 0.001$) and dye (z
226 $= 4.70, p < 0.001$) overall (Figure 3b). There was a significant ‘duckweed \times dye’ interaction
227 ($z = 2.27, p = 0.02$), reflecting a strong synergistic effect by the two treatments for mosquito
228 oviposition attraction here.

229 *Natural colonisation*

230 On average, 0.50 ± 0.17 (\pm SE) larval mosquitoes were found in duckweed-treated
231 mesocosms, whilst a mean of 8.31 ± 1.91 (\pm SE) were found in duckweed-free water (Figure
232 3). Overall, significantly fewer larval *C. pipiens* were found in duckweed-treated water ($z =$
233 $4.95, p < 0.001$). There was no significant effect of dye on larval mosquito abundances ($z =$
234 $1.22, p = 0.22$). Additionally, there was no significant ‘dye \times duckweed’ interaction ($z = 0.52,$
235 $p = 0.60$), and therefore the effects of duckweed in reducing mosquito abundances were
236 consistent across levels of the dye treatment. Further, larval mosquito numbers did not differ
237 significantly over the observation period ($z = 0.81, p = 0.42$; Figure 4).

238 **Discussion**

239 Ovipositional habitat selectivity by adult mosquitoes is strongly linked to gradients of fitness
240 risk and reward (Pintar et al. 2018). In recent years, as both insecticide resistance and
241 environmental degradation continue to increase worldwide, alongside an escalated prevalence
242 of mosquito-borne disease, interest in natural biological and environmental measures to
243 control mosquito populations has grown (Cameron and Lorenz, 2013; Shaalan and Canyon,
244 2018). However, more conclusive evidence is urgently required as to the implications of
245 plant-mosquito interactions in a vector control context (Stone et al. 2018). In particular,
246 duckweed-treated waters have been shown to reduce larval mosquito survivorship through
247 mechanical and chemical effects (Hobbs and Molina, 1983; Eid et al. 1992a, b). However,
248 ovipositional responses of adults to duckweed remain poorly understood.

249 The present study has demonstrated that, contrary to previous reports on the same
250 species complex (e.g. O'Meara et al. 1989; Eid et al. 1992a; Yang et al. 2005), ovipositing
251 mosquitoes exhibit preferential selection of *L. minor* colonised habitats, a common duckweed
252 species, in comparison to duckweed-free water. Furthermore, our results indicate that pond
253 dyes have the capacity to interact with the presence of duckweed to synergistically enhance
254 attractiveness to duckweed-treated habitats, likely by darkening water between leaves.
255 However, although duckweed was observed to be a significant attractant, results from our
256 outdoor mesocosms empirically demonstrate that *L. minor* duckweed alone can profoundly
257 decrease the natural population numbers of mosquitoes, whilst the effects of pond dye were
258 undiscernible. Accordingly, the use of duckweed such as *L. minor* may facilitate a population
259 sink for mosquitoes, characterised by high levels of oviposition coupled with reductions in
260 larval abundances. Importantly, although the presence of black pond dye is likely to reduce
261 the growth rate of submerged aquatic plants, it is unlikely to affect floating duckweeds.

262 Previous research has demonstrated the density-dependent nature of mosquito
263 ovipositional deterrence by predator cues (Silberbush and Blaustein, 2011). Through the use

264 of different densities of duckweed, the present study did not find any evidence for density-
265 specific modulations in ovipositional preferences towards duckweed-treated water.
266 Mosquitoes demonstrate strong and sustained preference for duckweed-treated water, even up
267 to high density surface coverage which could act as a mechanical barrier for larval mosquito
268 respiration or egg hatching (Baz, 2017). Although not examined here, it is possible that
269 greater ovipositional preferences could exist for higher rather than lower densities of
270 duckweed, in scenarios where a choice between different densities is available. Our results
271 contrast to other experimentations, which have demonstrated a lack of oviposition in water
272 covered by duckweed by mosquitoes within the *C. pipiens* complex (O'Meara et al. 1989; Eid
273 et al. 1992a; Yang et al. 2005). Although these studies were conducted in a different
274 geographical area from the present study, it is unlikely that different coevolutionary histories
275 caused the variations in results found in the present study, given the high abundance and wide
276 distribution of both duckweed and *C. pipiens* across Great Britain and Ireland (Coughlan et
277 al. 2015b; Townroe and Callaghan, 2015). As oviposition attraction was sustained in the
278 presence of ground duckweed, it is probable that attraction is largely driven by chemical cues,
279 which can have larvicidal effects *via* water-borne synomones (i.e. allelochemicals which
280 evoke a response in mosquitoes) (Angerilli and Beirne, 1974; Eid et al. 1992a, b), as oppose
281 to the visual presence of intact duckweed plantlets. Further, given a lack of significant
282 difference between paired duckweed and dye treatments, the attraction of gravid mosquitoes
283 to duckweed may be deemed similar in strength to the attractiveness of black pond dye
284 previously demonstrated, although the drivers of this attraction between treatments likely
285 differ (Ortiz-Perea and Callaghan, 2017).

286 The present study corroborates with Ortiz-Perea and Callaghan (2017), with black
287 pond dye significantly enhancing the attractiveness of aquatic habitats to gravid adult
288 mosquitoes, which are often reliant on visual cues in habitat selection (e.g. Collins and

289 Blackwell, 2000). Perceived coloration is known to significantly affect ovipositional
290 preferences of container-breeding mosquitoes (e.g. Beehler and DeFoliart, 1990; Beehler et
291 al. 1993; Li et al. 2009; Oliva et al. 2014;), while pond dye effects have been only recently
292 assessed (Ortiz-Perea and Callaghan, 2017; Cuthbert et al. 2019b). Given such strong
293 evidence for colour-based attraction in mosquitoes, it is likely that the attraction shown in the
294 present study is based on the black water coloration itself, rather than effects of specific dye
295 ingredients. Further, dyes from the same manufacturer, but of different colours, have been
296 shown to have no effects of oviposition (see Ortiz-Perea et al. 2018). Therefore, it is unlikely
297 that there are specific organic compounds within pond dyes which further alter selectivity.

298 In the present study, the use of dye strongly interacted with the presence of duckweed,
299 further enhancing oviposition selectivity in duckweed-treated waters. Whilst dye likely
300 facilitates attraction through the darkening of water and the creation of greater perceived
301 nutrient loads (Williams, 1962; Hoel et al. 2011), it is likely that duckweed emits additional
302 attractive cues, given the particularly profound selectivity towards ground duckweed
303 treatments evidenced here. Thus, the use of pond dye and duckweed in synergy may further
304 aid the control of mosquito populations by facilitating higher rates of oviposition in risky
305 habitats. Indeed, both pond dyes and duckweed have been shown to reduce mosquito larval
306 survivorship (Eid et al. 1992a; Ortiz-Perea and Callaghan, 2017), and dye has been shown to
307 not affect interaction strengths between native predators and larval mosquitoes (Cuthbert et
308 al. 2018a). In particular, duckweed has been shown to have larvicidal effects on mosquitoes
309 (e.g. Eid et al. 1992a), and therefore it likely was the main driver of larval abundance
310 reductions in the outdoor colonisation experiment. This combination is attractive from a
311 biological control perspective, as it may draw mosquitoes away from low-risk sites and
312 towards those which are potentially lethal to progeny.

313 Despite the high oviposition attractiveness of duckweed observed in the laboratory,
314 abundances of larval mosquitoes in outdoor mesocosms treated with duckweed were
315 significantly and consistently reduced compared to duckweed-free treatments over the entire
316 experimental period. Where duckweed was absent, dye trended towards reducing larval
317 mosquito abundances, corroborating with Ortiz-Perea and Callaghan (2017); however this
318 effect was small when paralleled with the substantial main effects of *L. minor*. It is likely that
319 the ovipositional preference towards duckweed demonstrated in the laboratory persisted
320 within the outdoor mesocosms, given that wild-caught mosquitoes were used in all
321 oviposition choice tests. Although, egg rafts were not quantified in the colonisation
322 experiment. Nevertheless, reductions in larval mosquito abundances within the outdoor
323 mesocosms may have resulted from toxicities associated with duckweed chemicals (Eid et al.
324 1992a, b), or mechanical effects which inhibit respiration by larvae and the hatching of egg
325 *via* desiccation (Baz, 2017). Moreover, Eid et al. (1992b) report non-lethal effects such as
326 larval malformations in mosquitoes associated with duckweed. Additional environmental
327 context-dependencies as to the impacts of duckweed necessitate further examination,
328 alongside assessments of effects at different times of the year when mosquito populations
329 peak (see Ewing et al. 2019).

330 In conclusion, the present study shows that *L. minor*, a common duckweed species,
331 impacts on the behaviour and survival of mosquitoes. These effects can be further modulated
332 by the presence of pond dyes. Our results imply that duckweeds are lethal to mosquitoes
333 through either chemical or mechanical mechanisms, and may be applied for mosquito control
334 in both temperate and tropical regions. Indeed, novel pond dyes have also demonstrated
335 toxicities to larval mosquitoes over longer exposure times (Ortiz-Perea and Callaghan, 2017;
336 Ortiz-Perea et al. 2018). The present study suggests that both duckweeds and dye could be
337 used to improve the biological control of mosquitoes through the formation of mosquito

338 population sinks that are characterised by high levels of oviposition and mortality. However,
339 further work is required to elucidate the influence of duckweed spp. and pond dyes on
340 population dynamics and community interactions within aquatic ecosystems. Equally, there is
341 a need to refine the specific drivers of mosquito larval mortality when exposed to duckweeds
342 and pond dyes.

343 **Acknowledgements**

344 This study was funded through a Department for the Economy, Northern Ireland studentship.
345 NEC and JTAD are supported by the Irish Environmental Protection Agency research grant
346 2015-NC-MS-4. Thanks go to Matthew Mullins and Josh Spuyman for assistance with data
347 collection. The authors declare that they have no conflicts of interest.

348 **References**

- 349 Åbjörnsson, L., Brönmark, C. and Hansson, L. 2002. The relative importance of lethal and
350 non-lethal effects of fish on insect colonization of ponds. *Freshwater Biology*, 47:
351 1489–1495.
- 352 Albeny-Simões, D., Murrell, E.G., Elliot, S.L., Andrade, M.R., Lima, E., Juliano, S.A, and
353 Vilela, E.F. 2014. Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with
354 predator-killed conspecifics. *Oecologia*, 175: 481–492.
- 355 Angerilli, N.P. and Beirne, B.P. 1974. Influence of some freshwater plants on the
356 development and survival of mosquito larvae in British Columbia. *Canadian Journal*
357 *of Zoology*, 52: 813–815.
- 358 Bates, D., Maechler, M., Bolker, B. and Walker, S. 2015. Fitting linear mixed-effects models
359 using lme4. *Journal of Statistical Software*, 67: 1–48.

360 Baz, M.M. 2017. Influence of the aquatic plant, *Lemna minor* on the development and
361 survival of *Culex pipiens* mosquito immature. *Egyptian Academic Journal of*
362 *Biological Sciences*, 10: 87–96.

363 Beehler, J. and DeFoliart, G. 1990. Spatial distribution of *Aedes triseriatus* eggs in a site
364 endemic for La Crosse encephalitis virus. *Journal of the American Mosquito Control*
365 *Association*, 6: 254–257.

366 Beehler, J., Millar, J. and Mulla, M. 1993. Synergism between chemical attractants and visual
367 cues influencing oviposition of the mosquito, *Culex quinquefasciatus* (Diptera:
368 Culicidae). *Journal of Chemical Ecology*, 19: 635–644.

369 Benelli, G. and Mehlhorn, H. 2016. Declining malaria, rising of dengue and Zika virus: in-
370 sights for mosquito vector control. *Parasitology Research*, 115: 1747–1754.

371 Bentley, M.D. and Day, J.F. 1989. Chemical ecology and behavioural aspects of mosquito
372 oviposition. *Annual Review of Entomology*, 34: 401–421.

373 Cameron, M.M. and Lorenz, L.M. 2013. *Biological and Environmental Control of Disease*
374 *Vectors*. CABI, Wallingford.

375 Chesson, J. 1984. Effect of notonectids (Hemiptera: Notonectidae) on mosquitoes (Diptera:
376 Culicidae): predation or selective oviposition? *Environmental Entomology*, 13: 531-
377 538.

378 Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of*
379 *Ecology and Systematics*, 31: 343–366.

380 Collins, L.E. and Blackwell, A. 2000. Colour cues for oviposition behaviour in
381 *Toxorhynchites moctezuma* and *Toxorhynchites mboinensis* mosquitoes. *Journal of*
382 *Vector Ecology*, 25: 127–135.

383 Coughlan, N.E., Kelly, T.C., Davenport, J. and Jansen, M.A.K. 2015a. Humid microclimates
384 within the plumage of mallard ducks (*Anas platyrhynchos*) can potentially facilitate
385 long distance dispersal of propagules. *Acta Oecologia* 65–66: 17–23

386 Coughlan, N.E., Kelly, T.C. and Jansen, M.A.K. 2015b. Mallard duck (*Anas platyrhynchos*)-
387 mediated dispersal of Lemnaceae: a contributing factor in the spread of invasive
388 *Lemna minuta*? *Plant Biology*, 17: 108–114.

389 Coughlan, N.E., Kelly T.C. and Jansen M.A.K. 2017. “Step by step”: high frequency short-
390 distance epizoochorous dispersal of aquatic macrophytes. *Biological Invasions*, 19,
391 625–634.

392 Coughlan, N.E., Cuthbert, R.N., Kelly, T.C. and Jansen, M.A.K. 2018. Parched plants:
393 survival and viability of invasive aquatic macrophytes following exposure to various
394 desiccation regimes. *Aquatic Botany*, 150: 9–15.

395 Cuthbert, R.N., Callaghan, A. and Dick, J.T.A. 2018a. Dye another day: the predatory impact
396 of cyclopoid copepods on larval mosquito *Culex pipiens* is unaffected by dyed
397 environments. *Journal of Vector Ecology*, 43: 334–336.

398 Cuthbert, R.N., Dalu, T., Mutshekwa, T. and Wasserman, R.J. 2019a. Leaf inputs from
399 invasive and native plants drive differential mosquito abundances. *Science of the*
400 *Total Environment*, 689: 652–654.

401 Cuthbert, R.N., Dick, J.T.A. and Callaghan, A. 2018b. Interspecific variation, habitat
402 complexity and ovipositional responses modulate the efficacy of cyclopoid copepods
403 in disease vector control. *Biological Control*, 121: 80–87.

404 Cuthbert, R.N., Dick, J.T.A., Callaghan, A. and Dickey, J.W.E. 2018c. Biological control
405 agent selection under environmental change using functional responses, abundances

406 and fecundities; the Relative Control Potential (RCP) metric. *Biological Control*, 121:
407 50–57.

408 Cuthbert, R.N., Ortiz-Perea, N., Dick, J.T.A. and Callaghan, A. 2019b. Elusive enemies:
409 Consumptive and ovipositional effects on mosquitoes by predatory midge larvae are
410 enhanced in dyed environments. *Biological Control*, 132: 116–121.

411 Douglas, S.E., Raven, J.A. and Larkum, A.W. 2003. The algae and their general
412 characteristics. In: Larkum, A.W.D., Douglas, S.E. and Raven, J.A. (Eds.) *Advances*
413 *in photosynthesis and respiration*. Springer, Dordrecht, pp. 1–10.

414 Eid, M.A.A., Kandil, M.A.E., Moursy, E.B. and Sayed, G.E.M. 1992a. Effect of the duck-
415 weed, *Lemna minor* vegetations on the mosquito, *Culex pipiens pipiens*. *International*
416 *Journal of Tropical Insect Science*, 13: 357–361.

417 Eid, M.A.A., Kandil, M.A.E., Moursy, E.B. and Sayed, G.E.M. 1992b. Bioassays of duck
418 weed vegetation extracts. *International Journal of Tropical Insect Science*, 13: 741–
419 748.

420 Elango, G., Abdul Rahuman, A., Bagavan, A., Kamaraj, C., Abdul Zahir, A., Rajakumar, G.,
421 Marimuthu, S. and Santhoshkumar, T. 2010. Studies on effects of indigenous plant
422 extracts on malarial vector, *Anopheles subpictus* Grassi (Diptera: Culicidae). *Tropical*
423 *Biomedicine*, 27: 143–154.

424 Ewing, D., Purse, B.V., Cobbold, C.A., Schäfer, S.M. and White, S.M. 2019. Uncovering
425 mechanisms behind mosquito seasonality by integrating mathematical models and
426 daily empirical population data: *Culex pipiens* in the UK. *Parasites and Vectors*, 12:
427 74.

428 Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M.N., Nielsen,
429 A. and Sibert, J. 2012. ADModel Builder: using automatic differentiation for
430 statistical inference of highly parameterized complex nonlinear models. *Optimization*
431 *Methods and Software*, 27: 233–249.

432 Hemingway, J., Beaty, B.J., Rowland, M., Scott, T.W. and Sharp, B.L. 2006. The Innovative
433 Vector Control Consortium: improved control of mosquito-borne diseases. *Trends in*
434 *Parasitology*, 22: 308–312.

435 Hobbs, J.H. and Molina, P.A. 1983. The influence of the aquatic fern *Salvinia auriculata* on
436 the breeding of *Anopheles albimanus* in coastal Guatemala. *Mosquito News*, 43: 456-
437 457.

438 Hoel, D.F., Obenauer, P.J., Clark, M., Smith, R., Hughes, T.H., Larson, R.T., Diclaro, J.W.
439 and Allan, S.A. 2011. Efficacy of ovitrap colors and patterns for attracting *Aedes*
440 *albopictus* at suburban field sites in North-Central Florida. *Journal of the American*
441 *Mosquito Control Association*, 27: 245–251.

442 Kershenbaum, A., Spencer, M., Blaustein, L. and Cohen, J.E. 2012. Modelling evolutionarily
443 stable strategies in oviposition site selection, with varying risks of predation and
444 intraspecific competition. *Evolutionary Ecology*, 26: 955–974.

445 Kramer, W.L. and Mulla, M.S. 1979. Oviposition attractants and repellents of mosquitoes:
446 oviposition responses of *Culex* mosquitoes to organic infusions. *Environmental*
447 *Entomology*, 8: 1111–1117.

448 Li, J., Deng, T., Li, H., Chen, L. and Mo, J. 2009. Effects of water color and chemical com-
449 pounds on the oviposition behavior of gravid *Culex pipiens pallens* females under
450 laboratory conditions. *Journal of Agricultural and Urban Entomology*, 26: 23–30.

- 451 MacArthur, R.H. and Wilson, E.O. 1967. *The theory of island biogeography*. Princeton
452 University Press, Princeton.
- 453 McPeck, M.A. 2004. The growth/predation risk trade-off: So what is the mechanism? *The*
454 *American Naturalist*, 163: E88–E111.
- 455 Naqqash, M.N., Gökçe, A., Bakhsh, A. and Salim, M. 2016. Insecticide resistance and its
456 molecular basis in urban insect pests. *Parasitology Research*, 115: 1363-1373.
- 457 Nonacs, P. and Dill, L.M. 1990. Mortality risk vs. food quality trade-offs in a common
458 currency: ant patch preferences. *Ecology*, 71: 1886–1892.
- 459 O’Meara, G.F., Vose, F.E. and Carlson D.B. 1989. Environmental factors influencing
460 oviposition by *Culex* (*Culex*) (Diptera: Culicidae) in two types of traps. *Journal of*
461 *Medical Entomology*, 26: 528–534.
- 462 Oladipupo, S.O., Callaghan, A., Holloway, G.J. and Gbaye, O. 2019. Variation in the
463 susceptibility of *Anopheles gambiae* to botanicals across a metropolitan region of
464 Nigeria. *PLoS One*, 14: e0210440.
- 465 Oliva, L., Correia, J. and Albuquerque, C. 2014. How mosquito age and the type and color of
466 oviposition sites modify skip-oviposition behavior in *Aedes aegypti* (Diptera:
467 Culicidae)? *Journal of Insect Behavior*, 27: 81–91.
- 468 Ortiz-Perea, N. and Callaghan, A. 2017. Pond dyes are *Culex* mosquito oviposition
469 attractants. *PeerJ*, 5: e3361.
- 470 Ortiz-Perea, N., Gander, R., Abbey, O. and Callaghan, A. 2018. The effect of pond dyes on
471 oviposition and survival in wild UK *Culex* mosquitoes. *PLoS One*, 13: e0193847.

472 Pintar, M.R., Bohenek, J.R., Eveland, L.L. and Restarits Jr, W.J. 2018. Colonization across
473 gradients of risk and reward: Nutrients and predators generate species-specific
474 responses among aquatic insects. *Functional Ecology*, 32: 1589–1598.

475 R Core Development Team 2018. R: A language and environment for statistical computing.
476 R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>.

477 Reiter, P. 1987. A revised version of the CDC gravid mosquito trap. *Journal of the American*
478 *Mosquito Control Association*, 3: 325–327.

479 Rosenzweig, M.L. 1981. A theory of habitat selection. *Ecology*, 62: 327–335.

480 Shaalan, E.A. and Canyon, D.V. 2018. Mosquito oviposition deterrents. *Environmental*
481 *Science and Pollution Research*, 25: 10207–10217.

482 Shaalan, E.A., Canyon, D., Younes, M.W.F., Abdel-Waheb, H. and Mansour, A.-H. 2005. A
483 review of botanical phytochemicals with mosquitocidal potential. *Environment*
484 *International*, 31: 1149–1166.

485 Silberbush, A. and Blaustein, L. 2011. Mosquito females quantify risk of predation to their
486 progeny when selecting an oviposition site. *Functional Ecology*, 25: 1091–1095.

487 Stone, C.M., Witt, A.B.R., Walsh, G.C., Foster, W.A. and Murphy, S.T. 2018. Would the
488 control of invasive alien plants reduce malaria transmission? A review. *Parasites and*
489 *Vectors*, 11: 76.

490 Townroe, S. and Callaghan, A. 2014. British container breeding mosquitoes: the impact of
491 urbanisation and climate change on community composition and phenology. *PLoS*
492 *One* 9: e95325.

493 Townroe, S. and Callaghan, A. 2015. Morphological and fecundity traits of *Culex* mosquitoes
494 caught in gravid traps in urban and rural Berkshire, UK. *Bulletin of Entomological*
495 *Research*, 105: 615–620.

496 Vezzani, D., Rubio, A., Velazquez, S., Schweigmann, N. and Wieg, T. 2005. Detailed
497 assessment of microhabitat suitability for *Aedes aegypti* (Diptera: Culicidae) in
498 Buenos Aires, Argentina. *Acta Tropica*, 95: 123–131.

499 Vonesh, J. and Blaustein, L. 2010. Predator-induced shifts in mosquito oviposition site
500 selection: a meta-analysis and implications for vector control. *Israel Journal of*
501 *Ecology and Evolution*, 56: 263–279.

502 Vonesh, J.R., Kraus, J.M., Rosenberg, J.S. and Chase, J.M. 2009. Predator effects on aquatic
503 community assembly: disentangling the roles of habitat selection and post-
504 colonization processes. *Oikos*, 118: 1219–1229.

505 Werner, E.F. and Gilliam, J.F. 1984. The ontogenic niche and species interactions in size-
506 structured populations. *Annual Review of Ecology and Systematics*, 15: 383–425.

507 World Health Organisation, 2018. Mosquito-borne diseases.
508 http://www.who.int/neglected_diseases/vector_ecology/mosquito-borne-diseases/en/
509 (accessed 12 September 2018).

510 Williams, R.E. 1962. Effect of coloring oviposition media with regard to the mosquito *Aedes*
511 *triseriatus* (Say). *The Journal of Parasitology*, 48: 919–925.

512 Yang, P.J., Manuchei, D. and Takekuma, C. 2007. Impact of *Lemna minor* Linnaeus (1753)
513 and *Azolla filiculoides* Lamarck (1783) on mosquito breeding in Kauai taro fields. *The*
514 *Pan-Pacific Entomologist*, 81: 159–163.

515

516 **Figure legends**

517 Figure 1. Diagrammatic illustration of three experiments used to examine the influence of
518 intact and ground duckweed *Lemna minor* on *Culex pipiens* oviposition (Experiment 1:
519 Paired choice tests, and Experiment 2: Factorial choice tests; 1.a–1.e and 2a – 2.b,
520 respectively), and larval colonisation (Experiment 3: Natural colonisation; 3.a). Shaded
521 containers represent black liquid pond dye, 0.3 g L⁻¹.

522 Figure 2. Oviposition activity index (OAI) values resulting from pairwise oviposition choice
523 tests with gravid adult female *Culex pipiens* and different treatments of *Lemna minor*
524 duckweed with pond dye. Duckweed and non-dye controls include: a), 5 g duckweed; b), 20
525 g duckweed; c), 50 g duckweed; and d), 5 g ground duckweed; while duckweed with a dyed
526 control is: e), 5 g ground duckweed. Values above indicate significance levels for each
527 treatment pair ($p < 0.001$, ***; $p < 0.01$, **; $p < 0.05$, *; $p \geq 0.05$, NS). Means are ± 1 SE.
528 The solid line indicates null preference, whilst values close to 1 indicate increasing
529 preference for duckweed-treated water; values closed to -1 indicate avoidance behaviour.

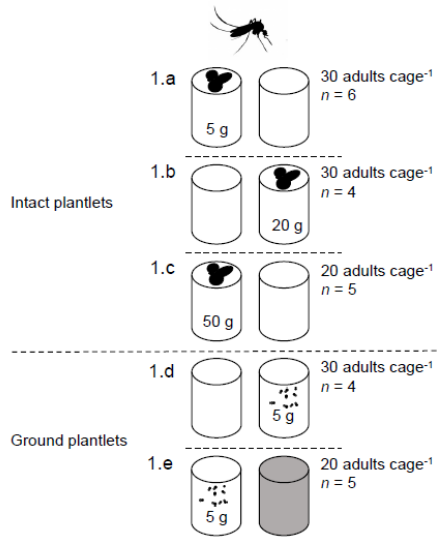
530 Figure 3. Mean (± 1 SE) number of egg rafts laid under factorial treatments of *Lemna minor*
531 duckweed and pond dye by adult female *Culex pipiens* in the presence of: a) 5 g intact
532 duckweed; and b), 5 g ground duckweed.

533 Figure 4. Mean (± 1 SE) number of *Culex pipiens* larvae in mesocosms under different dye
534 treatments, in complete absence (a) and presence, i.e. complete surface coverage, (b) of
535 *Lemna minor* duckweed over a nine week observation period. Lines are locally-weighted
536 scatterplot smoothing (LOWESS) with 9/10 smoother span. Note differences in y axes
537 scaling.

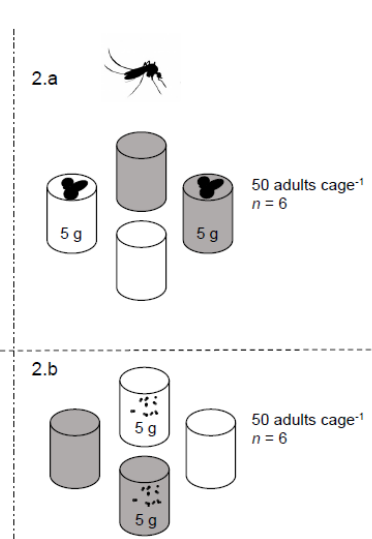
538

539

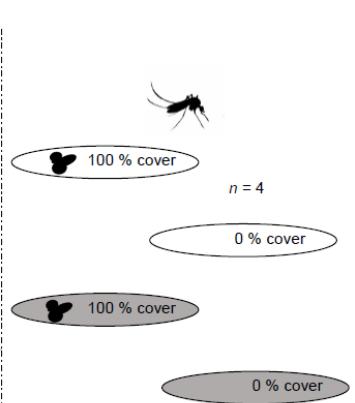
Experiment 1: Paired choice tests



Experiment 2: Factorial choice tests



Experiment 3: Natural colonisation



540

541 Figure 1.

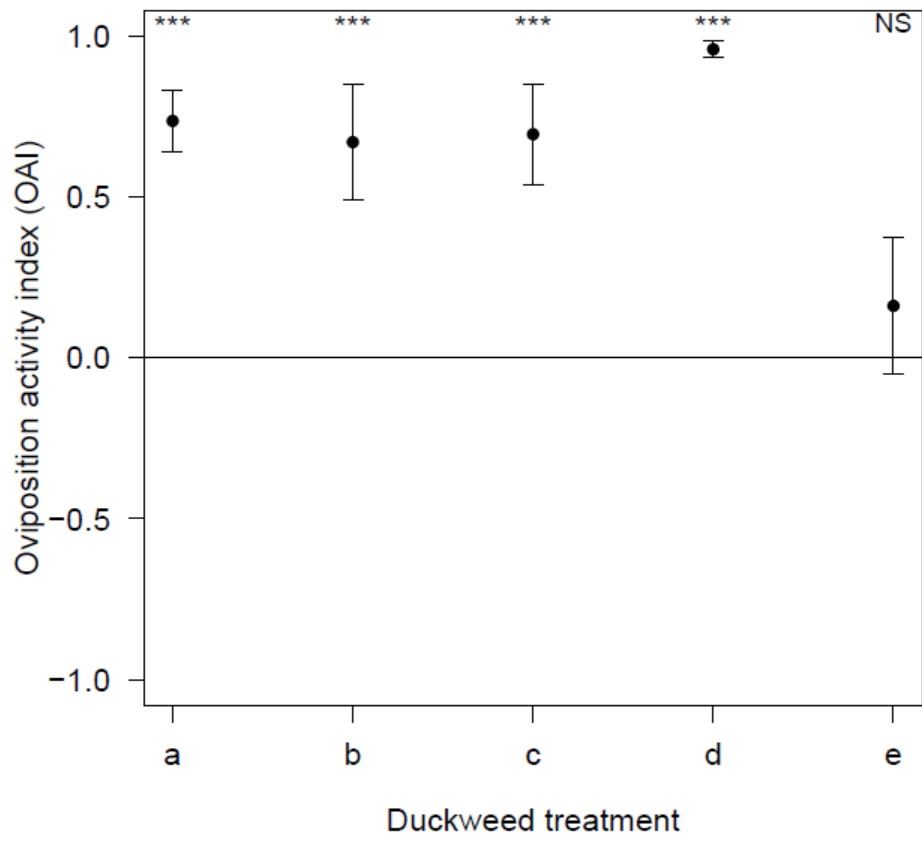
542

543

544

545

546



547

548 Figure 2.

549

550

551

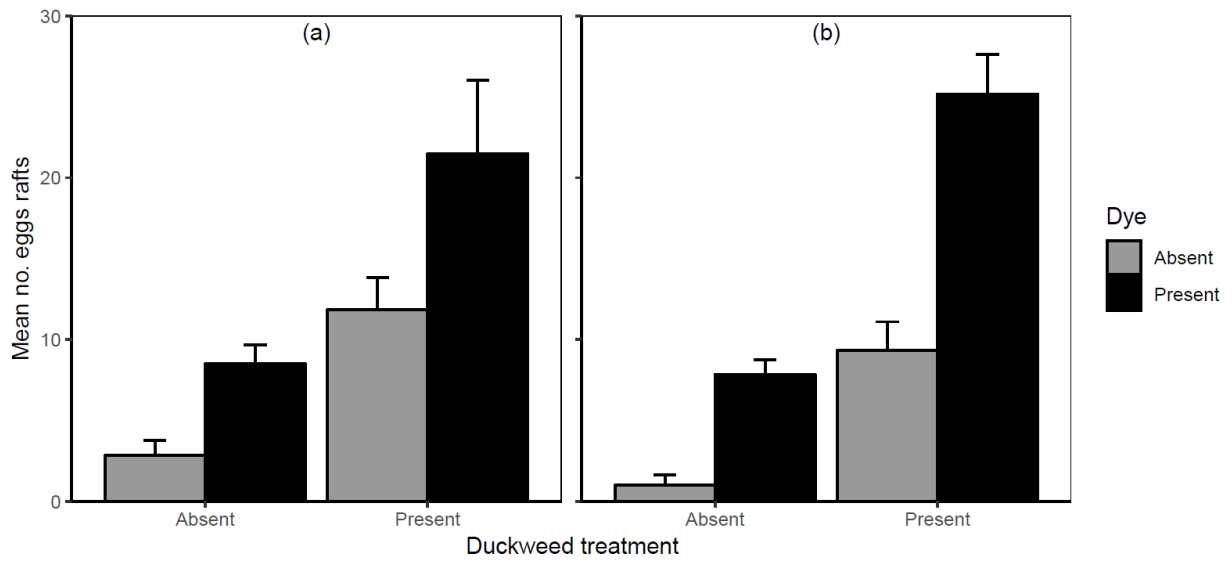
552

553

554

555

556



557

558 Figure 3.

559

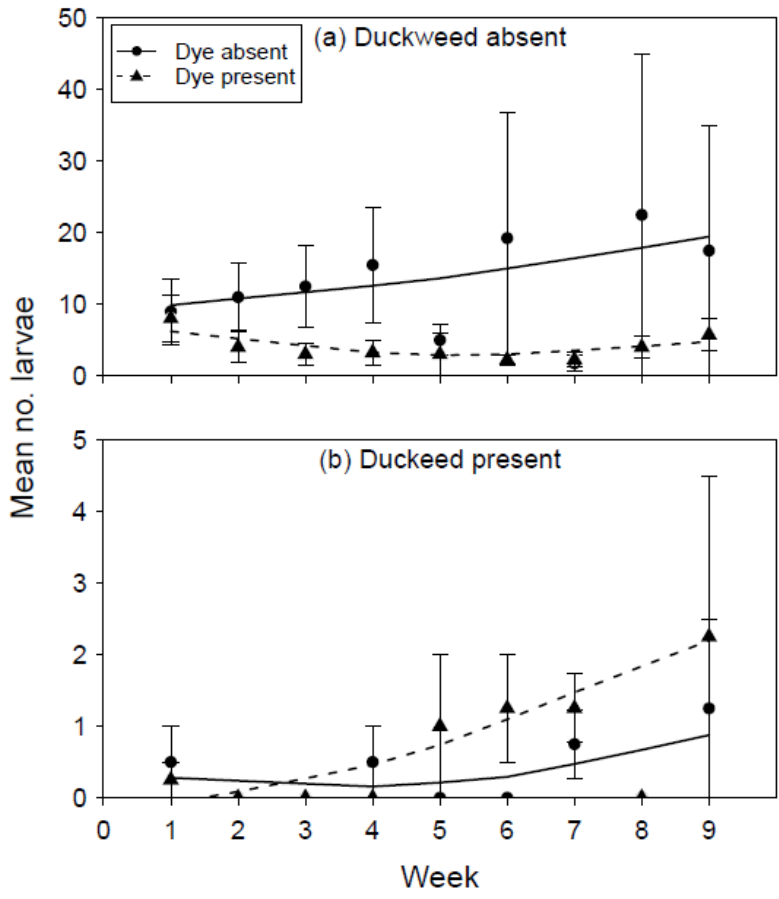
560

561

562

563

564



565

566 Figure 4.