

Elusive enemies: consumptive and ovipositional effects on mosquitoes by predatory midge larvae are enhanced in dyed environments

Article

Accepted Version

Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0

Cuthbert, R. N., Ortiz-Perea, N., Dick, J. T. A. and Callaghan, A. ORCID: <https://orcid.org/0000-0002-2731-3352> (2019) Elusive enemies: consumptive and ovipositional effects on mosquitoes by predatory midge larvae are enhanced in dyed environments. *Biological Control*, 132. pp. 116-121. ISSN 1049-9644 doi: <https://doi.org/10.1016/j.biocontrol.2019.02.008> Available at <https://centaur.reading.ac.uk/82445/>

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

Published version at: <https://www.sciencedirect.com/science/article/pii/S1049964418308065?via%3Dihub>

To link to this article DOI: <http://dx.doi.org/10.1016/j.biocontrol.2019.02.008>

Publisher: Elsevier

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 *Research Article*

2

3 **Elusive enemies: consumptive and ovipositional effects on mosquitoes by predatory**
4 **midge larvae are enhanced in dyed environments**

5

6 Ross N. Cuthbert*^{1,2}, Natali Ortiz-Perea², Jaimie T.A. Dick¹, Amanda Callaghan²

7

8 ¹Institute for Global Food Security, School of Biological Sciences, Queen's University
9 Belfast, Medical Biology Centre, Belfast BT9 7BL, Northern Ireland

10 ²Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading,
11 Harborne Building, Reading RG6 6AS, England

12

13 *Corresponding author: rcuthbert03@qub.ac.uk

14

15

16

17

18

19

20

21 **Abstract**

22 Mosquito-borne disease incidences continue to proliferate and cause enormous mortality and
23 debilitation rates. Predatory natural enemies can be effective in population management
24 strategies targeting medically-important mosquito species. However, context-dependencies
25 and target organism behavioural responses can impede or facilitate biological control agents.
26 Black pond dye has been shown to be a strong mosquito oviposition attractant, and could
27 potentially be used alongside predatory agents to create mosquito population sink effects.
28 Here, we thus examine the predatory impact of larvae of the non-biting chaoborid midge
29 *Chaoborus flavicans* towards larvae of the West Nile virus vector mosquito complex *Culex*
30 *pipiens* in the presence and absence of black pond dye. We then examine the ovipositional
31 responses of *C. pipiens* to predation risk and dye in laboratory-, semi-field- and field-based
32 trials. Larval *C. flavicans* exhibited potentially population destabilising type II functional
33 responses towards mosquito larvae irrespective of the presence of pond dye. Neither
34 consumption rates nor functional response parameters (attack rates, handling times) were
35 significantly influenced by the presence of dye, indicating a use of hydromechanics to detect
36 mosquito prey by chaoborids. Wild-caught adult *C. pipiens* did not avoid predatory
37 chaoborids when ovipositing, however they were significantly more attracted to oviposit in
38 dye-treated water regardless of the presence of predators. We thus demonstrate high
39 predatory impact towards mosquito larvae by non-biting chaoborid midges during their
40 predaceous aquatic larval stages, and proliferations of such predators may assist or augment
41 control efforts for mosquitoes. Our results suggest a lack of influence of predatory dipterans
42 on oviposition selectivity by *C. pipiens* mosquitoes, and that pond dye may enhance the
43 efficacy of select predatory biological control agents through the creation of population sinks,
44 characterised by high rates of oviposition and subsequent predation.

46 **Key words**

47 predator-prey; functional response; biological control; trait mediation; *Chaoborus*; *Culex*

48 **1. Introduction**

49 Pathogens and parasites vectored by mosquitoes continue to threaten the well-being of
50 humans and wildlife (Ferguson, 2018). For instance, West Nile virus, spread principally by
51 *Culex pipiens* complex mosquitoes, is increasingly prevalent across Europe (Napp et al.
52 2018) and has spread rapidly across the United States in recent decades (Murray et al. 2010).
53 Further, proliferations of mosquitoes are being further exacerbated by changing domestic
54 water storage practices and changing climates (Townroe and Callaghan, 2014). Natural
55 enemies, such as predators and parasites, can assist in population-level suppression of
56 medically-important mosquitoes (e.g. Nam et al. 2012), however, the potential contributions
57 of many resident enemies towards mosquito control remain unquantified. In particular,
58 coupled quantifications of density- and trait-mediated ecological impacts towards target
59 mosquito prey of predators remain elusive (Abrams et al. 1996; Zuharah and Lester, 2011;
60 Cuthbert et al. 2018a). Further, predator avoidance behaviour by gravid adult mosquitoes has
61 been shown to impede the efficacy of natural enemies in control efforts *via* positive selection
62 towards predator-free habitats (e.g. Vonesh and Blaustein, 2010; Cuthbert et al. 2018b). This
63 effect is particularly problematic for the control of mosquitoes which breed in small, patchy
64 aquatic systems, wherein an abundance of habitats may facilitate a high degree of choice (e.g.
65 container-style environments; Townroe and Callaghan, 2014). Therefore, quantifications of
66 both predatory impact alongside avoidance behaviours mediated by predators are paramount
67 for understanding biocontrol efficacies. Moreover, the identification of measures to offset
68 such avoidance behaviours could enhance biocontrol success.

69 *Culex pipiens* complex mosquitoes are globally widespread and colonise minute
70 aquatic habitats in high abundances (Townroe and Callaghan, 2014). Many natural enemies
71 are unable to succeed in such habitats due to issues with overland dispersal or size-related
72 limitations (e.g. fish, Azevedo-Santos et al. 2016). Thus, rapid colonisation of small and often
73 ephemeral habitats by mosquitoes following inundation with water often renders such
74 systems enemy-free and ensures high mosquito recruitment. However, other dipterans are
75 also capable of colonising small natural or artificial aquatic habitats, and many of these are
76 predatory in their larval form (e.g. Sunahara et al. 2002; Griswold and Lounibos, 2006). In
77 particular, larvae of widespread non-biting chaoborid midges are known to prey upon larval
78 mosquitoes and can proliferate in high abundances in aquatic habitats (Borkent, 1980;
79 Liljendahl-Nurminen et al. 2003; Cuthbert et al. 2019). Given that adult midges disperse
80 through flight, there is a high potential for coexistence with larval mosquitoes in aquatic
81 ecosystems which are inaccessible to less mobile predators. However, whilst gravid adult
82 mosquitoes are generally evasive of both invertebrate and vertebrate predators when
83 ovipositing (Vonesh and Blaustein, 2010; Cuthbert et al. 2018b), responsiveness to the
84 presence of other such predatory dipterans remains largely untested (Albeny-Simões et al.
85 2014). Furthermore, the extent of predatory impacts of many coexisting dipterans on
86 mosquitoes remains unquantified.

87 Black pond dye may be a strong attractant to gravid mosquitoes when ovipositing
88 (Ortiz-Perea and Callaghan, 2017; Cuthbert et al. 2018b; but see Ortiz-Perea et al. 2018). The
89 presence of dye has been suggested to elicit attraction through greater perceived depth or
90 higher concentration of organic matter, which may benefit progeny (Williams, 1962; Hoel et
91 al. 2011). Alternatively, the presence of dye may mimic shading, which can help to reduce
92 deleterious effects associated with temperature extremes (Vezzani et al. 2005). However,
93 whilst dye can mimic shading, the effects of pond dye on thermal regimes within waterbodies

94 is uncertain. The use of such pond dye is increasing in urban areas to enhance the aesthetics
95 of waterbodies such as garden ponds (see Ortiz-Perea and Callaghan, 2017). Although the
96 effects of dye products on broader aquatic community compositions remains unclear,
97 predatory impacts of natural enemies, such as copepods, on mosquitoes have been shown
98 previously to be unaffected by water clarity regimes (e.g. Cuthbert et al. 2018c, d). In
99 particular, aquatic predators which do not rely on visual cues to detect and capture prey could
100 be more efficacious in mosquito population management when present in dyed environments,
101 given heightened rates of mosquito oviposition in these habitats coupled with potentially
102 unhampered, or enhanced, rates of killing.

103 The present study thus examines predatory impacts of larvae of the non-biting
104 phantom midge *Chaoborus flavicans* towards larvae of medically-important *C. pipiens*
105 mosquitoes. Furthermore, we examine whether the presence of predatory midge larvae
106 mediates changes in oviposition behaviour by wild adult *C. pipiens* mosquitoes. We firstly
107 use comparative functional responses to quantify density-dependent predation of mosquitoes
108 by larval chaoborids between dyed and undyed environments. Functional responses are
109 widely used in biological control agent selection (O'Neill, 1990; Cuthbert et al. 2018a, b),
110 and typically are considered to conform to one of three types (Solomon, 1949; Holling,
111 1959): (1) type I functional responses are linear, wherein prey consumption increases in line
112 with prey density up to a given threshold; (2) type II functional responses are hyperbolic,
113 characterised by inversely density-dependent consumption, wherein consumption rates are
114 high at low prey densities; (3) type III functional responses are sigmoidal, wherein
115 consumption rates are reduced at low prey densities, potentially due to prey switching
116 behaviour or significant search times where prey are rare (Hassell, 1978). Importantly, the
117 form and magnitude of the functional response have implications for the stability of prey
118 populations and thus functional responses are of particular utility in biocontrol agent

119 selection. For instance, whilst type III functional responses are considered more likely to
120 impart stability to populations, type II functional responses are commonly assumed to be
121 destabilising, owing to high rates of consumption even where prey are relatively scarce (Dick
122 et al. 2014). Further, we use laboratory-, semi-field- and field-based oviposition selectivity
123 experiments to examine behaviours by adult mosquitoes in response to the presence of larval
124 predatory chaoborids and pond dye.

125 **2. Methods**

126 *2.1. Animal collection and maintenance*

127 Fourth instar larvae of *Chaoborus flavicans* (1.0 – 1.2 mm) were purchased commercially
128 (Northampton Reptile Centre, Northampton, England). Larvae were maintained in a
129 laboratory at the University of Reading (19 ± 2 °C, 16:8 light:dark regime) in 5 L tanks
130 containing dechlorinated tap water and fed *ad libitum* with larvae of the mosquito *Culex*
131 *pipiens*.

132 *Culex pipiens* were collected from the Whiteknights campus of the University of
133 Reading. Larval cultures were initiated from egg rafts collected from artificial container-style
134 habitats, and reared in the laboratory (19 ± 2 °C, 16:8 light:dark regime) in 2 L arenas
135 containing dechlorinated tap water. Mosquito larvae were fed *ad libitum* with crushed guinea
136 pig food pellets. Gravid wild adult female *Culex pipiens* were collected using Reiter ovitraps
137 (Reiter, 1987) overnight, as per Townroe and Callaghan (2015), during July and August
138 2018. Here, gravid female mosquitoes were attracted to a hay and yeast infusion, and then
139 were pulled into a collection chamber *via* a duct using a fan. The fan created negative air
140 pressure in the collection chamber and was connected to a 12 V lead-acid battery. The ovitrap
141 comprised two parts: the upper portion was a modified toolbox containing a fan connected to
142 a battery and a collection chamber, and the lower portion was a 5 L tray containing 3 L of the

143 hay and yeast infusion (i.e. bait). The hay and yeast infusion comprised 0.5 kg of hay and 2.5
144 g fast-action dried yeast, fermented for 7 days in sealed 80 L water butts containing tap water.
145 The bait was strained before use through a 1000 μm mesh. *Culex pipiens* comprised >99 % of
146 the trapped mosquitoes, however, individuals of *Culiseta annulata* and *Anopheles plumbeus*
147 were also caught and dispatched.

148 2.2. Functional responses

149 *Chaoborus flavicans* were haphazardly selected from the laboratory stock and individually
150 starved for 24 h in 20 mL glass beakers containing dechlorinated tap water ($19 \pm 2^\circ\text{C}$, 16:8
151 light:dark regime). Early instar larvae of *C. pipiens* (2.0 – 3.0 mm) were presented at 6
152 densities (1, 3, 6, 20, 30 or 40; $n = 4$ per experimental group) in 40 mL glass beakers
153 containing dechlorinated tap water and allowed to settle for 1 h. Water was either dyed (0.3
154 g/L black liquid pond dye, Dyofix, Leeds, England) or undyed (i.e. 0.0 g/L). This dye
155 concentration is as recommended by the manufacturer and fully impedes visibility from the
156 water surface. Individual predators were then added to experimental beakers and allowed to
157 feed for 24 h, after which they were removed and numbers of prey killed enumerated.
158 Controls consisted of four replicates at each prey density and dye treatment in the absence of
159 predators.

160 All statistical analyses were performed using R (v3.4.4; R Core Development Team
161 2017). Generalised linear models were used to examine overall prey consumption with
162 respect to the ‘dye’ and ‘prey density’ factors using a quasi-Poisson error distribution as
163 counts were overdispersed (residual deviance greater than degrees of freedom). *F* tests were
164 used for stepwise model simplification (Crawley, 2007). Functional response analyses were
165 performed phenomenologically using the ‘frair’ package in R (Pritchard et al. 2017).
166 Functional response types were inferred categorically using logistic regression of the

167 proportion of prey eaten as a function of prey density. A significantly negative first-order
168 term is indicative of a type II functional response (Trexler et al. 1988; Juliano, 2001). As prey
169 were not replaced as they were eaten, Rogers' random predator equation was used to model
170 functional responses (Rogers, 1972):

$$171 \quad N_e = N_0(1 - \exp(a(N_e h - T))) \quad (1)$$

172 where N_e is the number of prey killed or consumed, N_0 is the starting prey supply, a is the
173 attack rate, h is the handling time and T is the total experimental time. The Lambert W
174 function was used in fitting the random predator equation (Bolker, 2008). A non-parametric
175 bootstrapping procedure ($n = 2000$) was followed to generate 95% bias corrected and
176 accelerated confidence intervals around functional response curves. The delta method
177 (Juliano, 2001) was used to test for differences in functional response attack rates (a) and
178 handling times (h) between the two dye treatments. Maximum feeding rates ($1/h$) over the
179 experimental period were also derived between treatment groups.

180 2.3. Ovipositional responses

181 All ovipositional choice experiments were conducted between June and August 2018 in
182 Reading, England. Paired oviposition choice tests were used to decipher predator avoidance
183 behaviour of wild gravid adult female *C. pipiens* towards larval *C. flavicans*, which were not
184 starved prior to experimentation. Twenty adult mosquitoes were released into each replicate
185 cage (30 cm³) in a laboratory at the University of Reading (25 ± 2°C, 16:8 light:dark regime)
186 and given the choice of two polypropylene 200 mL arenas (9.5 cm dia.) in which to oviposit.
187 Each cage contained a control arena containing dechlorinated tap water alone, and an arena
188 containing dechlorinated tap water and a given predator treatment. Predator treatments
189 comprised either one or ten *C. flavicans* ($n = 10$ per pair), or ten *C. flavicans* with dyed water
190 (0.3 g/L black liquid pond dye) ($n = 4$ per pair). The treatment pairs (control, predator) were

191 randomly assigned to opposing corners of each cage. Adult mosquitoes were allowed to
192 oviposit for three days, with egg rafts removed and enumerated daily. Each cage additionally
193 contained a cup with cotton pads soaked in a 10% sucrose solution for sustenance. Replicate
194 groups for each experimental pair were conducted simultaneously.

195 Factorial oviposition choice experiments were conducted using a tent (350 × 305 ×
196 170 cm) placed in a wooded area outdoors on the Whiteknights campus of the University of
197 Reading (51°26'10.9"N 0°56'35.0"W). Approximately 200 wild gravid adult female *C.*
198 *pipiens* were released into the tent and presented with four treatment choices in 2 L arenas
199 (surface area: 14 × 21 cm) containing dechlorinated tap water in which to oviposit.
200 Treatments were fully crossed with respect to 'dye' and 'predator'. For dye treatments, either
201 dye was added (0.3 g/L black liquid pond dye), or arenas remained undyed (i.e. 0.0 g/L). For
202 predator treatments, either ten *C. flavicans* were added, or none. Treatments were replicated
203 five times in a completely randomised, uniform array in the tent (i.e. 4 × 5 containers).
204 Mosquitoes were allowed to oviposit for seven days, with egg rafts removed and enumerated
205 daily. A container with 10 % sucrose solution-soaked cotton was additionally provided in the
206 tent for sustenance. The experiment was executed on two separate occasions using the same
207 tent in the same location.

208 The crossed 'dye' and 'predator' experiment was repeated as before, outdoors in the
209 periphery of the wooded area using a randomised block design ($n = 5$ per treatment group)
210 with identical 2 L arenas. Bait from a yeast and hay infusion was additionally added to all
211 treatment groups outdoors to increase the attractiveness of the arenas to wild gravid
212 mosquitoes.

213 For each treatment pair in the oviposition choice test, generalised linear mixed models
214 were used to examine ovipositional preferences of mosquitoes (Bates et al. 2015). The total

215 number of egg rafts oviposited over the experimental period was modelled with ‘treatment’
216 as a fixed effect and ‘cage number’ as a random effect, to account for the paired design. A
217 Poisson distribution of error was used if counts were not overdispersed (deviation smaller than
218 mean). Where counts were found to be overdispersed (deviation larger than mean), a negative
219 binomial distribution was employed. Ovipositional activity indices (OAI) were calculated for
220 illustration of each treatment pair as per Kramer and Mulla (1979):

$$221 \quad OAI = (NT - NC)/(NT + NC) \quad (2)$$

222 where the oviposition activity index (OAI) is a function of the number of egg rafts laid in
223 treated water (NT) in relation to controls (NC). The OAI range is from -1 to 1, where 0
224 corresponds to no preference, values closer to 1 indicate increasing preference for predator
225 treatments and values closer to -1 indicate increasing preference for control treatments (i.e.
226 predator avoidance).

227 Factorial oviposition counts in the tent and outdoors were separately analysed using
228 generalised linear mixed models assuming a negative binomial error distribution to account
229 for overdispersion of residuals. Total numbers of egg rafts were analysed with respect to the
230 fixed ‘dye’ and ‘predator’ treatments, and their interaction. For oviposition in the tent, ‘trial
231 number’ was included as a random effect, whilst ‘block number’ was included as a random
232 effect in the outdoor experiment. In all models, non-significant terms were removed stepwise
233 to maximise parsimony (Crawley, 2007).

234 **3. Results**

235 *3.1. Functional responses*

236 There was no mortality of *C. pipiens* larvae in control groups, and so experimental deaths
237 were deemed to result from predation by *C. flavicans*. Overall prey consumption was not
238 significantly influenced by the presence of dye ($F_{1, 46} = 0.54$, $P = 0.47$). Prey consumption

239 was significantly greater under increasing prey supplies ($F_{5, 42} = 20.51, P < 0.001$), and there
240 was no significant interaction between dye treatment and prey supply ($F_{5, 36} = 2.08, P = 0.09$).

241 Type II functional responses were displayed by *C. flavicans* towards *C. pipiens* larvae
242 in both dyed and undyed treatments, evidenced by significantly negative first order terms and
243 high proportional consumption at low prey densities (Table 1; Figure 1). There was no
244 significant difference in attack rates ($z = 1.10, P = 0.27$) or handling times ($z = 0.29, P =$
245 0.77) between dyed and undyed treatment groups. This lack of difference was further
246 evidenced by overlapping of 95% confidence intervals across all prey supplies between both
247 treatment groups (Figure 1). Maximum feeding rates ($1/h$) were additionally similar between
248 dye treatments (Table 1; Figure 1).

249 3.2. Ovipositional responses

250 In the pairwise choice tests, gravid adult wild *C. pipiens* did not exhibit ovipositional
251 selectivity in response to *C. flavicans*, either where predators were present individually ($z =$
252 $0.16, P = 0.88$) or in groups of ten ($z = 0.20, P = 0.85$; Figure 2). However, where dye was
253 present alongside ten *C. flavicans*, significant preferential selection towards predator-treated
254 water was demonstrated over the undyed, predator-free controls by gravid mosquitoes ($z =$
255 $5.42, P < 0.001$; Figure 2).

256 In the tent, significantly more egg rafts were oviposited in dyed compared to undyed
257 water overall ($z = 3.38, P < 0.001$; Figure 3). The presence of *C. flavicans* did not
258 significantly influence oviposition by gravid mosquitoes ($z = 1.45, P = 0.15$), and there was
259 no significant interaction effect between the dye and predator treatments ($z = 0.22, P = 0.83$).
260 Similarly, outdoors, significantly greater numbers of egg rafts were oviposited in dyed water
261 overall ($z = 2.67, P = 0.008$; Figure 4), whilst predators did not induce selectivity by gravid

262 mosquitoes ($z = 0.15$, $P = 0.88$). There was no significant interaction between dye and
263 predator treatments here ($z = 0.40$, $P = 0.69$).

264 **4. Discussion**

265 Larval chaoborids effectively preyed upon larval *C. pipiens* mosquitoes irrespective of the
266 presence of dye in the present study. Individual predators exhibited maximum feeding rates
267 of approximately 20 larval mosquitoes per day, which is similar to other dipteran predators
268 which are known to regulate mosquito populations (e.g. Griswold and Lounibos, 2005).
269 Whilst chaoborid larvae have been previously suggested as biocontrol agents for mosquitoes
270 (Bay, 1974; Borkent, 1980; Helgen, 1989), there has been little work to quantify *per capita*
271 offtake rates of this predator towards mosquito prey (but see Cuthbert et al. 2019). Here,
272 chaoborids exhibited type II functional responses in both dyed and undyed environments,
273 indicating effective prey intake even at low prey densities. Thus, water clarity does not impart
274 a visual refuge effect to mosquito prey. Accordingly, neither the attack rate nor handling time
275 parameters were significantly altered by the presence of dye. Chaoborids are ambush
276 predators which strike prey as they pass close by, with detection *via* hydromechanical
277 receptors (Horridge, 1966; Riessen et al. 1984); therefore, it is not surprising that dye did not
278 significantly influence the impact of this predator through inhibition of visual cues (see also
279 Cuthbert et al. 2018c). The marked *per capita* impact of *C. flavicans* on mosquitoes in the
280 present study corroborates with the known field-impacts of this abundant group on other
281 invertebrates (Dodson, 1974; von Ende and Dempsey, 1981), and chaoborids have been
282 shown to positively select larval mosquitoes over other forms of prey (Helgen, 1989).

283 Understanding the implications of context-dependencies, such as water clarity, for the
284 efficacy of biocontrol agents is imperative for robust derivations of ecological impacts on
285 target organisms in varied environments (see Cuthbert et al. 2018a, b). Whilst other abiotic

286 contexts, such as turbulence, have been shown to negatively influence feeding rates of
287 chaoborids (Härkönen et al. 2014), variabilities such as these are unlikely to be encountered
288 within stagnant habitats colonised by mosquitoes. Furthermore, whilst functional responses
289 quantify *per capita* ecological impacts of predators, explicit integrations of population-level
290 responses to resources (i.e. numerical response) of predators such as *C. flavicans* could
291 further assist in the assessment of such biocontrol agents across environmental contexts (see
292 Cuthbert et al. 2018a). Considerations for how differential water clarities influence
293 behavioural responses of mosquitoes to predators additionally requires consideration by
294 researchers.

295 Mosquito oviposition is selective across gradients of perceived risk and reward (e.g.
296 Pintar et al. 2018). As such, adult mosquitoes are generally evasive of predators with which
297 they have coevolved (Vonesh and Blaustein, 2010; Ohba et al. 2012). Unlike the strong
298 avoidance behaviour of mosquitoes to many invertebrate and vertebrate predators (e.g.
299 notonectids, odonates, fish; see Vonesh and Blaustein, 2010), in the present study wild gravid
300 adult female *C. pipiens* did not demonstrate selective behaviour when ovipositing in habitats
301 holding midge predators. This lack of selectivity persisted across laboratory, field and semi-
302 field conditions, and regardless of predator density. Whilst studies into the influence of
303 predatory dipterans on mosquito oviposition are scarce, Albeny-Simões et al. (2014) found
304 *Aedes aegypti* to prefer habitats where the larvae of the predatory non-biting mosquito
305 *Toxorhynchites theobaldi* had fed. Indeed, Vonesh and Blaustein (2010) found a weak or
306 non-existent response of mosquitoes to predatory dipterans in their meta-analysis,
307 corroborating with observations in the present study. Furthermore, whilst other research has
308 found density-dependent patterns of avoidance of aquatic predators by mosquitoes
309 (Silberbush and Blaustein, 2011), the present study found that mosquitoes did not exhibit
310 selectivity even as predator densities increased markedly. The drivers of this lack of response

311 to predator cues requires further investigation, however it is plausible that the presence of
312 other dipterans is indicative of good quality aquatic habitats which may support mosquito
313 development. Thus, predatory dipterans, which too are capable of aerial dispersal and
314 colonisation of ephemeral aquatic habitats, may be particularly efficacious predators of larval
315 mosquitoes across the landscape.

316 Pond dyes are increasingly popular in urban waterbodies and have recently been
317 shown to have significant influence on the ovipositional selectivity of gravid *C. pipiens*
318 complex mosquitoes; pond dye alone can also increase mortality rates between the larval and
319 adult stage in mosquitoes (Ortiz-Perea and Callaghan, 2017; but see Ortiz-Perea et al. 2018).
320 The perception of greater nutritional loads, depth or shading is likely to prompt positive
321 selection towards these habitats because of the potentially positive effects to progeny (see
322 Ortiz-Perea and Callaghan, 2017). However, the influence of pond dye on thermal regimes
323 within waterbodies requires further investigation, as mosquito proliferations are additionally
324 heavily influenced by temperature (Vezzani et al. 2005; Townroe and Callaghan, 2014). In
325 contrast to the weak effect of predators on ovipositional selectivity behaviours by adult *C.*
326 *pipiens* complex mosquitoes, the presence of black pond dye had strong and consistent
327 positive effects on oviposition here. Indeed, in pairwise laboratory tests, the presence of
328 pond dye synergised with predators facilitated an attraction effect towards predator-treated
329 habitats. Cuthbert et al. (2018c) found a similar effect, with dye reversing avoidance
330 behaviours of *C. pipiens* complex mosquitoes towards cyclopoid copepods. In the present
331 study, the attraction towards dyed habitats irrespective of predators persisted across field- and
332 semi-field conditions. Therefore, the presence of pond dye alongside predatory midge larvae
333 could facilitate population sinks associated with high rates of mosquito oviposition alongside
334 unimpeded predation rates. However, the influence of pond dyes on oviposition selectivity
335 behaviours of chaoborids was not examined in the present study. As such, longer term studies

336 are required to further understand and quantify recruitment of both mosquitoes and predatory
337 dipterans to waterbodies. Given that mosquitoes can be attracted to conspecifics killed by
338 predators (Albeny-Simões et al. 2014), it is possible that the presence of chemical cues or
339 bacterial abundances associated with predation could further drive attraction to aquatic
340 habitats containing predatory chaoborids. In turn, this could contribute to potential population
341 sink effects, yet also requires additional investigation.

342 In conclusion, encouraging the proliferation of non-biting dipterans which have a
343 predatory larval life history stage in aquatic habitats could further contribute to mosquito
344 population management. High maximum feeding rates that are robust to context-
345 dependencies such as water clarity, a lack of ovipositional deterrence and capacity for aerial
346 dispersal to patchy aquatic habitats make chaoborids potentially highly efficacious natural
347 enemies of mosquitoes. The use of domestic pond dyes is increasingly common in urban
348 areas in order to improve the aesthetics of waterbodies such as ponds, and the ecological
349 ramifications of such products remain largely unknown (but see Ortiz-Perea and Callaghan,
350 2017; Ortiz-Perea et al. 2018). However, the deliberate use of pond dye could further enhance
351 biological control through the establishment of population sinks wherein mosquitoes are
352 attracted to habitats inoculated with predators *in situ*, and particularly *via* predators which
353 rely on hydromechanical signalling for prey detection and which are unaffected by dyes.
354 However, further research is required to elucidate the effects of pond dyes on broader
355 community interactions within aquatic ecosystems, and particularly in understanding the
356 direct effects of dyes on resident natural enemies which assist in the management of
357 medically-important mosquito populations.

358 **Acknowledgements**

359 This study was funded through a Department for the Economy, Northern Ireland studentship.

360 **References**

361 Abrams. P.A., Menge, B.A., Mittelbach, G.G., Spiller, D., Yodzis, P., 1996. The role
362 of indirect effects in food webs. In: G.A. Polis, K.O. Winemiller (Eds.) Food Webs:
363 Integration of Patterns and Dynamics. Chapman & Hall, New York, pp. 371–395.

364 Albeny-Simões, D., Murrell, E.G., Elliot, S.L., Andrade, M.R., Lima, E., Juliano,
365 S.A., Vilela, E.F., 2014. Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with
366 predator-killed conspecifics. *Oecologia* 175, 481–492.

367 Azevedo-Santos, V.M., Vitule, J.R.S., Pelicice, F.M., García-Berthou, E., Simberloff,
368 D., 2017. Nonnative fish to control *Aedes* mosquitoes: a controversial, harmful tool.
369 *BioScience* 67, 84–90.

370 Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed- effects
371 models using lme4. *J. Stat. Softw.* 67, 1–48.

372 Bay, E.C., 1974. Predation-prey relationships among aquatic insects. *Ann. Rev.*
373 *Entomol.* 19, 441–453.

374 Bolker, B.M., 2008. *Ecological Models and Data in R*. Princeton University Press,
375 Princeton.

376 Borkent, A. 1980. The potential use of larvae of *Chaoborus cooki* Sakther (Diptera:
377 Chaoboridae) as a biological control of mosquito larvae. *Mosq. News* 40, 634–635.

378 Crawley, M.J., 2007. *The R Book*. John Wiley, Chichester.

379 Cuthbert, R.N., Dick, J.T.A., Callaghan, A., Dickey, J.W.E., 2018a. Biological control
380 agent selection under environmental change using functional responses, abundances and
381 fecundities; the Relative Control Potential (RCP) metric. *Biol. Control* 121, 50–57.

382 Cuthbert, R.N., Dick, J.T.A., Callaghan, A., 2018b. Interspecific variation, habitat
383 complexity and ovipositional responses modulate the efficacy of cyclopoid copepods in
384 disease vector control. *Biol. Control* 121, 80–87.

385 Cuthbert, R.N., Callaghan, A., Dick, J.T.A., 2018c. Dye another day: the predatory
386 impact of cyclopoid copepods on larval mosquito *Culex pipiens* is unaffected by dyed
387 environments. *J. Vector Ecol.* 43, 334–336.

388 Cuthbert, R.N., Dalu, T., Wasserman, R.J., Coughlan, N.E., Callaghan, A., Weyl,
389 O.L.F., Dick, J.T.A., 2018d. Muddy waters: efficacious predation of container-breeding
390 mosquitoes by a newly-described calanoid copepod across differential water clarities. *Biol.*
391 *Control* 127, 25–30.

392 Cuthbert, R.N., Al-Jaibachi, R., Dalu, T., Dick, J.T.A., Callaghan, A., 2019. The
393 influence of microplastics on trophic interaction strengths and oviposition preferences of
394 dipterans. *Sci. Total Environ.* 651, 2420–2423.

395 Dick, J.T.A., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J.,
396 Robinson, T.B., Kumschick, S., Weyl, O.L.F., Dunn, A.M., Hatcher, M.J., Paterson, R.A.,
397 Farnsworth, K.D., Richardson, D.M., 2014. Advancing impact prediction and hypothesis
398 testing in invasion ecology using a comparative functional response approach. *Biol. Invasions*
399 16, 735–753.

400 Dodson, S.I., 1974. Zooplankton competition and predation: an experimental test of
401 the size-efficiency hypothesis. *Ecology* 55, 605–613.

402 Ferguson, N.M., 2018. Challenges and opportunities in controlling mosquito-borne
403 infections. *Nature* 559, 490–497.

404 Griswold, M.W., Lounibos, L.P., 2006. Predator identity and additive effects in a
405 treehole community. *Ecology* 87, 987–995.

406 Härkönen, L., Pekcan-Hekim, Z., Hellén, N., Horppila, J., 2014. Feeding efficiency of
407 *Chaoborus flavicans* (Insecta, Diptera) under turbulent conditions. *Hydrobiologia* 722, 9–17.

408 Hassell, M.P., 1978. The dynamics of arthropod predator- prey systems. Princeton
409 University Press, Princeton, New Jersey.

410 Helgen, J.C., 1989. Larval mosquitoes as vulnerable prey: *Chaoborus* predation. *Can.*
411 *J. Fish Aquat. Sci.* 46, 1642– 1650.

412 Hoel, D.F., Obenauer, P.J., Clark, M., Smith, R., Hughes, T.H., Larson, R.T., Diclaro,
413 J.W., Allan, S.A., 2011. Efficacy of ovitrap colors and patterns for attracting *Aedes*
414 *albopictus* at suburban field sites in North-Central Florida. *J. Am. Mosq. Control Assoc.* 27,
415 245–251.

416 Holling, C.S., 1959. Some characteristics of simple types of predation and parasitism.
417 *Can. Entomol.* 91 (7), 385–398.

418 Horridge, G.A., 1966. Some recently discovered underwater vibration receptors in
419 invertebrates. In Barnes, H. (Ed.), *Some Contemporary Studies in Marine Science*. Allen and
420 Undwin, London, pp. 395–405.

421 Juliano, S.A., 2001. Non-linear curve fitting: predation and functional response
422 curves. In: Scheiner, S.M., Gurevitch, J. (Eds.), *Design and Analysis of Ecological*
423 *Experiments*. Oxford University Press, Oxford, pp. 178–196.

424 Kramer, W.L., Mulla, M.I.R.S., 1979. Oviposition attractants and repellents of
425 mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environ.*
426 *Entomol.* 8, 1111–1117.

427 Liljendahl-Nurminen, A., Horppila, J., Malinen, T., Eloranta, P., Vinni, M., Alajarvi,
428 E., Valtonen, S., 2003. The supremacy of invertebrate predators over fish — factors behind
429 the unconventional seasonal dynamics of cladocerans in Lake Hiidenvesi. Arch. Hydrobiol.
430 158, 75–96.

431 Murray, K.O., Mertens, E., Despres, P., 2010. West Nile virus and its emergence in
432 the United States of America. Vet. Res. 41, 67.

433 Nam, V.S., Yen, N.T., Duc, H.M., Tu, T.C., Thang, V.T., Le, N.H., Le Loan, L.,
434 Huong, V.T.Q., Khanh, L.H.K., Trang, H.T.T., Lam, L.Z.Y., Kutcher, S.C., Aaskov, J.G.,
435 Jeffery, J.A.L., Ryan, P.A., Kay, B.H., 2012. Community-based control of *Aedes aegypti* by
436 using *Mesocyclops* in Southern Vietnam. Am. J. Trop. Med. Hyg. 86 (5), 850–859.

437 Napp, S., Petrić, D., Busquets, N., 2018. West Nile virus and other mosquito-borne
438 viruses present in Eastern Europe. Pathog. Glob. Health 112, 233–248.

439 O’Neil, R.J., 1990. Functional response of arthropod predators and its role in the
440 biological control of insect pests in agricultural systems. In: Dunn, P.E., Baker, R.R. (Eds.),
441 New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and
442 Diseases. Alan R. Liss, Inc., New York, pp. 83–96.

443 Ohba, S-Y., Ohtsuka, M., Sunahara, T., Sonoda, Y., Kawashima, E., Takagi, M.,
444 2012. Differential responses to predator cues between two mosquito species breeding in
445 different habitats. Ecol. Entomol. 37, 410–418.

446 Ortiz-Perea, N., Callaghan, A., 2017. Pond dyes are *Culex* mosquito oviposition
447 attractants. PeerJ 5, e3361.

448 Ortiz-Perea, N., Gander, R., Abbey, O., Callaghan, A., 2018. The effect of pond dyes
449 on oviposition and survival in wild *Culex* mosquitoes. PLOS One 3, e0193847.

450 Pintar, M.R., Bohenek, J.R., Eveland, L.L. and Restarits Jr, W.J. 2018. Colonization
451 across gradients of risk and reward: Nutrients and predators generate species-specific
452 responses among aquatic insects. *Funct. Ecol.*, 32: 1589–1598.

453 Pritchard, D.W., Paterson, R.A., Bovy, H.C., Barrios-O’Neill, D., 2017. Frair: an R
454 package for fitting and comparing consumer functional responses. *Methods Ecol. Evol.* 8,
455 1528–1534.

456 R Core Development Team., 2015. A language and environment for statistical
457 computing. R Foundation for Statistical Computing, Vienna.

458 Riessen, H P., O’Brien, W.J., Loveless, B., 1984. An analysis of the components of
459 *Chaoborus* predation on zooplankton and the calculation of relative prey vulnerabilities.
460 *Ecology* 65, 514–522.

461 Reiter, P., 1987. A revised version of the CDC gravid mosquito trap. *J. Am. Mosq.*
462 *Control Assoc.* 3, 325–327.

463 Rogers, D., 1972. Random search and insect population models. *J. Anim. Ecol.* 41,
464 369–383.

465 Silberbush, A. and Blaustein, L. 2011. Mosquito females quantify risk of predation to
466 their progeny when selecting an oviposition site. *Funct. Ecol.* 25, 1091–1095.

467 Solomon, M.E., 1949. The natural control of animal populations. *J. Anim. Ecol.* 18,
468 1–35.

469 Sunahara, T., Ishizaka, K., Mogi, M., 2002. Habitat size: a factor determining the
470 opportunity for encounters between mosquito larvae and aquatic predators. *J. Vector Ecol.*
471 27, 8–20.

472 Townroe, S., Callaghan, A., 2014. British container breeding mosquitoes: the impact
473 of urbanisation and climate change on community composition and phenology. PLoS ONE 9,
474 e95325.

475 Townroe, S., Callaghan, A., 2015. Morphological and fecundity traits of *Culex*
476 mosquitoes caught in gravid traps in urban and rural Berkshire, UK. Bull. Entomol. Res. 105,
477 615–620.

478 Trexler, J.C., McCulloch, C.E., Travis, J., 1988. How can the functional response best
479 be determined? Oecologia 76, 206–214.

480 von Ende, C.N., Dempsey, D.O., 1981. Apparent exclusion of the cladoceran
481 *Bosmina longirostris* by the invertebrate predator *Chaoborus americanus*. Am. Midl. Nat.
482 105, 240–248.

483 Vonesh, J.R., Blaustein, L., 2010. Implications of predator-induced shifts in mosquito
484 oviposition site selection for vector control: a meta-analysis. Israel J. Ecol. Evol. 56, 263–
485 279.

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

489 Williams, R.E., 1962. Effect of coloring oviposition media with regard to the
490 mosquito *Aedes triseriatus* (Say). J. Parasitol. 48, 919–925.

491 Zuharah, W.F., Lester, P.J., 2010. Can adults of the New Zealand mosquito *Culex*
492 *pervigilans* (Bergorth) detect the presence of a key predator in larval habitats? J. Vector Ecol.
493 35, 100–105.

494 Table 1. First order terms and functional response parameters (attack rate, a ; handling time,
495 h ; maximum feeding rate, $1/h$) of larval *Chaoborus flavicans* feeding on larval *C. pipiens* in
496 presence and absence of dye, alongside associated significance levels.

Dye	First order term, P	a, P	h, P	$1/h$
Present	-0.04, < 0.001	0.96, < 0.001	0.05, < 0.001	19.46
Absent	-0.02, 0.04	0.67, < 0.001	0.05, 0.01	22.08

497

498

499

500

501

502

503

504

505

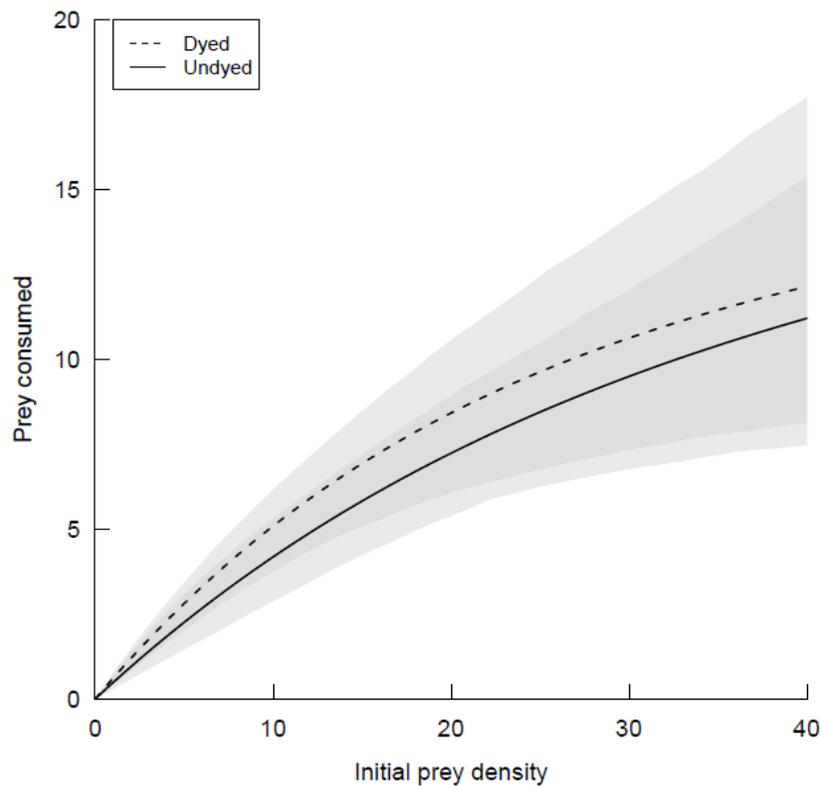
506

507

508

509

510



511

512 Figure 1. Functional responses of larval *Chaoborus flavicans* towards larval *Culex pipiens* in
513 the presence and absence of black dye. Shaded areas are bootstrapped 95% confidence
514 intervals. Note differences in scaling between x and y axes.

515

516

517

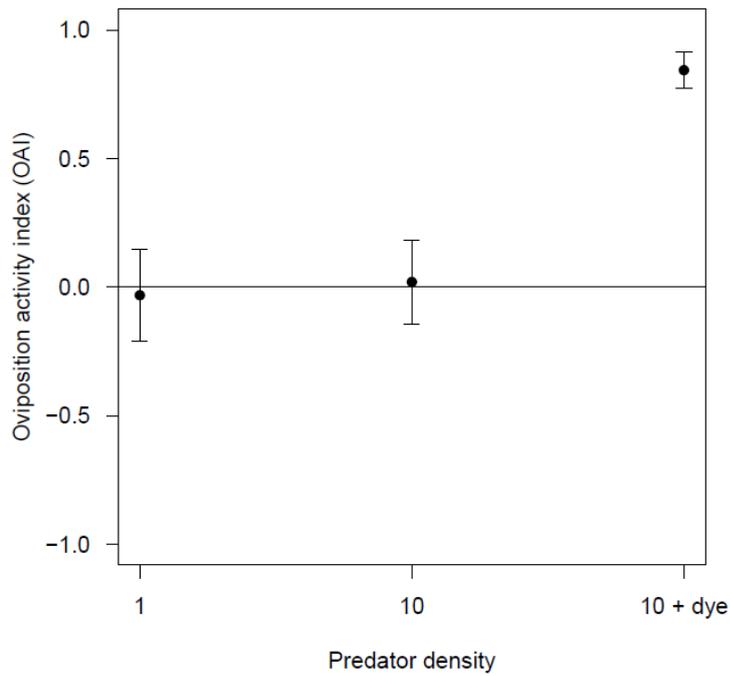
518

519

520

521

522



523

524 Figure 2. Pairwise oviposition activity index (OAI) values of wild gravid adult female *Culex*
525 *pipiens* towards larval *Chaoborus flavicans* at different predator density and black dye
526 treatments in laboratory. Values closer to 1 indicate increasing preference towards predator-
527 treated water. Means are ± 1 SE.

528

529

530

531

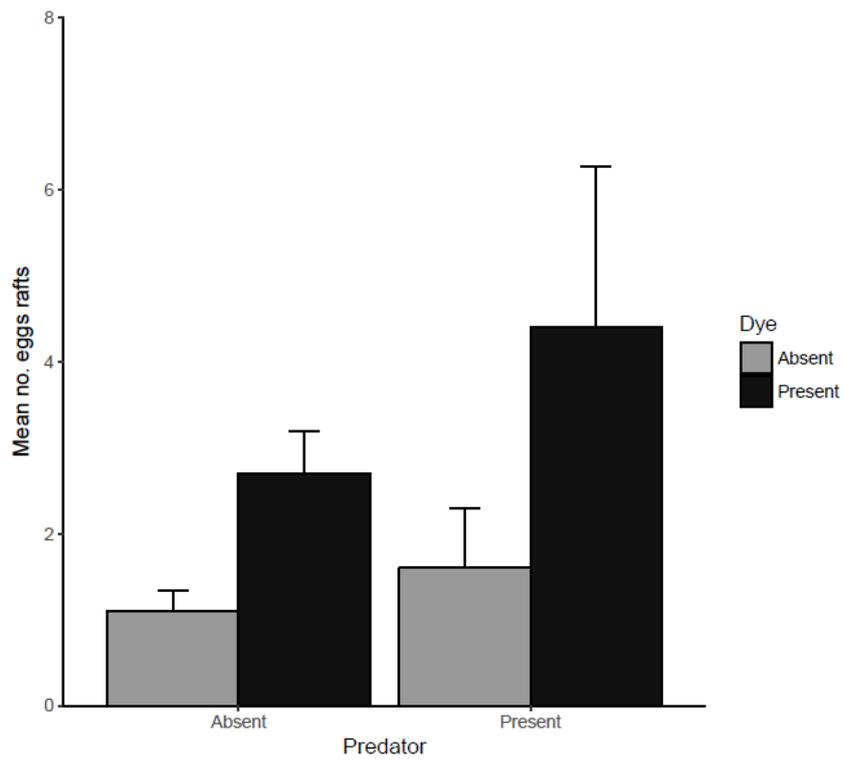
532

533

534

535

536



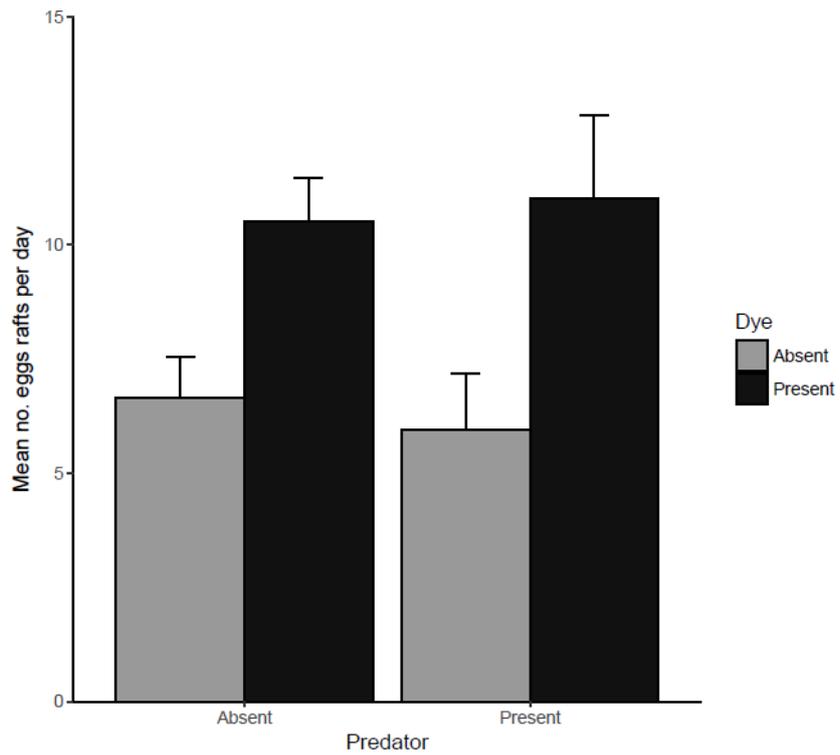
537

538 Figure 3. Numbers of egg rafts oviposited in semi-field tent trial by wild gravid adult *Culex*

539 *pipiens* in response to black dye and predatory *Chaoborus flavicans*. Means are + 1 SE.

540

541



542

543 Figure 4. Numbers of egg rafts oviposited per day in outdoor trial by wild gravid adult *Culex*
544 *pipiens* in response to black dye and predatory *Chaoborus flavicans*. Means are + 1 SE.

545

546

547

548

549

550

551

552