

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

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

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## Abstract

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

## Key words

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

## 1. Introduction

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

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

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

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

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

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

## 2. Methods

### 2.1. Animal collection and maintenance

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

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



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

## 2.2. Functional responses

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

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

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

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

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

### 2.3. Ovipositional responses

All ovipositional choice experiments were conducted between June and August 2018 in Reading, England. Paired oviposition choice tests were used to decipher predator avoidance behaviour of wild gravid adult female *C. pipiens* towards larval *C. flavicans*, which were not starved prior to experimentation. Twenty adult mosquitoes were released into each replicate cage (30 cm<sup>3</sup>) in a laboratory at the University of Reading (25 ± 2°C, 16:8 light:dark regime) and given the choice of two polypropylene 200 mL arenas (9.5 cm dia.) in which to oviposit. Each cage contained a control arena containing dechlorinated tap water alone, and an arena containing dechlorinated tap water and a given predator treatment. Predator treatments comprised either one or ten *C. flavicans* ( $n = 10$  per pair), or ten *C. flavicans* with dyed water (0.3 g/L black liquid pond dye) ( $n = 4$  per pair). The treatment pairs (control, predator) were

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

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

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

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

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

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

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

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

### **3. Results**

#### *3.1. Functional responses*

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

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

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

### 3.2. Ovipositional responses

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

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

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

#### 4. Discussion

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

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

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

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

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

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



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

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

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Table 1. First order terms and functional response parameters (attack rate,  $a$ ; handling time,  $h$ ; maximum feeding rate,  $1/h$ ) of larval *Chaoborus flavicans* feeding on larval *C. pipiens* in 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



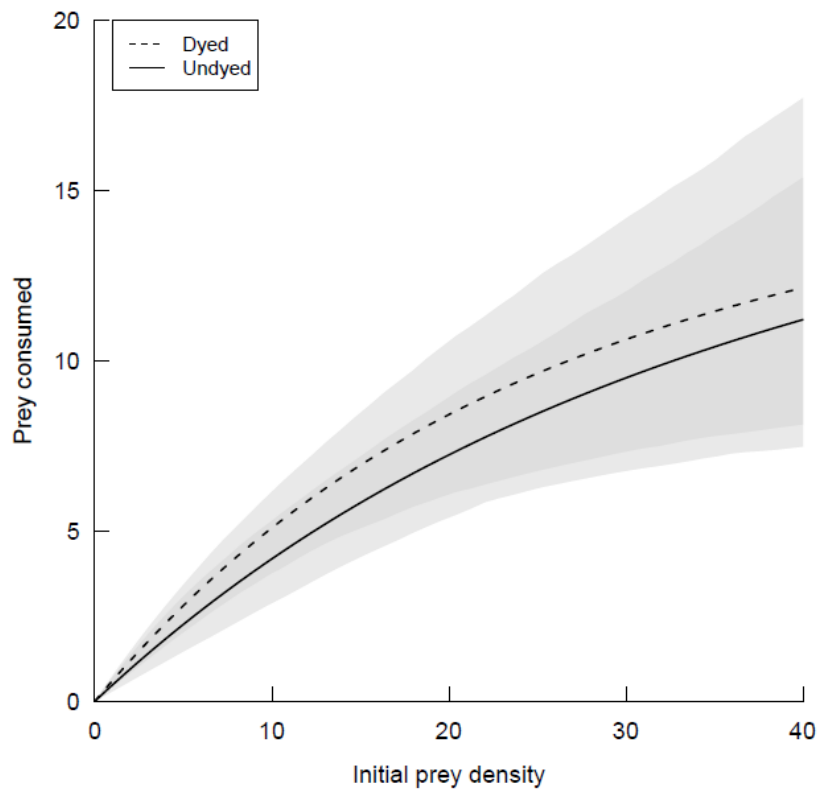


Figure 1. Functional responses of larval *Chaoborus flavicans* towards larval *Culex pipiens* in the presence and absence of black dye. Shaded areas are bootstrapped 95% confidence intervals. Note differences in scaling between x and y axes.

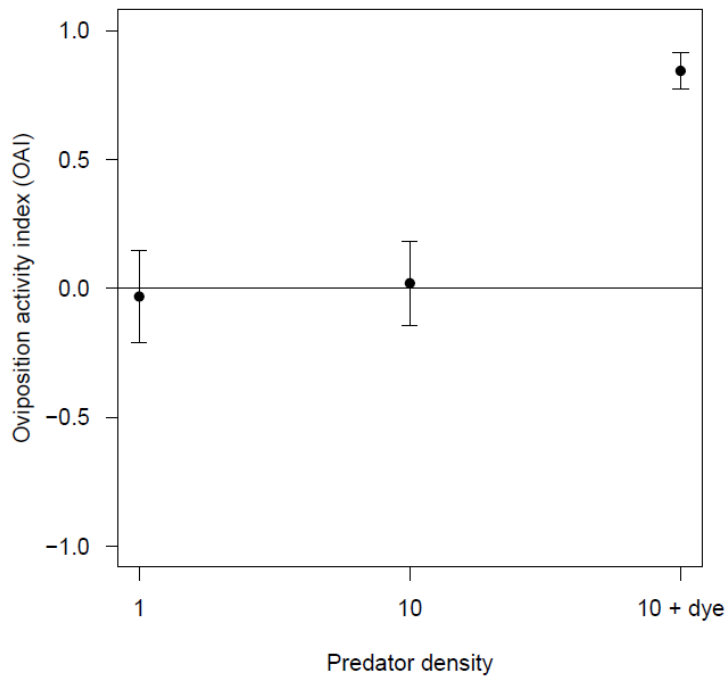


Figure 2. Pairwise oviposition activity index (OAI) values of wild gravid adult female *Culex pipiens* towards larval *Chaoborus flavicans* at different predator density and black dye treatments in laboratory. Values closer to 1 indicate increasing preference towards predator-treated water. Means are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

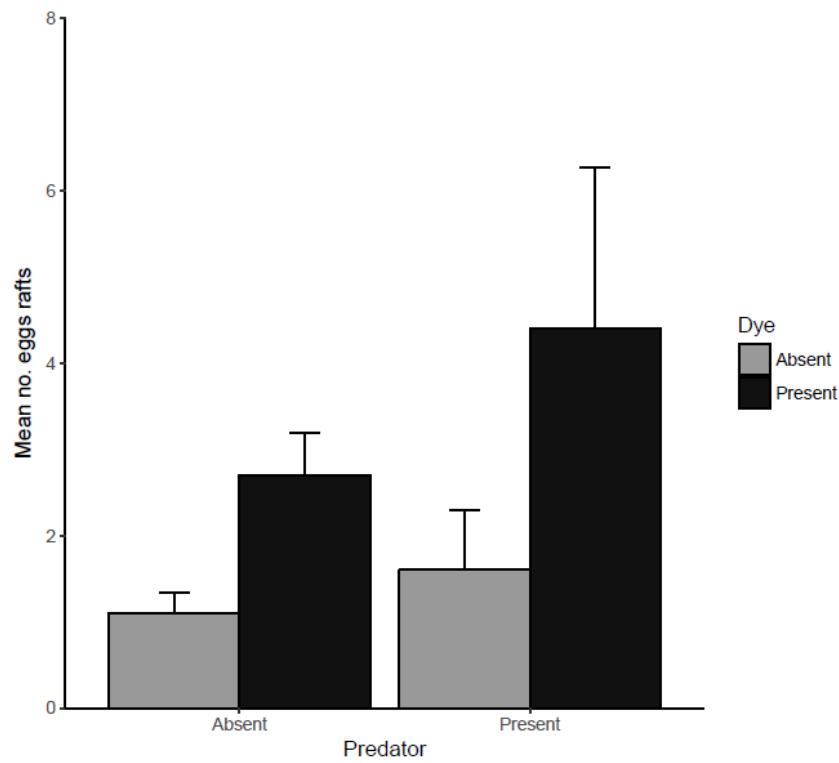


Figure 3. Numbers of egg rafts oviposited in semi-field tent trial by wild gravid adult *Culex pipiens* in response to black dye and predatory *Chaoborus flavicans*. Means are + 1 SE.

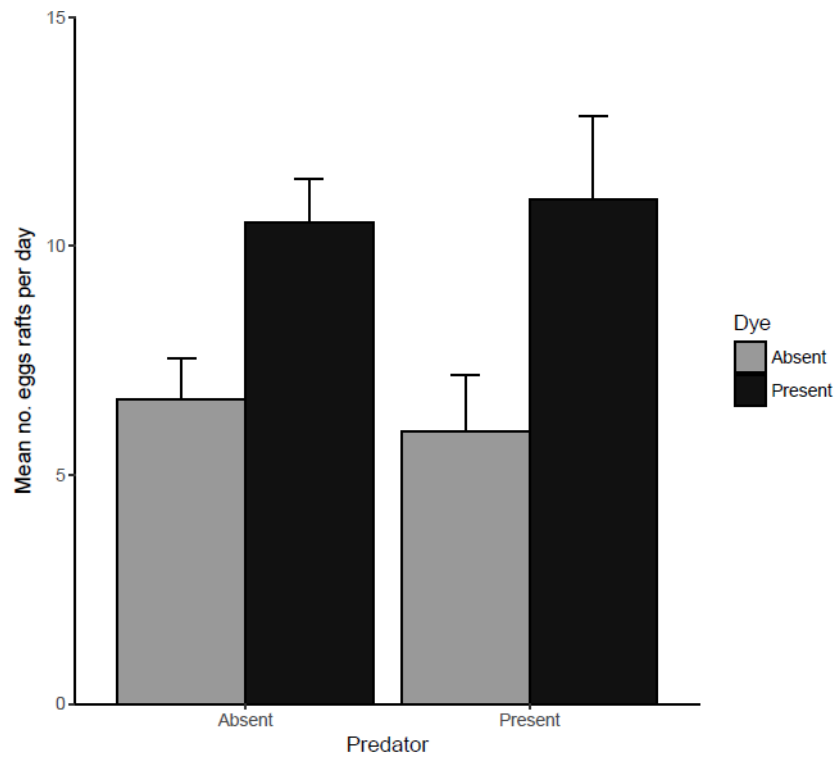


Figure 4. Numbers of egg rafts oviposited per day in outdoor trial by wild gravid adult *Culex pipiens* in response to black dye and predatory *Chaoborus flavicans*. Means are + 1 SE.