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Original Article

Sink trap: duckweed and dye attractant reduce mosquito populations

Duckweed and dye cause mosquito sink

Ross N. Cuthbert[*1,2], Neil E. Coughlan[1], Jaimie T.A. Dick[1], Amanda Callaghan[2]

[1] *Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, Northern Ireland, United Kingdom*

[2] *Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading, Harborne Building, Reading RG6 6AS, England, United Kingdom*

[*] Corresponding author: Ross N. Cuthbert (email, rcuthbert03@qub.ac.uk; telephone, +44(0)28 4272 8230)

Abstract

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

Keywords

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

Introduction

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

Recently, in response to substantial environmental damage and increasing levels of pesticide resistance resulting from the use of synthetic insecticides (Naqqash et al. 2016), there has been an increasing scientific interest in the use of plants or plant extracts for insect pest control (Shaalan and Canyon, 2018; Oladipupo et al. 2019). Indeed, insecticidal properties have been identified across a range of plant species within both aquatic and terrestrial habitats, and many pesticides are based on plant allelochemicals (Shaalan et al. 2005). Accordingly, this may also represent an alternative strategy for the control of disease vector insect species, such as mosquitoes (Elango et al. 2010). Yet, ovipositional responses of pest species may modulate the efficacy of such plants or plant compounds in the control of mosquito populations through selective avoidance behaviours; however, these effects have remained largely unquantified (but see Shaalan and Canyon, 2018). Indeed, if mosquitoes avoid plants which have deleterious effects on their aquatic progeny, this could impede population-level control efficacies.

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

The presence of floating aquatic plants can act as a physical barrier which mechanically inhibits larval mosquito respiration and egg hatchability on the water surface (e.g. Hobbs and Molina, 1983). Duckweed spp. (Lemnaceae) are common free-floating aquatic plants which form dense monospecific mats on surface waters. Many species of duckweed are widespread due to an extensive variety of dispersal mechanisms (e.g. Coughlan et al. 2015b, 2017), coupled with high levels of environmental resilience that facilitate long-distance movement by mobile vectors (Coughlan et al. 2015a, b, 2018). Indeed, duckweed spp. have also been found to colonise container-style habitats (Cuthbert pers. obs.), where vectorially important mosquitoes proliferate in peri-urban and urban areas (Townroe and Callaghan, 2014). Duckweed extracts have insecticidal properties which cause high rates of mortality in larval mosquito populations (Eid et al. 1992b). In addition, such plants have been reported to repel female mosquitoes from ovipositing, whilst also favouring mosquito predators such as copepods (Eid et al. 1992a; Yang et al. 2005; Cuthbert et al. 2018c). Accordingly, to enhance mosquito control effects, the development of measures to counteract such ovipositional avoidance behaviour is vital.

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

The present study therefore examines the effects of duckweed and black pond dye on oviposition selectivity behaviour and natural colonisation of aquatic habitats by wild *Culex pipiens* mosquitoes. First, using a series of laboratory ovipositional choice tests, the responsiveness of gravid mosquitoes to the presence of duckweed and duckweed extract is determined. Second, whether the presence of dye further influences observed mosquito oviposition behaviour in response to duckweed is examined. Finally, in an array of outdoor mesocosms, natural colonisation by mosquitoes under factorial duckweed and dye treatments is quantified over time by monitoring larval mosquito abundances. Specifically, whether mosquitoes will avoid ovipositing in the presence of duckweed or duckweed cues, given its reported mechanical and larvicidal effects, and whether the presence of dye will further modulate behavioural responses of mosquitoes to duckweed through enhanced attraction of dye-treated habitats is assessed. Further, whether the presence of duckweed and dye will act in synergy to reduce wild population numbers of larval mosquitoes in aquatic habitats is tested.

Materials and Methods

Experimental organisms

All experimental organisms were obtained on the University of Reading Whiteknights campus (51°26'12.8"N 0°56'31.8"W). Gravid adult female *C. pipiens* were collected using modified Reiter gravid box traps (Reiter 1987; Townroe and Callaghan 2015). The trap consists of separable upper and lower components. The upper component contains a motor, fan and lead acid battery which creates an air vacuum to draw adult mosquitoes into a collection chamber. The lower portion comprises a tray containing 3 L of bait, a hay and yeast infusion, prepared in advance by fermenting 300 g of hay with 2.5 g of fast-action dried yeast in sealed 80 L outdoor containers for 7 days, stirring occasionally. The bait was then strained and decanted into the lower tray portions of the gravid traps. Trapping occurred overnight, with fresh bait used on each sampling occasion. The following morning, adult mosquitoes were transferred into 30 cm^3 cages and were maintained at 25 °C (± 1 °C) within a laboratory and under a 16 h light:8 h dark photoperiod. Each cage contained 10 % sucrose-soaked cotton for sustenance. Although *C. pipiens* comprised > 99 % of the mosquitoes trapped, individuals of *Culiseta annulata* (Schrank) (Diptera:Culicidae) and *Anopheles plumbeus* Stephens (Diptera:Culicidae) were also collected and dispatched. Duckweed, *Lemna minor* was collected from artificial container-style aquatic habitats by trawling a 1 mm mesh net along the surface waters, before being transferred to the same laboratory, where it was rinsed and stored in 5 L dechlorinated tap water.

Oviposition preferences

During July-August 2018, in the laboratory (25 ± 1 °C, 16:8 light:dark), oviposition responses of wild-caught gravid adult female *C. pipiens* to the presence of duckweed were determined using choice tests. Groups of adult mosquitoes were released into 30 cm^2 cages and given a choice of 200 mL arenas (9.5 cm dia.) in which to oviposit. Arenas were positioned in random corners of each cage to avoid positional effects. In all oviposition experiments, egg rafts were removed and enumerated daily from each cage, over a total of 3 days.

Laboratory paired choice tests

In paired choice tests (treatment/control), treatment cups contained either 5 g (30 adults $cage^{-1}$, n = 6 cages), 20 g (30 adults $cage^{-1}$, n = 4 cages), 50 g (20 adults $cage^{-1}$, n = 5 cages) of intact duckweed plantlets (Figure 1; Experiment 1a–c), or 5 g of ground (30 adults $cage^{-1}$, n = 4 cages; Figure 1; Experiment 1d) duckweed plantlets in dechlorinated tap water from an aerated source, paired with a control treatment of dechlorinated tap water alone. The 5 g ground duckweed treatment was also separately paired with a dyed control treatment (Dyofix black liquid pond dye, 0.3g L^{-1}; 20 adults $cage^{-1}$, n = 5 cages: Figure 1; Experiment 1e). Ground duckweed was prepared using a pestle and mortar until it was a paste. Oviposition activity indices (OAI) were calculated for each treatment pair as per Kramer and Mulla (1979):

$$\mathrm{OAI} = (NT - NC)/(NT + NC) \tag{1}$$

where 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 duckweed treatments and values closer to -1 indicate increasing preference for control treatments (i.e. duckweed avoidance).

Laboratory factorial choice tests

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

Natural colonisation

Between August and October 2014, sixteen 40 L mesocosms (48 cm dia.) were established in the experimental gardens of the University of Reading Whiteknights campus (51°26'12.8"N 0°56'31.8"W). These mesocosms consisted of artificial containers which had been dug into the ground. In a completely randomised factorial design, mesocosms were treated with duckweed (present/absent) and dye (present/absent) (n = 4 per experimental group; Figure 1 Experiment 3). Duckweed treatments comprised total coverage of the pond surface by duckweed, whilst dye treatments comprised 0.3g L^{-1} black liquid pond dye (Dyofix). Each pond was sampled weekly for nine weeks, using a 250 µm mesh dipping net (6 × 12 cm). The net was moved in four figure-of-eight sweeps on each sampling occasion from the top to bottom of mesocosms (as per Ortiz-Perea et al. 2018). Larval *C. pipiens* were identified and then enumerated on a sampling tray before reintroduction into their respective source mesocosms.

Statistical analyses

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

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

Results

Oviposition preferences

In pairwise choice tests, significantly greater numbers of egg rafts were oviposited by *C. pipiens* in the presence of duckweed than duckweed-free controls, irrespective of duckweed 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 g of ground duckweed was present with an undyed control, significantly more egg rafts were oviposited in the presence of ground duckweed ($z = 5.62$, $p < 0.001$). However, when paired with a dyed control, there was no significant preference towards ground duckweed-treated water ($z = 0.83$, $p = 0.41$). These trends were further reflected in OAI values, where preference was significantly displayed towards duckweed-treated water as compared to undyed control groups (Figure 2).

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

Natural colonisation

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

Discussion

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

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

Previous research has demonstrated the density-dependent nature of mosquito ovipositional deterrence by predator cues (Silberbush and Blaustein, 2011). Through the use of different densities of duckweed, the present study did not find any evidence for density-specific modulations in ovipositional preferences towards duckweed-treated water. Mosquitoes demonstrate strong and sustained preference for duckweed-treated water, even up to high density surface coverage which could act as a mechanical barrier for larval mosquito respiration or egg hatching (Baz, 2017). Although not examined here, it is possible that greater ovipositional preferences could exist for higher rather than lower densities of duckweed, in scenarios where a choice between different densities is available. Our results contrast to other experimentations, which have demonstrated a lack of oviposition in water covered by duckweed by mosquitoes within the *C. pipiens* complex (O'Meara et al. 1989; Eid et al. 1992a; Yang et al. 2005). Although these studies were conducted in a different geographical area from the present study, it is unlikely that different coevolutionary histories caused the variations in results found in the present study, given the high abundance and wide distribution of both duckweed and *C. pipiens* across Great Britain and Ireland (Coughlan et al. 2015b; Townroe and Callaghan, 2015). As oviposition attraction was sustained in the presence of ground duckweed, it is probable that attraction is largely driven by chemical cues, which can have larvicidal effects *via* water-borne synomones (i.e. allelochemicals which evoke a response in mosquitoes) (Angerilli and Beirne, 1974; Eid et al. 1992a, b), as oppose to the visual presence of intact duckweed plantlets. Further, given a lack of significant difference between paired duckweed and dye treatments, the attraction of gravid mosquitoes to duckweed may be deemed similar in strength to the attractiveness of black pond dye previously demonstrated, although the drivers of this attraction between treatments likely differ (Ortiz-Perea and Callaghan, 2017).

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

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

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

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

In conclusion, the present study shows that *L. minor*, a common duckweed species, impacts on the behaviour and survival of mosquitoes. These effects can be further modulated by the presence of pond dyes. Our results imply that duckweeds are lethal to mosquitoes through either chemical or mechanical mechanisms, and may be applied for mosquito control in both temperate and tropical regions. Indeed, novel pond dyes have also demonstrated toxicities to larval mosquitoes over longer exposure times (Ortiz-Perea and Callaghan, 2017; Ortiz-Perea et al. 2018). The present study suggests that both duckweeds and dye could be used to improve the biological control of mosquitoes through the formation of mosquito population sinks that are characterised by high levels of oviposition and mortality. However, further work is required to elucidate the influence of duckweed spp. and pond dyes on population dynamics and community interactions within aquatic ecosystems. Equally, there is a need to refine the specific drivers of mosquito larval mortality when exposed to duckweeds and pond dyes.

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Figure legends

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

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

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

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

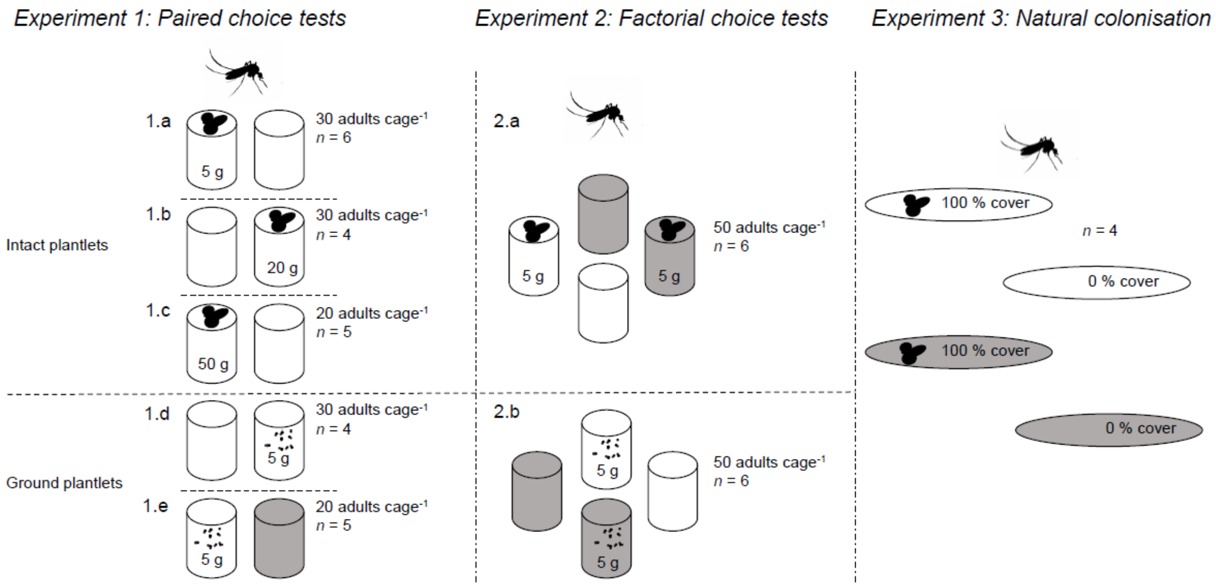
Experiment 1: Paired choice tests
Experiment 2: Factorial choice tests
Experiment 3: Natural colonisation
Intact plantlets
Ground plantlets
1.a
5 g
30 adults cage-1
n = 6
1.b
20 g
30 adults cage-1
n = 4
1.c
50 g
20 adults cage-1
n = 5
1.d
5 g
30 adults cage-1
n = 4
1.e
5 g
20 adults cage-1
n = 5
2.a
5 g
5 g
50 adults cage-1
n = 6
2.b
5 g
5 g
50 adults cage-1
n = 6
100 % cover
n = 4
0 % cover
100 % cover
0 % cover

Figure 1.

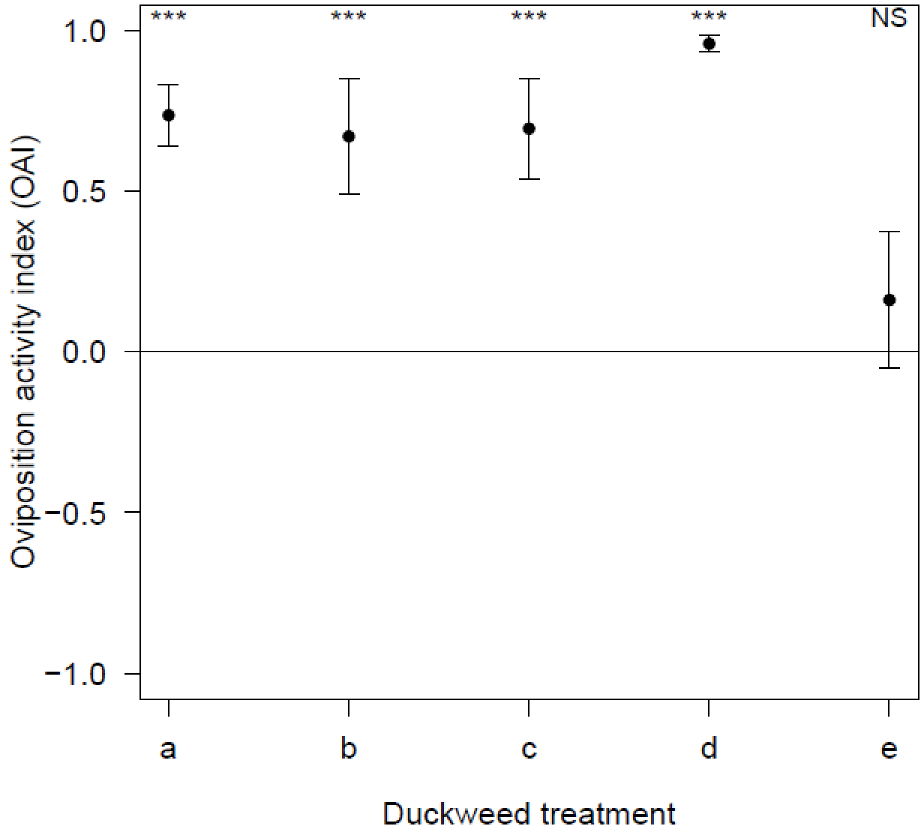
***
***
***
***
NS
1.0
0.5
0.0
−0.5
−1.0
Oviposition activity index (OAI)
a
b
c
d
e
Duckweed treatment

Figure 2.

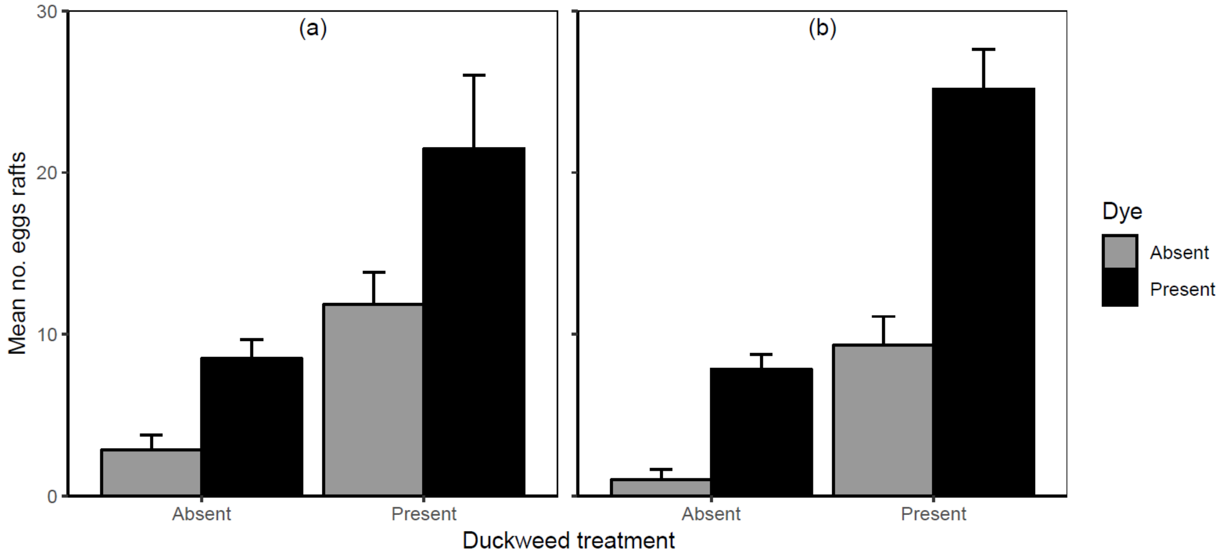
(a)
(b)
Mean no. eggs rafts
30
20
10
0
Absent
Present
Absent
Present
Duckweed treatment
Dye
Absent
Present

Figure 3.

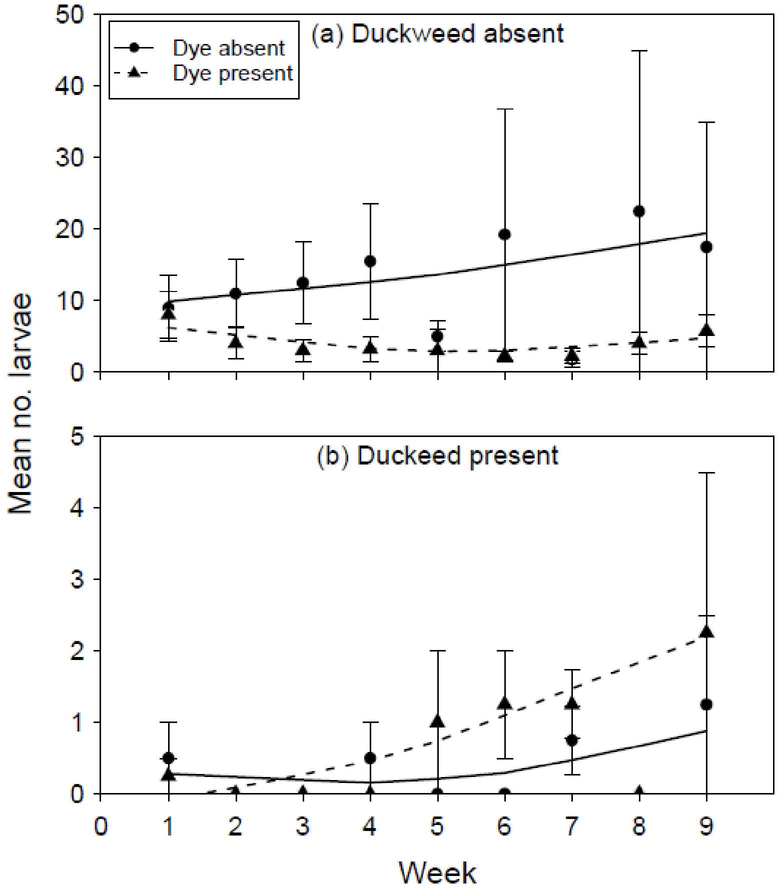
(a) Duckweed absent
Dye absent
Dye present
(b) Duckeed present
Mean no. larvae
Week
50
40
30
20
10
0
5
4
3
2
1
0
0
1
2
3
4
5
6
7
8
9

Figure 4.