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Research Article

Aquatic plant extracts and coverage mediate larval mosquito survivorship and development

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Abstract

Environmental concerns and insecticide resistance threaten the sustained efficacy of mosquito control approaches which remain reliant on synthetic chemicals. Plant-based extracts may be an environmentally sustainable and effective alternative to contemporary mosquito control approaches; however, the efficacies of many possible plant-based extracts remain untested. The present study examines the effects of extracts from three floating and three submerged aquatic plants on larval mosquito *Culex pipiens* mortality, and development to pupal and adult stages. Physical impacts of floating plant species on mosquito mortality and development are also examined. Extracts of *Lagarosiphon major* and *Lemna minuta* were toxic, causing significantly increased mosquito mortality compared to plant-free controls. Effects of *Azolla filiculoides*, *Crassula helmsii, Elodea canadensis* and *Lemna minor* were statistically unclear, yet in some cases tended to increase pupal and larval numbers at high extract concentrations. Surface coverage of all floating *Lemna* species drove significant mosquito mortality through mechanical processes which likely impeded surface respiration by larval mosquitoes. In particular, high-density mats of *L. minuta* consistently caused total larval mortality. The present study thus suggests that targeted use of specific aquatic plants could assist in mosquito control protocols. However, as the chemical composition of botanic material will differ across spatial and temporal gradients, even for a singular species, localised assessment of the efficacy of plant-based extracts from within areas experiencing problematic mosquito control is required. The application of aquatic plants that are both toxic to larvae and are effective physical control agents presents an economic and effective method of mosquito control.

Keywords

Biological control, *Culex pipiens*, disease vector ecology, invasive alien species, lethal effects

Introduction

Many disease-causing pathogens and parasites are commonly vectored by mosquitoes, and can have profound negative impacts on human and wildlife populations (World Health Organisation, 2019). Globally, increasing incidence of insecticide resistance presents a growing problem for the management of problematic mosquito populations (Lima et al., 2011; Duong et al., 2016; Ishak et al., 2017), with the potential to render existing chemical control tools ineffective (World Health Organisation, 2015; Griffin et al., 2016; Hemingway et al., 2016). Accordingly, there is an urgent need to identify novel, efficacious and safe means of facilitating or augmenting vector population suppression. In particular, biological control may play an important role in future management measures for mosquito populations (Thomas, 2018), whilst microbial pathogens such as *Bacillus thuringiensis israelensis* (B.t.i.) and *B. sphaericus* (B.s.) are already particularly useful control options (Becker, 2010). Natural enemies such as arthropods (Cuthbert et al., 2018a), fish (Deacon et al., 2019) and fungal pathogens (Alkhaibari et al., 2016), have been shown to cause lethal effects in mosquito species, and effects of multiple biological control agents can interact to enhance lethal effects (de Beeck et al., 2016). Increasingly, interest is developing in the use of plant extracts for vector mosquito control, given that some plant-based compounds may provide efficient and environmentally friendly insecticidal effects towards target species (Nogueira de and Palmerio, 2001; Benelli, 2016; Shaalan and Canyon, 2018; Oladipupo et al., 2019; Pavela et al., 2019; Cuthbert et al., 2019a). Such approaches might be more sustainable than presently-used synthetical chemicals in mosquito control, which may be damaging to health and the environment (Lowe et al., 2019).

Certain aquatic or riparian plants have been shown to significantly alter mosquito colonisation dynamics and directly influence survivorship of immature stages (Cuthbert et al., 2019a; 2020a). In particular, floating aquatic plants such as duckweed (*Lemna* spp.) and water fern (*Azolla* spp.) have been linked to increased larval mortality rates and physical deformations (Eid et al., 1992a, b; Ravi et al., 2018; Cuthbert et al., 2020a). Indeed, the presence of duckweed has been suggested to impart a mosquito population sink effect, owing to combined oviposition attraction and larval mortality (Cuthbert et al., 2020a). The insecticidal properties of floating aquatic plants may be either driven by their physical characteristics, whereby they form a mechanical barrier which prevents mosquito respiration or egg hatching at the surface (Hobbs and Molina, 1983), or through chemical processes which directly induce mortality via toxicities (Eid et al., 1992a, b; Cuthbert et al., 2020a). Yet these processes often remain undifferentiated and could also be density- or concentration-dependent.

Floating aquatic plants are especially common in stagnant waterbodies which mosquitoes colonise, and the efficient dispersal of such plants via vegetative propagules among habitat patches can be aided by a range of natural vectors (Coughlan et al. 2017a, b). Owing to this high dispersal capacity and rapid growth, many floating aquatic plants have become invasive and thus are associated with adverse ecological impacts (James, 1998; Maguire et al., 2011; Ceschin et al., 2019), and some species proliferate in anthropogenically nutrient-enriched conditions (Carbiener et al., 1990; Paolacci et al., 2016). In an invasion context, if insecticidal effects on mosquitoes are plant species-specific, patterns of co-occurrence and replacement among native and non-native aquatic vegetation may drive differential lethal effects towards mosquitoes. Understanding interspecific differences in plant efficacies among competing and co-existing species is thus warranted to better understand environmental change implications for vector control through, for example, biological invasions.

In contrast to floating aquatic plants, effects of chemical extracts from submerged aquatic macrophytes have remained unexplored in a mosquito control context. A range of aquatic macrophyte species are associated with mosquito habitats (Walton and Workman, 1998; Russell, 1999). In recent decades, invasive submerged macrophyte species have established and proliferated in waterbodies worldwide, with resultant negative effects on ecosystems (Schultz and Dibble, 2012; Getsinger et al., 2014; Lu et al., 2018), with many species capable of spreading effectively via vegetative fragments (Heidbüchel and Hussner, 2020). Submerged plants can also provide some refuge from predators of mosquitoes (Cuthbert et al., 2020b). Owing to the reported development of dense monospecific macrophyte swards in aquatic ecosystems (Caffrey et al., 2010), the presence of submerged plants may have direct larvicidal effects on mosquitoes where they coexist.

Accordingly, the present study examines the chemical effects of a selection of common native and non-native aquatic plants on survivorship and development rates of the vector mosquito *Culex pipiens*. *Culex pipiens* is a widespread and abundant mosquito complex which colonises a range of aquatic habitat types, including artificial containers in urban and peri-urban environments (Townroe and Callaghan, 2014). This species complex is an important vector of West Nile virus from bird to human populations (Becker et al., 2010), and has been previously shown to be sensitive to cues from aquatic plants when selecting oviposition sites (Turnipseed et al., 2018; Cuthbert et al., 2020a). Further, mortality in this species has been shown to be associated with the presence of floating aquatic plants, such as duckweeds (Eid et al., 1992a, b; Cuthbert et al., 2020a); however, whether these effects arise from physical or chemical processes remains largely unclear.

To investigate the potential for aquatic plants to cause larval mosquito mortality and negatively impact the development to pupal and adult life stages, our first experiment examined the effect of chemical extracts at different concentrations derived from six plant species: *Azolla filiculoides*, *Crassula helmsii*, *Elodea canadensis*, *Lagarosiphon major*, *Lemna minor* and *Lemna minuta*. Floating plant species included *A. filiculoides*, *L. minor* and *L. minuta*, whilst *C. helmsii*, *E. canadensis* and *L. major* are submerged macrophytes. In many regions worldwide, *A. filiculoides*, *C. helmsii*, *E. canadensis L. major* and *L. minuta* are considered noxious invasive species (Table 1), whilst *L. minor* is a relatively benign non-native in some regions. In a second experiment, we examined whether different surface coverages of mono-/polycultures for both *L. minor* and *L. minuta* can directly cause larval mosquito mortality through the formation of a mechanical barrier at the water surface.

Based on previous studies (e.g., Cuthbert et al., 2019a; 2020a) and piloting, overall, we hypothesise that: (1) extracts from selected aquatic plants will drive larval mosquito mortality and reduce development to subsequent life stages; (2) higher concentrations of plant extracts will enhance any larvicidal effects associated with efficacious species; (3) surface coverage of *Lemna* species will cause high mosquito mortality rates and reduced development through the presence of a physical barrier to respiration, and; (4) higher levels of plant coverage will enhance the efficacy of physical effects in driving mortality, irrespective of mono- or polyculture treatments.

Methods and materials

Experimental organisms and preparation

All plant species were collected from *in situ* locations in advance of the experiments (Table 1). Prior to use, plant species were separately maintained for a four-to-six week period in stock cultures using dechlorinated tap water at Queen's University Marine Laboratory (QML), Portaferry, Northern Ireland. All plants appeared healthy and displayed sustained growth over the maintenance period. For floating plantlets (i.e. *A. filiculoides*, *L. minor* and *L. minuta*), entire leaf material and attached roots were utilised, whilst apical sections of stem were used in cases of submerged macrophytes (i.e. *C. helmsii*, *E. canadensis* and *L. major*). Plantlets and stem sections were separately rinsed prior to experimentation to detach nonplant material and rotated using a handheld centrifugal spinner to remove excess liquid. Larvae of the *C. pipiens* mosquito complex were obtained from a colony maintained at QML in a laboratory maintained at 25 ± 2 °C and under a 16:8 light:dark photoperiod regime (for full rearing details, see Cuthbert et al. 2018).

Plant extract effects on mosquito mortality and development

A fully factorial experimental design was designed and implemented to test the effects of plant extracts (7 levels: control, *A. filiculoides*, *C. helmsii*, *E. canadensis*, *L. major*, *L. minor* and *L. minuta*) and concentrations (2 levels: low and high) on mosquito mortality and development. Each plant species was separately emulsified using a pestle and mortar, with two masses (0.5 g or 1 g, i.e. low and high) subsequently decanted in solution into 120 mL plastic experimental arenas containing dechlorinated tap water from a continuously aerated source. Ten first instar larval *C. pipiens* (1 – 2 mm), hatched within one day from egg rafts sourced from the laboratory mosquito colony, were added to each arena withholding the allocated treatment. First instar stages were chosen to enable observations of lethal effects across each subsequent ontogeny stage. Each arena also received 0.05 g of ground guinea pig food pellets as a supplemental food resource to prevent mosquito starvation and subsequent mortality/ontogenetic effects. Experiments were undertaken in the laboratory (see before) and lasted for two weeks, after which total mortality, pupal numbers and adult numbers were assessed. Each experimental group was replicated four times, with four replicates of plant-free controls performed for each concentration to quantify background mortality and development rates. All treatment groups were completely randomised to eliminate potential positional effects within the laboratory.

Physical duckweed effects on mosquito mortality and development

The effects of live floating duckweed (4 levels: control, *L. minor*, *L. minuta*, mixture) and coverage (2 levels: 100 % and 200 %, i.e. single or double layer) on mosquito mortality and development were examined in the laboratory (see above conditions). Each plant coverage treatment was established in 120 mL plastic arenas containing dechlorinated tap water from a continuously aerated source based on plant wet biomasses (*L. minor*: 100 %, 0.89 g; 200 %, 1.79 g; *L. minuta*: 100 %, 0.67 g; 200 %, 1.33 g). The mixed treatment group comprised a 1:1 ratio of *L. minor* and *L. minuta*, based on the single-species masses (i.e. 50 % of both species for 100 % coverage; 100 % of both species for 200 % coverage). Pilot studies indicated the quantities of plant groups needed to obtain the allotted surface coverages. Ten first instar larval *C. pipiens* mosquitoes were added to each experimental arena as before, alongside 0.05 g of ground guinea pig pellets as a supplemental food resource (as above). Four replicates were undertaken per experimental group in a randomised array, alongside four plant-free controls containing only the supplemental food source and dechlorinated tap water. After two weeks, total mortality, numbers of pupae and numbers of adults were recorded in each experimental replicate.

Statistics

In both the plant extract and physical experiments, overall mortality rates were analysed using binomial generalised linear models with logit links as a function of plant treatments (including controls as separate treatments levels). Bias reductions were implemented in cases of complete separation (Kosmidis, 2018). Numbers of individuals reaching the pupal and adult stages over the allotted experimental period were similarly analysed using Poisson generalised linear models as a function of plant treatments (including controls as above). Models were examined for overdispersion and zero inflation via simulation comparisons (Hartig, 2019). Where applicable, *post-hoc* pairwise tests were performed using estimated marginal means with Tukey-style adjustments for multiplicity (Lenth, 2018). All statistical analyses were performed in R v3.4.4 (R Core Development Team, 2018).

Results

Plant extract effects on mosquito mortality and development

A significant interaction term indicated that plant extract effects on mosquito mortality differed significantly according to extract concentration ($\chi^2 = 13.52$, df = 6, $p < 0.05$) (Figure 1a). At the low concentration (i.e. 0.5 g 120 mL^{-1}), only *L. major* drove significantly greater mortality rates compared to plant-free controls ($p < 0.05$). At the high extract concentration (i.e. 1 g), however, both *L. major* and *L. minuta* drove significant mosquito mortality relative to controls (both $p < 0.01$). Pupal numbers were significantly affected by plant extract type ($\chi^2 = 14.11$, df = 6, $p < 0.05$), but not concentration ($\chi^2 = 0.14$, df = 1, $p > 0.05$) nor the interaction term ($\chi^2 = 5.96$, df = 6, $p > 0.05$) (Figure 1b); proportions reaching the pupal stage were reduced most in *L. major* treatments, although pairwise comparisons with controls lacked statistical clarity ($p > 0.05$). Conversely, high concentrations of *A. filiculoides*, *C. helmsii* and *L. minor* tended to increase pupation rates over the experimental period. Numbers reaching the adult stage were not significantly affected by plant extracts ($\chi^2 = 10.42$, df = 6, $p > 0.05$), concentration ($\chi^2 = 0.79$, df = 1, $p > 0.05$) nor their interaction ($\chi^2 = 6.72$, df = 6, $p > 0.05$) (Figure 1c). However, numbers reaching the adult stage were generally most reduced under *L. major* treatments, yet tended to increase under higher concentrations of *A. filiculoides* and *L. minor*.

Physical duckweed effects on mosquito mortality and development

Duckweed coverage treatment caused significant mortality in immature mosquitoes ($\chi^2 = 67.26$, df = 6, $p < 0.001$) (Figure 2a). Total mortality was consistently evidenced under 200 %

coverage (i.e. double layer) levels of *L. minuta*, whilst all treatments drove significant mortality compared to plant-free controls (all $p < 0.05$), excepting 200 % cover under the mixed treatment ($p > 0.05$). Plant treatments also significantly affected pupal numbers ($\chi^2 = 16.87$, df = 6, $p < 0.01$), although pairwise comparisons lacked statistical clarity (all $p > 0.05$) (Figure 2b). Similarly, adult numbers were significantly affected by plant coverage ($\chi^2 = 13.87$, df = 6, $p < 0.05$) (Figure 2c). In particular, no instances of pupation or adult emergence were reported under 200 % *L. minuta* coverages, owing to total prepupal mosquito mortality rates.

Discussion

The present study demonstrates interspecific differences in the effects of aquatic plants in mediating larval mosquito mortality and development. Relative to plant-free controls, emulsifications of submerged *L. major* drove significantly increased larval *C. pipiens* mortality irrespective of concentration, and generally reduced numbers developing to subsequent pupal or adult life stages. Further, extracts from floating *L. minuta* also drove significant increases in mortality under higher concentrations, suggesting that the presence of these submerged and floating aquatic plants could influence mosquito survivorship. Contrastingly, extracts from aquatic plants such as floating *A. filiculoides*, submerged *C. helmsii*, submerged *E. canadensis* and floating *L. minor* had no significant effects on larval mosquito survivorship and development, and in some cases tended to promote development. However, whilst the plant chemical extracts never consistently drove total mortality in larval mosquitoes, high density surface coverage of *L. minuta* drove sustained 100 % mortality rates. Lower density coverage, as well as native *L. minor* or mixed-species cover, also significantly heightened larval mortality, yet to a lesser extent than *L. minuta*. These differential effects may be driven by changeable leaf sizes and shapes between species, in turn altering the resolution of surface coverage. Accordingly, aquatic plants have shown interspecific differences here in lethal effects towards mosquitoes, with *L. major* and *L. minuta* particularly efficacious chemically, and *L. minuta* physically.

Larval mosquito stages are often targeted in vector control strategies. Recent interest in the use of botanical derivatives for mosquito population management has arisen as a result of the development of resistance to artificial insecticides (Shaalan et al., 2005; Shaalan and Canyon, 2018). Synthetic larvicides may have harmful non-target effects on diversities of other organisms in the natural environment (Marina et al., 2014; Lowe et al., 2019). Botanical or phytochemical compounds, however, may provide an effective and environmental-friendly alterative to these synthetic larvicides, with the additional advantages of being biodegradable and relatively inexpensive (Ghosh et al., 2012; Abutaha et al., 2018). However, the effects of such extracts on other organisms requires elucidation, and studies should increasingly seek to report the chemical composition of botanical insecticides tested (Isman and Grieneisen, 2014). Comprehensive field-based evaluations are thus still required for many candidate species or compounds before their integration into management approaches, especially from within spatial areas where both these plant species and problematic mosquito populations reside. Nonetheless, this is the first study to test the efficacies of the focal submerged aquatic macrophytes for mosquito control. In particular, we demonstrate significant lethal effects associated with emulsifications of *L. major*, which is an invasive submerged macrophyte, endemic to South Africa. Whilst we do not suggest further introduction of this ecologically-damaging species to control mosquito populations, our results suggest that empirically-reported competitive replacement of plants such as *E. canadensis* by *L. major* may be detrimental to mosquito populations owing to plant cue effects (James et al., 1999; Stiers et al., 2011). However, extracts of these plants could potentially be used to help control mosquito populations without risking introduction of intact specimens, and generate economic activity in marginalised and disadvantaged areas (e.g. *L. major* harvesting by rural South African communities). Nevertheless, the specific chemical compounds that drive mortality in these and other plant species should be further investigated, as well as whether these properties differ among populations across spatial and temporal scales. However, efforts towards the assessment of chemical compounds are best focused on plant specimens found residing in areas experiencing problematic mosquito populations, such as *L. major* sourced directly within South Africa. The relevance of origin, as well as the extent to which these effects emanate from living plant stands, needs to be elucidated in further research.

Here, *L. minuta* also exhibited larvicidal effects towards mosquitoes at higher concentrations. Whilst *L. minuta* has not been previously examined for mosquito control, the congeneric *L. minor* has been suggested to reduce mosquito populations through chemical or physical effects (Eid et al., 1992a, b; Yang et al., 2007; Cuthbert et al., 2020a). Further, extracts from *L. minor* have been shown to cause non-lethal malformations in larvae and repel ovipositing females (Eid et al., 1992a, b; but see Cuthbert et al., 2020a). Thus, compounds from *L. minor* (e.g. fatty acid derivatives, Sun et al., 2016; hydroxycinnamic acids, Landolt, 1986) may play a role in defence against insects more generally (War et al., 2011). Contrastingly, our study demonstrated non-significant chemical effects of this plant species on mosquitoes overall. Similarly, whilst a congeneric *Azolla* species has previously demonstrated larvicidal effects (Ravi et al., 2018), *A. filiculoides* chemical effects were non-significant here. Other floating aquatic plants such as the water hyacinth *Eichhornia crassipes* have also been associated with larvicidal activity (Jayanthi et al., 2012; Annie et al., 2015). Where plant extract efficacies in driving mortality are unclear, the organic material associated may instead have been utilised as a food resource for mosquito development. Indeed, whilst not statistically clear, mosquito ontogenic progression rates tended to increase under certain plant extract treatments at higher concentrations in the present study, such as *A. filiculoides* and *L. minor*.

The physical presence of floating aquatic plants such as *Lemna* species has been shown to be a strong oviposition attracted for mosquitoes (Cuthbert et al., 2020a). These effects may also differ seasonally, with duckweeds more likely to form multilayer surface mats in the summer under warmer conditions. Accordingly, over the larval development stage, it is possible that conditions dynamically shift from suitable to unsuitable, and that effects depend on the seasonal ovipositional activities of adult mosquitoes. Other floating macrophytes have additionally displayed attractive properties for gravid mosquitoes (Turnipseed et al., 2018). Given that larvae of most mosquito species are reliant on the surface-air interface for respiration, high-density floating *Lemna* mats may reduce survivorship through the formation of a mechanical barrier. Indeed, in the present study, all *Lemna* species treatments caused significant mortality relative to control groups, suggesting that high-density surface coverage of these plants can help control mosquito populations. *Lemna* mats are, however, likely to have a detrimental impact on underwater ecosystems, blocking light and creating anaerobic conditions, and these effects should be further examined on a community-scale (but see e.g. Ceschin et al., 2019, 2020).

In particular, high-density surface coverage of *L. minuta* induced consistent total mortality of *C. pipiens*. Native to North and South America, *L. minuta* is an invasive duckweed species that has spread rapidly across European countries in recent years (Gassmann et al., 2006), and is known to compete with native *L. minor* for resources (Paolacci et al., 2018). The smaller leaves of *L. minuta* may enhance the resolution of surface-level coverage as compared to larger *L. minor*, making it more difficult for *C. pipiens* to access the surface for respiration. The relatively lower mortality detected for mats of *L. minor* could also be due to air pockets being enclosed under its larger frond surface area than that of *L. minuta*. Yet, the vertical layering of *Lemna* spp. at higher densities was shown to further enhance efficacies through the formation of a denser floating *Lemna* mat, which has also been observed to occur empirically (N. E. Coughlan, pers. obs.). Our results suggest that the replacement of *L. minor* by *L. minuta* could drive greater negative effects on mosquito survivorship, and particularly when viewed in parallel with the apparent adverse chemical effects of this species. Owing to total mortality, no larval mosquitoes experimentally reached the pupal or adult stages under high densities of this species. However, further research is required to examine these plant effects on other mosquito species and under emergent environmental contexts.

The development of novel methods to reduce mosquito populations remains a priority owing to continued circulation of mosquito-borne disease globally. The present study tests the potential efficacy of multiple submerged and floating aquatic plants for mosquito control, with significant larvicidal effects of *L. major* and *L. minuta* displayed. Empirically, if mosquitoes are attracted to oviposit in waters treated with these plants, the use of volatile chemicals from these species could assist with mosquito control strategies. However, further work is required to elucidate whether mosquito oviposition behaviour is modulated by the presence of these plants, in the context of potential population sink effects (Schorkopf et al., 2016; Cuthbert et al., 2020a). Further studies should also seek to better-characterise the chemical composition of tested materials in botanical insecticides (Isman and Grieneisen, 2014), and across a higher range of concentrations, as the chemical properties of plants might differ spatiotemporally or according to nutrient availability in the harvested area. Moreover, field-based evaluations are urgently required more generally in the contexts of botanical insecticides more generally (Pavela et al., 2019). Whilst we do not suggest deliberate introductions or applications of intact non-native species, our results suggest that transitions from native to invasive aquatic plant stands could also be associated with increased mosquito mortality rates in certain situations.

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Figure captions

Figure 1. Effects of chemical aquatic plant cues on *Culex pipiens* under different exposure concentrations of 0.5 g and 1 g. For clarity, grey shaded bars show 1 g treatments (or controls accounting for both concentrations). (A) Median percentage mortality, with interquartile ranges, and maximum and minimum values shown; (B) mean (± SE) pupation numbers, i.e. number of pupae hatched; and (C), mean (± SE) eclosion numbers i.e. number of adults emerged. In all cases, treatment groups are *n* = 4. The area between the median and first quartile is shaded black (A). The symbol * depicts significant differences between control and treatment groups.

Figure 2. Effects of physical surface cover on *Culex pipiens* under different surface coverages of 100 % and 200 % (i.e. double layer). For clarity, grey shaded bars show 200 % treatments. (A) Median percentage mortality, with interquartile range and maximum and minimum values shown; (B) mean (± SE) pupation numbers, i.e. number of pupae hatched; and (C), mean (± SE) eclosion numbers, i.e. number of adults emerged. In all cases, treatment groups are *n* = 4. The area between the median and first quartile is shaded black (A), while the symbol ■ is used to denote consistent values of 100 (A) or 0 (B & C). The symbol * depicts significant differences between control and treatment groups.

Figure 1:

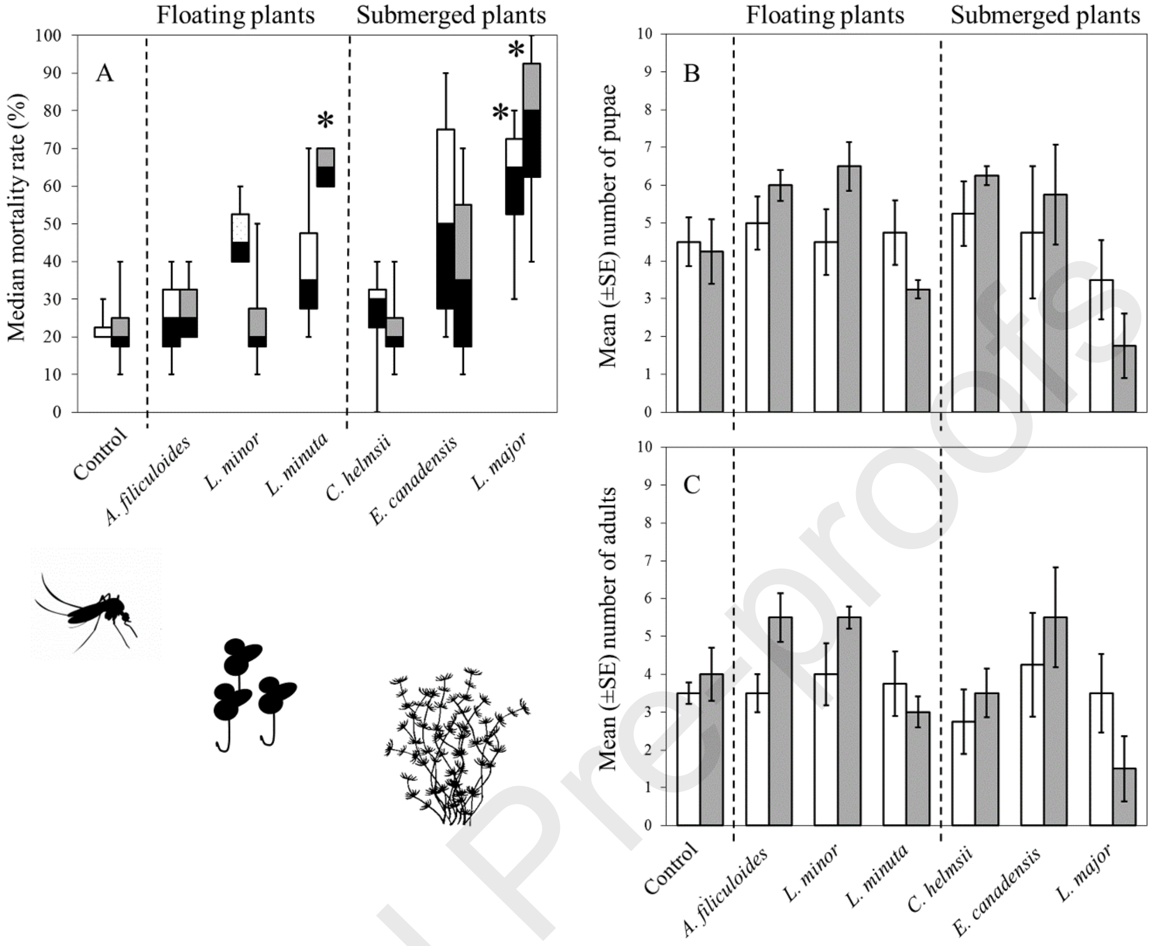
Floating plants
Submerged plants
A
Median mortality rate (%)
100
90
80
70
60
50
40
30
20
10
0
*
*
*
Control
A. filiculoides
L. minor
L. minuta
C. helmsii
E. canadensis
L. major
B
Mean (±SE) number of pupae
10
9
8
7
6
5
4
3
2
1
0
C
Mean (±SE) number of adults
Control
A. filiculoides
L. minor
L. minuta
C. helmsii
E. canadensis
L. major

Figure 2:

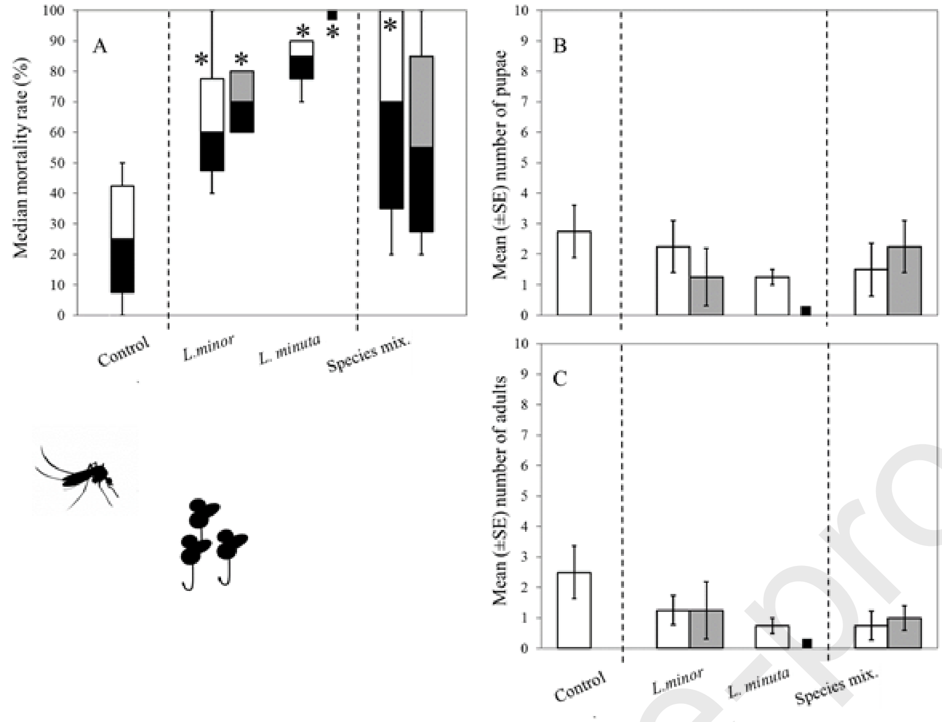
A
Median mortality rate (%)
0
10
20
30
40
50
60
70
80
90
100
*
*
*
*
*
Control
L.minor
L. minuta
Species mix.
B
Mean (±SE) number of pupae
0
1
2
3
4
5
6
7
8
9
10
C
Mean (±SE) number of adults
0
1
2
3
4
5
6
7
8
9
10
Control
L.minor
L. minuta
Species mix.

Table 1: Study species, source site locations, native and invaded ranges. See www.cabi.org/isc/ for further species specific information.

Species	Common name	Source site	Native Range	Invasion Range
Floating Species				
Azolla filiculoides Lam.	Water fern	Lee Road Ponds 51° 53' 50.9"N; 8° 31' 43.5"W	North America, South America	Europe, Africa, Asia, Oceania; potentially invasive in native range
Lemna minor L.	Common duckweed/Lesser duckweed	Upper Lough Erne 54° 13' 50.6"N; 7° 34' 14.2"W	Africa, Asia, Europe, North America.	Considered naturalised in Australasia and South America
Lemna minuta Kunth	Least duckweed/Minute duckweed	Lee Road Ponds 51° 53' 50.9"N; 8° 31' 43.5"W	North America, South America	Asia, Europe
Submerged Species				
Crassula helmsii (Kirk) Cockayne	Australian swamp stonecrop/ New Zealand Pigmyweed	Lough Beg 54° 47' 28.6"N; 6° 28' 27.1" W	Australia, New Zealand	Europe, North America; invasive in native range
Elodea canadensis Michx.	Canadian waterweed	Mill Pond, Tully Mill 54° 15′ 32.34″N; 7° 42′ 50.88″W	North America	Africa, Asia, Europe, South America, Oceania; invasive in native range

Lagarosiphon major (Ridl.) Moss	African elodea/ African curly waterweed	Portadown Golf Club Pond 54° 24' 14.6"N; 6° 24' 51.3"W	Africa	Australia, Europe, New Zealand; potentially invasive in native range

Author statement

Ross N. Cuthbert: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Supervision; Visualization; Roles/Writing - original draft. Gina Y. W. Vong: Data curation; Investigation; Methodology; Writing - review & editing. Simona Paolacci: Conceptualization; Resources; Writing - review & editing. Jaimie T. A. Dick: Project administration; Resources; Supervision; Writing - review & editing. Amanda Callaghan: Supervision; Writing - review & editing; Neil E. Coughlan: Conceptualization; Funding acquisition; Investigation; Methodology; Supervision; Visualization; Writing - review & editing.

- Aquatic plant extracts and presence affect mosquito mortality and development.
- *Lagarosiphon major* and *Lemna minuta* extracts are significantly toxic on *Culex.*
- Floating *Lemna minor* and *L. minuta* caused significant mortality physically.
- Larval mosquito pupation and adult emergence can be significantly reduced.
- Plant extracts and presence have species-specific effects on mosquitoes.