No impact of biocontrol agent's predation cues on development time or size of surviving *Aedes albopictus* under optimal nutritional availability

Marie C. Russell[1]* and Lauren J. Cator[1]

[1]Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot, UK

*Correspondence: marie.clare.russell@gmail.com

Graphical abstract

Abstract

Cyclopoid copepods have been applied successfully to limit populations of highly invasive *Aedes albopictus* mosquitoes that can transmit diseases of public health importance. However, there is concern that changes in certain mosquito traits, induced by exposure to

copepod predation, might increase the risk of disease transmission. In this study, third instar *Ae. albopictus* larvae, hereafter referred to as "focal individuals", were exposed to *Megacyclops viridis* predator cues associated with both consumption of newly-hatched mosquito larvae and attacks on focal individuals. The number of newly-hatched larvae surrounding each focal larva was held constant to control for density effects on size, and the focal individual's day of pupation and wing length were recorded for each replicate. Exposing late instar *Ae. albopictus* to predation decreased their chances of surviving to adulthood, and three focal larvae that died in the predator treatment showed signs of melanization, indicative of wounding. Among surviving focal *Ae. albopictus*, no significant difference in either pupation day or wing length was observed due to copepod predation. The absence of a significant sublethal impact from *M. viridis* copepod predation on surviving later-stage larvae in this analysis supports the use of *M. viridis* as a biocontrol agent against *Ae. albopictus*.

Keywords

predator-prey interactions, sublethal effects, vector traits

Introduction

Aedes albopictus is an important vector of dengue, chikungunya, yellow fever, and Zika [1]. This species is highly invasive due, in part, to its ability to lay desiccation-resistant eggs that can be transported across long distances, often by the shipment of used tires [2-6]. The northern limits of the global range of *Ae. albopictus* include North American populations in New York and Connecticut, USA [7], as well as European populations throughout Italy and France [8].

Several different methods have been proposed for controlling aedine mosquitoes in Europe, including chemical, genetic, and biological techniques [9]. Although space spraying with pyrethroid insecticide has been found to be effective against *Ae. albopictus* in Catalonia, this control strategy would require regular monitoring for insecticide resistance [10]. The Sterile Insect Technique (SIT) has previously been tested on *Ae. albopictus* mosquitoes from Italy, but it was not found to significantly reduce their population due to the reduced mating competitiveness of irradiated males [11]. Cyclopoid copepods have been used successfully as biocontrol agents against mosquito larvae in the US [12], Australia [13], Vietnam [14], and Italy [15]. Copepods are an especially convenient type of biocontrol because they are small enough to be distributed through a backpack sprayer [16]. However, exposure to predators over multiple generations can result in greater mosquito population performance, as measured by a composite index of performance (*r'*) that takes mosquito wing length into account [17, 18].

Copepod predators are much more effective at killing first and second instar than third and fourth instar *Ae. albopictus* [19]. Thus, after an application of copepod predators to *Ae. albopictus* larval habitats, it is likely that any immatures that were third and fourth instar larvae at the time of application would still emerge as adults. These individuals may have withstood both unsuccessful copepod attacks and exposure to the deaths of surrounding smaller conspecifics. Such indirect interactions between predator and prey species can strongly impact prey traits [20, 21].

In aquatic ecosystems, sublethal effects are governed by olfactory cues, including predator kairomones and chemical alarm cues from the prey [22]. For example, *Culex pipiens* larvae

significantly reduce their movement when exposed to conspecifics that have been killed by notonectid predators [23]. In *Cx. restuans*, both freezing (hanging at the water's surface or drifting in the water column) and fleeing (moving for nearly a full minute without resting) behaviors were observed as responses to conspecific alarm cues [24]. *Cx. restuans* larvae have also learned to recognize a salamander predator's odor as a threat, after experiencing the predator's odor paired with conspecific alarm cues [25]. When *Ae. triseriatus* larvae were exposed to chemical cues of *Toxorhynchites rutilus* predation until adult emergence, females exhibited significantly shorter development times under high nutrient availability [26].

Adult body size is an important vector trait that can be affected by predation. For example, exposure to a backswimmer predator, *Anisops jaczewskii,* was found to reduce the size of *Anopheles coluzzi*, one of the main vectors of the human malaria parasite *Plasmodium falciparum* in Africa [27]. Similarly, larval exposure to chemical cues from the predatory fish *Hypseleotris galii* reduces adult size of *Ae. notoscriptus*, the main vector of canine heartworm, *Dirofilaria immitis* in Australia [28]. However, in comparison to how other mosquito species respond to predators, studies on *Ae. albopictus* indicate this species may be less sensitive to predator cues [29, 30].

As an invader, *Ae. albopictus* is less likely to share an evolutionary history with the predators that it encounters. A lack of a shared evolutionary history between predator and prey species has been shown to weaken non-consumptive predator effects in arthropods [31]. Previous work has shown that second instar *Ae. albopictus* reduce their movement in response to cues from the act of predation by *C. appendiculata*, which likely include dead conspecifics and predator feces, but not in response to cues from a non-feeding predator [32]. However, larger

fourth instar *Ae. albopictus* are less vulnerable to predation and show no behavioral response to cues from *C. appendiculata*, even to cues from the act of predation [33].

If copepods are to be used as biocontrol agents, it is important to understand how predation may alter the adult traits of larvae that are too large to be consumed at the time of application. The body size of aedine mosquitoes can be altered by predation cues [28, 34], and changes in size can have important and complex consequences for population dynamics and disease transmission. In *Ae. albopictus*, larger adults have greater reproductive success [35, 36]. Larger males have been shown to produce more sperm [36], and in larger females, more spermathecae have been observed to contain sperm [37]. Larger female body size consistently correlates with higher fecundity, when measured as the number of mature follicles in the ovaries [38], or as the number of eggs laid [35]. Thus, larger emerging *Ae. albopictus* could lead to increased mosquito abundance.

In addition, larger *Ae. albopictus* adults have displayed significantly longer median lifespans, when controlling for differences due to sex and diet [39]. Vector lifespan is a particularly important trait for predicting disease transmission because older vectors have had more opportunities for exposure to pathogens, and they are more likely to be infectious because they are more likely to have survived a pathogen's extrinsic incubation period [40]. However, smaller *Ae. albopictus* females are more likely to become infected with dengue and to disseminate the virus than larger females [41]. Small females have also been shown to be more likely than large females to take multiple bloodmeals within a single gonotrophic cycle [42]. This higher contact frequency with hosts could increase the risk for disease transmission by small-sized *Ae. albopictus* vectors.

116 Previous studies have shown an increase in *Ae. albopictus* adult size after exposure to
117 predation, but these studies were not designed to control for the greater per capita nutrition or
118 decreased intraspecific competition that often occur when the population density has been
119 significantly lowered due to successful predation [29, 43-45]. The phenomenon of increasing
120 animal body size with decreasing population density has been documented across taxa [46].
121 However, impacts on the feeding behavior of insect prey have been observed in response to
122 predator-associated cues alone [47]. This study is designed to test whether *Ae. albopictus* that
123 have been exposed to predator-associated cues during the later larval stages experience
124 sublethal effects on development time or adult size, independent of density effects. Due to the
125 suitability of some areas in southeast England for *Ae. albopictus* populations [48], we used
126 *Megacyclops viridis*, a likely copepod species for future biocontrol applications [49],
127 collected from Longside Lake in Egham, UK, and *Ae. albopictus* that were originally
128 collected in Montpellier, France. Our results show that while cyclopoid copepod predation by
129 *M. viridis* significantly increases mortality of late-instar *Ae. albopictus*, the related predation
130 cues do not significantly change development time or adult size of those late-instar *Ae.*
131 *albopictus* that survive, assuming optimal nutritional availability.
132

133 **Materials and Methods**

134

135 Local copepod collections:
136 One hundred thirty adult female *M. viridis* copepods were collected during the third week of
137 September in 2019 from the edge of Longside Lake in Egham, Surrey, UK (N 51° 24.298',
138 W 00° 32.599'). Copepods were identified as *M. viridis* (Jurine, 1820) by morphology. The
139 copepods were kept in ten 1 L containers, each holding approximately 500 mL of spring
140 water (Highland Spring, UK) at a 12:12 light/dark cycle, and 20 ± 1°C. *Paramecium*

caudatum were provided *ad libitum* as food for the copepods, and boiled wheat seeds were added to the containers to provide a food source for the ciliates [50].

Temperate *Ae. albopictus* colony care:

A colony of *Ae. albopictus* mosquitoes (original collection Montpellier, France 2016 obtained through Infravec2) was maintained at 27 ± 1°C, 70% relative humidity, and a 12:12 light/dark cycle. Larvae were fed fish food (Cichlid Gold Hikari®, Japan), and adults were given 10% sucrose solution and horse blood administered through a membrane feeding system (Hemotek®, Blackburn, UK). *Ae. albopictus* eggs were collected from the colony on filter papers and stored in plastic bags containing damp paper towels to maintain humidity.

Experimental procedure:

On the morning of the tenth day after the last *M. viridis* were collected from the field, *Ae. albopictus* larvae were hatched over a 3-hour period at 27 ± 1°C. The hatching temperature was kept high, relative to the temperature of the experiment (20 ± 1°C), to maximize the yield of larvae over a semi-synchronous period. Stored egg papers were submerged in 3 mg/L nutrient broth solution (Sigma-Aldrich © 70122 Nutrient Broth No 1) and oxygen was displaced by vacuum suction for 30 min. Immediately following oxygen displacement, ground fish food (Cichlid Gold Hikari®, Japan) was added *ad libitum*. After three hours, 500 larvae were counted and placed into spring water to dilute residual food from the hatching media. These focal larvae were then placed in a 1 L container with 600 mL of spring water and twelve pellets (each 50 mg) of fish food at 20 ± 1°C. The water and fish food were changed every other day.

The focal larvae were held at a constant temperature of 20°C because a previous median regression showed that summer temperatures in Southeast England rose from 14.9°C to 17.0°C between 1971 and 1997 [51]. This warming trend is very likely to continue, with the London climate projected to resemble that of present-day Barcelona by 2050 [52]. The average summer maximum temperature for the Greater London area from 1976 to 2003 was 22.3°C [53]; therefore, 20°C is within the range of realistic summer temperatures to be experienced in Southeast England during the next few decades.

On the sixth day of focal larvae development, 70 female *M. viridis* copepods were each placed in a Petri dish (diameter: 50 mm, height: 20.3 mm) holding 20 mL of spring water to begin a 24-hour starvation period. A second set of *Ae. albopictus* eggs were hatched according to the previously described method, except that the hatch was held at 27 ± 1°C for 18 hours.

On the seventh day of focal larvae development, 140 third instar focal *Ae. albopictus* larvae were each placed in a Petri dish holding 20 mL of spring water, a 50 mg pellet of fish food, and four first instar larvae from the hatch that was started on the previous day. A subset of 30 third instar focal larvae were preserved in 80% ethanol for head capsule width measurements [54]. Seventy starved *M. viridis* copepods were then introduced to 70 out of the 140 Petri dishes (Fig 1).

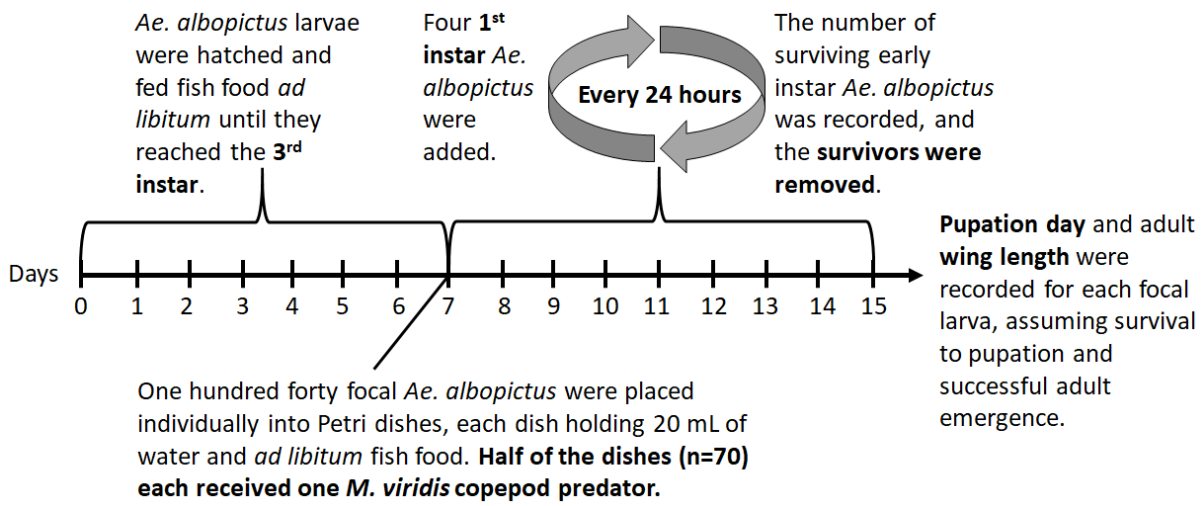

Fig 1. Experiment schedule

Each day, the number of surviving first or second instar larvae in each of the 140 Petri dishes was recorded, surviving first or second instars were removed, and four new first instars from the 18-hour hatch started on the previous day were added to each replicate (Fig 1). The status (dead or alive) of each focal larva was recorded daily, and in predator treatment replicates, the status of the copepod was also recorded. In the case of a focal larva death, the larva was preserved in 80% ethanol, and that replicate was removed from further observation. In the case of a copepod death, the copepod was preserved in 80% ethanol, and a new adult female *M. viridis* copepod from the September field collections was randomly chosen to replace it.

Pupation among focal individuals was recorded each day at 18:00 hrs. Pupae were transferred to 10 mL of spring water in a graduated cylinder with a mesh cover for emergence. Emerged adults were frozen at -20°C. Wings were removed and measured as a proxy for body size [55].

Data analysis:

All analyses were completed in R version 3.4.2 [56]. Welch two sample t-tests for samples of unequal variance were used to compare percent survival of first or second instar larvae between copepod absent and copepod present treatments. The possibility of a difference in the proportion of focal larvae emerging as adults based on copepod presence was examined using a Pearson's chi-squared test without Yates' continuity correction. Two Kruskal-Wallis tests, one for males and one for females, were used to compare adult wing lengths between copepod absent and copepod present treatments. A Kruskal-Wallis test was also used to compare pupation day distributions between predator present and predator absent treatments. A non-parametric local regression method ("loess", "ggplot2" package) was used to present the cumulative proportion of focal larvae pupated over time, by predator presence.

Results

Head capsule width measurements (mean = 0.59 mm, sd = 0.062 mm) of a subset of the focal mosquito larvae (n = 30) confirmed that they were third instars on the first day of exposure to copepod predation [54]. One hundred fifteen copepods (mean length = 1.75 mm, sd = 0.16 mm) were used throughout the experiment across the 70 predator treatment replicates.

Out of the 70 focal larvae that were not exposed to copepod predators, 68, or 97.1%, emerged successfully as adults; one died in the larval stage, and one died in the pupal stage. Out of the 70 focal larvae that were exposed to copepod predators, 61, or 87.1%, emerged successfully as adults; five died in the larval stage, and four died in the pupal stage. Results of a Pearson's chi-squared test showed that the probability of successful adult emergence was higher in the absence of copepod predators (p-value = 0.0279). Three of the five focal larvae that died in

the presence of a predator showed clear signs of melanization in the abdominal region, most likely due to wounding from copepod attacks (Fig 2).

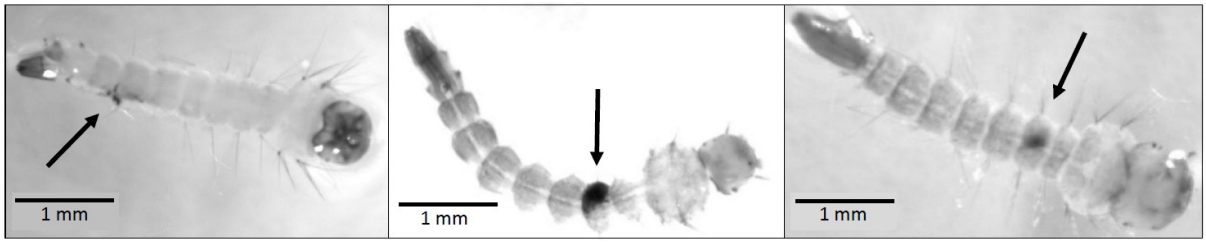

Fig 2. Evidence of melanization in focal larvae that died in the presence of a copepod predator on the eighth, ninth, and tenth days of observation, respectively

The percentage of first instar *Ae. albopictus* surviving each day was significantly lower in the presence of a copepod predator throughout the six days immediately following predator introduction (Table 1). No significant difference in first instar survival was observed on the last two days, when the number of remaining replicates was very low (Table 1).

Table 1. First instar percent survival by day and predator presence

Day of Experiment	**Predator Absent (mean ± se)**	**Number of Predator Absent Replicates**	**Predator Present (mean ± se)**	**Number of Predator Present Replicates**	**P-value[a]**
8	99.3 ± 0.5	70	6.8 ± 2.0	70	<0.0001
9	99.3 ± 0.5	70	30.1 ± 4.1	69	<0.0001
10	99.3 ± 0.5	70	47.8 ± 3.9	68	<0.0001
11	100 ± 0.0	70	42.9 ± 4.3	67	<0.0001
12	100 ± 0.0	42	55.9 ± 6.6	34	<0.0001
13	100 ± 0.0	12	58.3 ± 8.9	12	0.0007
14	100 ± 0.0	5	33.3 ± 33.3	3	0.1835
15	100	1	25 ± 14.4	3	NA

[a]P-value corresponds to a Welch two sample t-test for samples of unequal variance used to determine if there was a difference in first instar survival based on predator presence.

Ae. albopictus adult wing length data were left-skewed among both males and females. Female wing lengths (median = 2.87 mm) were significantly larger than male wing lengths (median = 2.33 mm, Kruskal-Wallis rank sum test, p-value < 0.0001). However, there were no significant differences in male wing length (Kruskal-Wallis rank sum test, p-value =

0.6387) or in female wing length (Kruskal-Wallis rank sum test, p-value = 0.1769), due to copepod presence (Fig 3a).

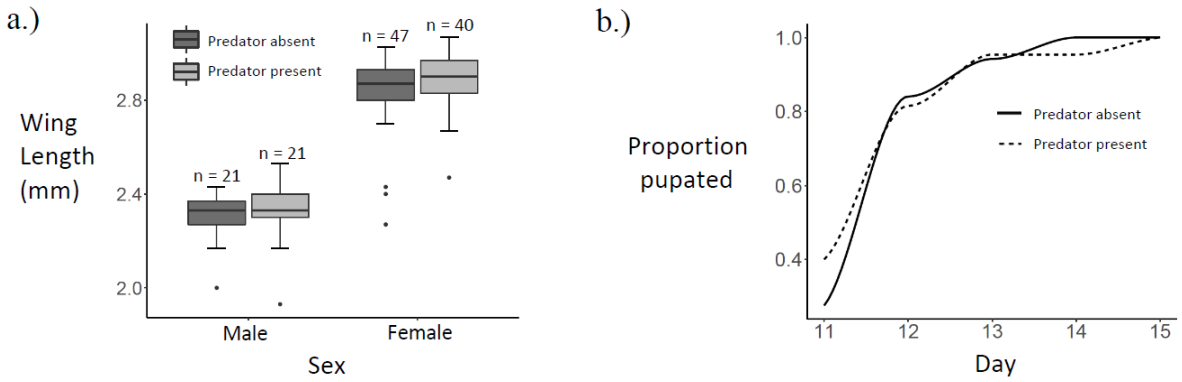

Fig 3. Size and development: a.) Boxplot of wing lengths by sex and predator presence, b.) Cumulative proportion pupated by predator presence

Pupation day data were right-skewed among both males and females. Sex did not affect the day of pupation (Kruskal-Wallis rank sum test, geometric mean ± sd: males = 11.8 ± 1.1, females = 11.9 ± 1.1; p-value = 0.2607). In addition, neither male pupation day (geometric mean ± sd: copepod present = 11.8 ± 1.1, copepod absent = 11.7 ± 1.1; Kruskal-Wallis rank sum test, p-value = 0.7819), nor female pupation day (geometric mean ± sd: copepod present = 11.8 ± 1.1, copepod absent = 12.0 ± 1.1; Kruskal-Wallis rank sum test, p-value = 0.1580), differed with copepod presence (Fig 3b).

Discussion

Although third and fourth instar *Ae. albopictus* larvae are generally less vulnerable to copepod predators than first and second instars [19], some of the third instar, fourth instar, and pupal stage deaths observed in this study were likely due to *M. viridis* copepod attacks. Three focal individuals that died in the larval stage showed signs of melanization (Fig. 2), a response triggered locally by cuticular wounding that results in the accumulation of melanin,

a brown-black pigment, at the wound site [57-59]. Melanization is an energetically costly response that is likely to be influenced by nutritional status in mosquitoes [57, 60, 61]. Since the focal larvae in this study were provided with fish food *ad libitum*, it is unlikely that their immune responses were limited due to poor nutritional status. The location of the three melanization sites in the abdominal region (Fig. 2) is consistent with a previous study, which found that the larval abdomen was attacked more frequently by cyclopoid copepods than either the head/thorax region or the last body segment, containing the siphon [62].

While successfully emerging males were spread evenly between the predator and control treatments, less females emerged successfully from the predator treatment. These observations are consistent with those of a previous study showing that *Ae. albopictus* survivorship is skewed towards males in response to predation by *Toxorhynchites rutilus* [43]. A longer (five-week) semi-field study found that the *Ae. albopictus* sex ratio was skewed towards females after extended exposure to cyclopoid copepod predation [44]. However, lower larval densities have been shown to produce lower proportions of males in *Ae. albopictus* rearing [63]. One possible explanation for this is that the increased nutrient availability for each larva at lower densities might better support the larger body size of females. Thus, lower larval density resulting from predation, is likely to be the main cause of the female-dominated sex ratio that was previously observed [44]. A small increase in mortality among late instar larvae due to predation, such as the 10% increase shown in this study, is unlikely to result in increased body size among the surviving late instars, especially when nutrients are available at high levels, as is often the case in *Ae. albopictus* larval habitats observed in the field [64].

In order for cyclopoid copepods to be effective biocontrols, enough adult female copepods need to be applied in order to quickly eliminate first and second instar mosquito larvae, which are the most vulnerable stages to copepod predation [19]. In some cases of incomplete control, *Ae. albopictus* adults that developed in the presence of predators emerged larger than adults that developed in control conditions because the lower larval density produced by predators resulted in less intraspecific competition and greater per capita nutrition [43-45]. It has also been suggested that first instar larvae benefit nutritionally from decomposing dead conspecifics [29]. First and second instar larvae are likely to benefit the most from increased nutrition because, based on head capsule widths, most larval growth occurs between the first and third instar stages [54]. Under the scenario of incomplete biocontrol leading to the emergence of larger *Ae. albopictus* adults, it is important to consider that *Ae. albopictus* females do not avoid copepods during oviposition [19, 44]. Therefore, the higher fecundity that is associated with larger female size [35, 38] is likely to be strongly counteracted by copepod predation against newly-hatched *Ae. albopictus* larvae of the next generation.

Among *Ae. albopictus* adults that emerged successfully in this experiment, there was a significant difference in wing length due to sex (Fig. 3a), but there was not a significant difference in pupation day between sexes. *Ae. albopictus* males have previously been observed to be 17-20% smaller than females [65]. Accordingly, the male median wing length in this study is 18.8% smaller than the female median wing length, and wing length is known to correlate positively with mass in *Ae. albopictus* [55]. Previous work has shown that while mass clearly differs due to sex, development time in *Ae. albopictus* is less sexually dimorphic [66].

There was no significant difference in wing length or pupation day due to *M. viridis* predation cues (Fig. 3). Therefore, neither the greater reproductive success [35-38] and longer lifespans [39] observed among larger *Ae. albopictus*, nor the higher chance of dengue infection [41] and higher biting rates [42] observed among smaller female *Ae. albopictus* are likely to result directly from cues associated with *M. viridis* predators. A similar lack of predator impact on mosquito size and development time was observed when *Toxorhynchites amboinensis* was tested against newly-hatched *Ae. polynesiensis* in coconuts at three different larval densities [67]. In addition, a study spanning four generations of *Ae. albopictus* did not find any evidence of an evolutionary response to predator exposure in the larval stage [68].

Conclusions

We found evidence of lethal attacks on late instar larvae that resulted in a small, but significant, reduction in the probability of emergence. However, we did not find any evidence that experiencing *M. viridis* predation, both directly and on smaller conspecifics, affects size or development time of surviving *Ae. albopictus* adults at optimal nutritional availability. Previous work has shown that *M. viridis* exhibits a type II functional response curve and a relatively high predation efficiency against *Ae. albopictus* prey at temperatures representative of UK larval habitats [49]. This study builds on recent work and further supports the use of *M. viridis* as a biocontrol agent for *Ae. albopictus* in the southeastern UK, at the northern edge of the vector's expanding global range.

Further research:

Ae. albopictus larvae are generally found in container habitats that have high levels of organic detritus [64]. In addition, ovipositing females prefer organic infusions to containers

holding only water [69]; they also prefer high levels of detritus to low levels, and high quality detritus to low quality [70]. Therefore, we found it most appropriate to test for sublethal effects under conditions of high nutrient availability. Further research is necessary to determine if the same results would be observed under low nutrient availability.

Declarations

Ethics approval and consent to participate:
Not applicable

Consent for publication:
Not applicable

Availability of data and materials:
All data that were collected during this study will be made publicly accessible from the Dryad Digital Repository.

Competing interests:
The authors declare that they have no competing interests.

Funding:
This project relied on resources funded by the European Union's Horizon 2020 research and innovation program under grant agreement No 731060 (Infravec2). The work was primarily funded by a President's PhD Scholarship from Imperial College London awarded to Marie C.

Russell. The funders had no role in study design, data collection and analysis, interpretation of results, decision to publish, or preparation of the manuscript.

Authors' contributions:

LJC and MCR conceptualized the experiment and determined the methodology, including the type of statistical analyses that were employed. MCR collected the data, performed the data analysis, produced the tables and figures, and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Acknowledgements:

We thank Alima Qureshi for her assistance with the mosquito wing measurements. We also thank Catalina Estrada, Martin Selby, and Paul Beasley for their technical support throughout the experiment.

References

1. Centers for Disease C, Prevention. Surveillance and Control of Aedes aegypti and Aedes albopictus in the United States. 2017. Contract No.: Report.
2. Eritja R, Escosa R, Lucientes J, Marques E, Molina R, Roiz D, et al. Worldwide invasion of vector mosquitoes: present European distribution and challenges for Spain. Biological Invasions. 2005;7(1):87-97.
3. Juliano SA, Lounibos LP. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecology Letters. 2005;8(5):558-74.
4. Lounibos LP. Invasions by insect vectors of human disease. Annual Review of Entomology. 2002;47:233-66.
5. Medlock JM, Avenell D, Barrass I, Leach S. Analysis of the potential for survival and seasonal activity of Aedes albopictus (Diptera : Culicidae) in the United Kingdom. Journal of Vector Ecology. 2006;31(2):292-304.
6. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: Global risk of invasion by the mosquito Aedes albopictus. Vector-Borne and Zoonotic Diseases. 2007;7(1):76-85.
7. Kache PA, Eastwood G, Collins-Palmer K, Katz M, Falco RC, Bajwa WI, et al. Environmental Determinants of Aedes albopictus Abundance at a Northern Limit of Its Range in the United States. American Journal of Tropical Medicine and Hygiene. 2020;102(2):436-47.

401 8. European Centre for Disease P, Control, cartographers. Aedes albopictus - current
402 known distribution: May 20202020.
403 9. Baldacchino F, Caputo B, Chandre F, Drago A, della Torre A, Montarsi F, et al.
404 Control methods against invasive Aedes mosquitoes in Europe: a review. Pest management
405 science. 2015;71(11):1471-85.
406 10. Paaijmans K, Brustollin M, Aranda C, Eritja R, Talavera S, Pages N, et al. Phenotypic
407 insecticide resistance in arbovirus mosquito vectors in Catalonia and its capital Barcelona
408 (Spain). Plos One. 2019;14(7).
409 11. Bellini R, Balestrino F, Medici A, Gentile G, Veronesi R, Carrieri M. Mating
410 Competitiveness of Aedes albopictus Radio-Sterilized Males in Large Enclosures Exposed to
411 Natural Conditions. Journal of Medical Entomology. 2013;50(1):94-102.
412 12. Marten GG. Elimination of Aedes-Albopictus from Tire Piles by Introducing
413 Macrocyclops-Albidus (Copepoda, Cyclopidae). Journal of the American Mosquito Control
414 Association. 1990;6(4):689-93.
415 13. Russell BM, Muir LE, Weinstein P, Kay BH. Surveillance of the mosquito Aedes
416 aegypti and its biocontrol with the copepod Mesocyclops aspericornis in Australian wells and
417 gold mines. Medical and Veterinary Entomology. 1996;10(2):155-60.
418 14. Kay BH, Nam VS, Van Tien T, Yen NT, Phong TV, Diep VTB, et al. Control of
419 Aedes vectors of dengue in three provinces of Vietnam by use of Mesocyclops (Copepoda)
420 and community-based methods validated by entomologic, clinical, and serological
421 surveillance. American Journal of Tropical Medicine and Hygiene. 2002;66(1):40-8.
422 15. Veronesi R, Carrieri M, MacCagnani B, Maini S, Bellini R. Macrocyclops Albidus
423 (Copepoda: Cyclopidae) for the Biocontrol of Aedes Albopictus and Culex Pipiens in Italy.
424 Journal of the American Mosquito Control Association. 2015;31(1):32-43.
425 16. Marten GG. Issues in the development of cyclops for mosquito control. Arbovirus
426 Research in Australia. 1990;5:159-64.
427 17. Livdahl TP, Sugihara G. Non-Linear Interactions of Populations and the Importance
428 of Estimating Per Capita Rates of Change. Journal of Animal Ecology. 1984;53(2):573-80.
429 18. Neale JT, Juliano SA. Predation yields greater population performance: What are the
430 contributions of density- and trait-mediated effects? Ecol Entomol. 2021;46(1):56-65.
431 19. Dieng H, Boots M, Tuno N, Tsuda Y, Takagi M. A laboratory and field evaluation of
432 Macrocyclops distinctus, Megacyclops viridis and Mesocyclops pehpeiensis as control agents
433 of the dengue vector Aedes albopictus in a peridomestic area in Nagasaki, Japan. Medical and
434 Veterinary Entomology. 2002;16(3):285-91.
435 20. Preisser EL, Bolnick DI, Benard MF. Scared to death? The effects of intimidation and
436 consumption in predator-prey interactions. Ecology. 2005;86(2):501-9.
437 21. Werner EE, Peacor SD. A review of trait-mediated indirect interactions in ecological
438 communities. Ecology. 2003;84(5):1083-100.
439 22. Ferrari MCO, Wisenden BD, Chivers DP. Chemical ecology of predator-prey
440 interactions in aquatic ecosystems: a review and prospectus. Canadian Journal of Zoology.
441 2010;88(7):698-724.
442 23. Sih A. Antipredator Responses and the Perception of Danger by Mosquito Larvae.
443 Ecology. 1986;67(2):434-41.
444 24. Ferrari MCO, Messier F, Chivers DP. Variable predation risk and the dynamic nature
445 of mosquito antipredator responses to chemical alarm cues. Chemoecology. 2007;17(4):223-
446 9.
447 25. Ferrari MCO, Messier F, Chivers DP. Threat-sensitive learning of predators by larval
448 mosquitoes Culex restuans. Behavioral Ecology and Sociobiology. 2008;62(7):1079-83.

26. Ower GD, Juliano SA. The demographic and life-history costs of fear: Trait-mediated effects of threat of predation on Aedes triseriatus. Ecology and Evolution. 2019;9(7):3794-806.
27. Roux O, Vantaux A, Roche B, Yameogo KB, Dabire KR, Diabate A, et al. Evidence for carry-over effects of predator exposure on pathogen transmission potential. Proceedings of the Royal Society B-Biological Sciences. 2015;282(1821):20152430.
28. van Uitregt VO, Hurst TP, Wilson RS. Reduced size and starvation resistance in adult mosquitoes, Aedes notoscriptus, exposed to predation cues as larvae. Journal of Animal Ecology. 2012;81(1):108-15.
29. Costanzo KS, Muturi EJ, Alto BW. Trait-mediated effects of predation across life-history stages in container mosquitoes. Ecological Entomology. 2011;36(5):605-15.
30. Ohba S-Y, Ohtsuka M, Sunahara T, Sonoda Y, Kawashima E, Takagi M. Differential responses to predator cues between two mosquito species breeding in different habitats. Ecological Entomology. 2012;37(5):410-8.
31. Buchanan AL, Hermann SL, Lund M, Szendrei Z. A meta-analysis of non-consumptive predator effects in arthropods: the influence of organismal and environmental characteristics. Oikos. 2017;126(9):1233-40.
32. Kesavaraju B, Juliano SA. Nature of Predation Risk Cues in Container Systems: Mosquito Responses to Solid Residues From Predation. Ann Entomol Soc Am. 2010;103(6):1038-45.
33. Kesavaraju B, Juliano SA. Behavioral Responses of Aedes albopictus to a Predator Are Correlated with Size-Dependent Risk of Predation. Annals of the Entomological Society of America. 2008;101(6):1150-3.
34. van Uitregt VO, Hurst TP, Wilson RS. Greater costs of inducible behavioural defences at cooler temperatures in larvae of the mosquito, Aedes notoscriptus. Evolutionary Ecology. 2013;27(1):13-26.
35. Blackmore MS, Lord CC. The relationship between size and fecundity in Aedes albopictus. Journal of Vector Ecology. 2000;25(2):212-7.
36. Hatala AJ, Harrington LC, Degner EC. Age and Body Size Influence Sperm Quantity in Male Aedes albopictus (Diptera: Culicidae) Mosquitoes. Journal of Medical Entomology. 2018;55(4):1051-4.
37. Carrasquilla MC, Lounibos LP, Honorio NA, Murr S. Spermathecal Filling in Aedes aegypti and Aedes albopictus: Effects of Female and Male Body Sizes and Species. Journal of Medical Entomology. 2019;56(2):334-40.
38. Armbruster P, Hutchinson RA. Pupal mass and wing length as indicators of fecundity in Aedes albopictus and Aedes geniculatus (Diptera : culicidae). Journal of Medical Entomology. 2002;39(4):699-704.
39. Xue RD, Barnard DR, Muller GC. Effects of Body Size and Nutritional Regimen on Survival in Adult Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology. 2010;47(5):778-82.
40. Cator LJ, Johnson LR, Mordecai EA, El Moustaid F, Smallwood TRC, LaDeau SL, et al. The Role of Vector Trait Variation in Vector-Borne Disease Dynamics. Frontiers in Ecology and Evolution. 2020;8.
41. Alto BW, Reiskind MH, Lounibos LP. Size Alters Susceptibility of Vectors to Dengue Virus Infection and Dissemination. American Journal of Tropical Medicine and Hygiene. 2008;79(5):688-95.
42. Farjana T, Tuno N. Multiple Blood Feeding and Host-Seeking Behavior in Aedes aegypti and Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology. 2013;50(4):838-46.

43. Alto BW, Griswold MW, Lounibos LP. Habitat complexity and sex-dependent predation of mosquito larvae in containers. Oecologia. 2005;146(2):300-10.
44. Cardôso HCB, da Silva BQ, de Assis TB, Lopez LCS. Effects of predation by the copepod Mesocyclops ogunnus on the sex ratios of mosquito Aedes albopictus. Hydrobiologia. 2013;705(1):55-61.
45. Dieng H, Boots M, Mwandawiro C, Satho T, Hasegawa M, Nyambura GJ, et al. Effects of a copepod predator on the survivorship and development of Aedes albopictus (Diptera: Culicidae). Medical Entomology and Zoology. 2003;54(2):187-92.
46. Peters RH. Animal abundance. In: Beck E, Birks HJB, Connor EF, editors. The ecological implications of body size. Cambridge: Cambridge University Press; 1983. p. 164-83.
47. Hermann SL, Thaler JS. Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. Oecologia. 2014;176(3):669-76.
48. Metelmann S, Caminade C, Jones AE, Medlock JM, Baylis M, Morse AP. The UK's suitability for Aedes albopictus in current and future climates. Journal of the Royal Society Interface. 2019;16(152).
49. Russell MC, Qureshi A, Wilson CG, Cator LJ. Size, not temperature, drives cyclopoid copepod predation of invasive mosquito larvae. PLOS ONE. 2021;16(2):e0246178.
50. Suarez MF, Marten GG, Clark GG. A Simple Method for Cultivating Fresh-Water Copepods used in Biological-Control of Aedes-Aegypti. Journal of the American Mosquito Control Association. 1992;8(4):409-12.
51. Donaldson GC, Keatinge WR, Nayha S. Changes in summer temperature and heat-related mortality since 1971 in North Carolina, South Finland, and Southeast England. Environmental Research. 2003;91(1):1-7.
52. Bastin JF, Clark E, Elliott T, Hart S, van den Hoogen J, Hordijk I, et al. Understanding climate change from a global analysis of city analogues. Plos One. 2019;14(7).
53. Gosling SN, McGregor GR, Paldy A. Climate change and heat-related mortality in six cities Part 1: model construction and validation. International Journal of Biometeorology. 2007;51(6):525-40.
54. Teng HJ, Apperson CS. Identification of larval instars of Aedes albopictus (Skuse) and Aedes triseriatus (Say) (Diptera: culicidae) based on head capsule size. Journal of Vector Ecology. 1996;21(2):186-91.
55. Siegel JP, Novak RJ, Ruesink WG. Relationship between wing length and dry weight of mosquitoes. Journal of the American Mosquito Control Association. 1994;10(2):186-96.
56. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020.
57. Christensen BM, Li JY, Chen CC, Nappi AJ. Melanization immune responses in mosquito vectors. Trends Parasitol. 2005;21(4):192-9.
58. Lai SC, Chen CC, Hou RF. Immunolocalization of prophenoloxidase in the process of wound healing in the mosquito Armigeres subalbatus (Diptera : Culicidae). Journal of Medical Entomology. 2002;39(2):266-74.
59. Nakhleh J, El Moussawi L, Osta MA. The Melanization Response in Insect Immunity. In: Ligoxygakis P, editor. Insect Immunity. Advances in Insect Physiology. 522017. p. 83-109.
60. Chapman RF, editor. The insects: structure and function: Cambridge University Press; 1998.
61. Lee KP, Simpson SJ, Wilson K. Dietary protein-quality influences melanization and immune function in an insect. Functional Ecology. 2008;22(6):1052-61.

62. Awasthi AK, Wu CH, Tsai KH, King CC, Hwang JS. How Does the Ambush Predatory Copepod Megacyclops formosanus (Harada, 1931) Capture Mosquito Larvae of Aedes aegypti? Zoological Studies. 2012;51(7):927-36.
63. Puggioli A, Carrieri M, Dindo ML, Medici A, Lees RS, Gilles JRL, et al. Development of Aedes albopictus (Diptera: Culicidae) Larvae Under Different Laboratory Conditions. Journal of Medical Entomology. 2017;54(1):142-9.
64. Yee DA. What Can Larval Ecology Tell Us About the Success of Aedes albopictus (Diptera: Culicidae) Within the United States? Journal of medical entomology. 2016;53(5):1002-12.
65. Briegel H, Timmermann SE. Aedes albopictus (Diptera : Culicidae): Physiological aspects of development and reproduction. Journal of Medical Entomology. 2001;38(4):566-71.
66. Wormington JD, Juliano SA. Sexually dimorphic body size and development time plasticity in Aedes mosquitoes (Diptera: Culicidae). Evolutionary Ecology Research. 2014;16(3):223-34.
67. Mercer DR, Wettach GR, Smith JL. Effects of larval density and predation by Toxorhynchites amboinensis on Aedes polynesiensis (Diptera : culicidae) developing in coconuts. Journal of the American Mosquito Control Association. 2005;21(4):425-31.
68. Kesavaraju B, Juliano SA. No Evolutionary Response to Four Generations of Laboratory Selection on Antipredator Behavior of Aedes albopictus: Potential Implications for Biotic Resistance to Invasion. Journal of Medical Entomology. 2009;46(4):772-81.
69. Trexler JD, Apperson CS, Schal C. Laboratory and field evaluations of Oviposition responses of Aedes albopictus and Aedes triseriatus (Diptera : Culicidae) to oak leaf infusions. Journal of Medical Entomology. 1998;35(6):967-76.
70. Fader JE, Juliano SA. Oviposition habitat selection by container-dwelling mosquitoes: responses to cues of larval and detritus abundances in the field. Ecological Entomology. 2014;39(2):245-52.