Evaluating the carnivorous efficacy of Utricularia aurea on the larval stages of Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti

Ajeet Kumar Mohanty (ajeet.nimr@gmail.com)
ICMR-National Institute of Malaria Research
Abhishek Govekar
ICMR-National Institute of Malaria Research
Charles Souza
ICMR-National Institute of Malaria Research
Abhipsa Mohapatra
Goa University
Malapati Kuppuswamy Janarthanam
Goa University
Raja Vukanti
Bhavan's Vivekananda College
Praveen Balabaskaran Nina
Central University of Kerala

Research Article

Keywords: Utricularia aurea, Carnivorous plants, Bladderwort, Vector borne diseases, Mosquito control agent

Posted Date: May 17th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2932771/v1

Abstract

Background

The emergence of insecticide resistance in mosquitoes necessitates the exploration, development and validation of alternative and sustainable biological strategies for controlling mosquitoes in their natural environments.

Methods

We assessed the predatory effect of *Utricularia aurea*, an aquatic carnivorous plant found in the Indian sub-continent, Japan, and Australia, on four instar (larval) stages of *Aedes aegypti, Anopheles stephensi*, and *Culex quinquefasciatus*, in the laboratory and field settings.

Results

In the laboratory, within twelve hours, *U. aurea* predated ~ 95% of the 1st, 2nd, and 3rd instars of the three mosquito species, ~ 80% of the 4th instars of *Ae. aegypti* and *An. stephensi* and ~ 60% of 4th instars of *Cx. quinquefasciatus*. The predatory effect of *U. aurea* significantly varied among the three mosquito species and their instar stages. The effect of *U. aurea* on the instars of *Cx. quinquefasciatus* differed significantly from that on the instars of *Ae. aegypti* and *An. stephensi*. The effect of *U. aurea* on the 4th instars was significantly lower than that on the 1st, 2nd, and 3rd instars of the mosquitoes. The effect of *U. aurea* was higher during the first hour when compared to the other time points; within the first hour, *U. aurea* predated 58, 45, 61% of 1st instars of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus*, respectively. In the field settings, at the end of 16 days, *U. aurea* predated 71 and 76% of the immature *Ae. aegypti* and *An. stephensi* respectively.

Conclusion

Our findings suggest *U. aurea* can be utilized as a potential bio-larval control agent in natural environments; however, the current claim warrants additional investigations in different natural transmission settings .

Background

Mosquito-borne diseases, such as malaria, dengue, chikungunya, Japanese encephalitis, and filariasis, cause most of the febrile illnesses in India [1]. Malaria is primarily spread by *Anopheles stephensi* (in western and southern India), *An. fluviatilis* and *An. culicifacies* (in central and eastern India), *An. baimaii* and *An. minimus* (in north-eastern India) [2]. In India, dengue and chikungunya are primarily spread by *Aedes aegypti* [3]. Filariasis and Japanese encephalitis are transmitted by species belonging to *Aedes, Culex*, and *Mansonia* genera. These mosquito vectors are present in all types of habitats ranging from urban polluted small water bodies to large brackish and freshwater bodies, making their control very difficult [4, 5].

Integrated vector control management in India recommends using temephos (an organophosphate insecticide) for controlling mosquito larvae, and pyrethrum spray and malathion fogging for controlling adult mosquitoes, especially during their breeding season [6]. However, the emergence of insecticide resistance in mosquitoes and the harmful effects of the insecticides on other non-targeted organisms such as silkworm moths, honey bees,

notonectid bugs, fishes, etc. warrants exploration, development, and validation of alternative and sustainable biological strategies for controlling mosquitoes in their natural environments [7, 8, 9, 10].

Sustainable biological methods to control mosquito larvae include larvivorous fishes, odonates, copepods, tadpoles, and aquatic carnivorous plants [8, 11, 12, 13]. A promising, but understudied biological control strategy for controlling mosquito larvae is the use of *Utricularia*, an aquatic carnivorous plant also known as bladderwort. It is an oligotrophic plant that partially derives nutrition from its prey [14] by capturing a wide range of organisms belonging to Tardigrada, Nematoda, Gastropoda, Acaridae, Rotifera, Ciliata and Crustaceae [15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. The suction trap of *Utricularia* is the fastest known trap among all carnivorous plants and can capture prey within 500 µm from the trapdoor [25]. The captured prey dies due to anoxia and is digested by a set of hydrolases and low pH environment [26]. Although the predation of mosquito larvae by *Utricularia* was reported in the early 1930s, the use of *Utricularia* for controlling mosquito larvae is relatively underexplored[18]. Under laboratory conditions within two days, *Utricularia reflexa* predated 100% of *An. gambiae*, the African malaria vector [27, 28]. Similarly, *Utricularia macrorhiza*, widely distributed in North America was shown to kill 100% of *Ae. aegypti* and 95% of *Ae. albopictus* larvae within 5 days [29]. Here, we report for the first time, the predatory ability of *U. aurea* that is widely distributed in the Indian sub-continent, Japan, and Australia [30, 31, 32, 33], against the larvae of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* over time in laboratory and field settings.

Methods

Collection of the mosquito larvae and determination of their size

The mosquito larvae were obtained from the insectary of the National Institute of Malaria Research, Goa. The body length of larvae belonging to the 1st, 2nd, 3rd, and 4th instar stages of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus*, was measured from preclypeus to the 8th abdominal segment using a micro-scale and stereo-microscope (Wild).

Collection and Identification of Utricularia aurea

U. area shoots of 10 cm length were collected from the lake at Band Bhat area, St. Cruz, Goa, India (15°28'11.4"N 73°50'26.2"E). The shoots were transported to the laboratory in a five-litre bucket filled with the same reservoir water. The plants were identified using the methods described by Taylor [33] and Janarthanam and Henry [30].

Predation of the larvae by U. aurea **in laboratory mesocosms**

The 10 cm shoots of *U. aurea* containing ~ 100–110 traps were added to cups containing 250 ml of RO water. Twenty-five larvae of each instar stage of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* were introduced into separate cups, in quadruplicates, containing the shoot of *U. aurea* (the ratio between the trap and larvae was 4:1). The experimental controls were larvae without the *U. aurea* shoot. The larvae were fed once with ~ 5 mg of fish food (Tetramin). Hourly capturing activity of the *U. aurea* bladder was recorded from 0–12 hours.

Predation of the larvae by U. aurea in field macrocosms

The curing waters at a construction site in the Ponda area in Goa were surveyed for the presence of immature (larvae and pupae) *Ae. aegypti* and *An. stephensi*. The size of these breeding sites was approximately of 1 m in length, 1.2 m in breadth, and 0.17 m in depth. Three sites were chosen for the introduction of *U. aurea* shoots. Ten big shoots (~ 30 cm long) of *U. aurea* were introduced into each experimental breeding site; two shoots per corner (four corners), and two shoots in the centre. Three cutting water sites without *U. aurea* shoots were kept in control. Using the dipper method, the density of the immature stages (larvae and pupae) was recorded before and after the introduction of *U. aurea* shoots. From each site, five dips were collected daily for 16 days; the density of immature *Ae. aegypti* and *An. stephensi* from each dip were counted and recorded. The percentage reduction in the density of immature *Ae. aegypti* and *An. stephensi* was determined daily for 16 days using Mulla's formula [34].

Statistical analyses

The data are presented as means and their ± 1 standard deviation. The lengths of the three mosquito species at each instar stage were compared using one-way ANOVA along with Tukey's post hoc tests. The predatory effect of *U. aurea* on the 1st, 2nd, 3rd, and 4th instar stages of each mosquito species was compared using one-way ANOVA along with Tukey's post hoc tests. In addition, to test whether the predatory effect of *U. aurea* varied with the mosquito species (irrespective of the instar stages) and the instar stage (irrespective of the mosquito species), the data were analyzed using two-way ANOVA along with Tukey's post hoc tests. Statistical tests were performed in SPSS version 23 (Statistical Package for Social Sciences, Inc., Chicago, IL, USA). The statistically significant difference between the means is reported when the *p*-value is less than 0.05.

Results

Overall, the lengths of different instars of *An. stephensi* were smaller than those of *Cx. quinquefasciatus*, which in turn were smaller than those of *Ae. aegypti* (Fig. 1). With the exception of the difference between the length of the 2nd instar of *Cx. quinquefaciatus* and *Ae. aegypti*, the length of all the instar stages significantly differed among the three mosquito species ($p < 0.05$). *U. aurea*, either completely or partially, captured the four instar stages of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* (Fig. 2). Specifically, the bladders of *U. aurea* completely engulfed the smaller mosquito larvae (Figs. 2A, B, E, F, G, I), and partially engulfed the bigger mosquito larvae (Figs. 2C, D, H, J, K, L).

In a 12-hour laboratory predation experiment, *U. aurea*, at different rates, predated the four instar stages of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* (Fig. 3 and Tables 1–3). No larval deaths occurred in the controls (waters with the larvae and without *U. aurea*).

Table 1
Stage-wise capture of *Ae. aegypti* larvae by *Utricularia aurea* during the first 12 hours of exposure.

Hours	1st instar				2nd instar				3rd instar				4th instar			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
1	13	14	13	18	23	22	20	23	8	5	10	11	6	5	4	7
2	22	20	16	23	25	24	24	25	12	9	13	11	6	5	6	7
3	22	23	20	24	-	24	25	-	14	13	15	12	7	7	7	8
4	23	23	20	24	-	25	-	-	18	16	17	14	8	11	8	10
5	23	25	20	25	-	-	-	-	19	17	18	16	11	14	9	12
6	23	-	20	-	-	-	-	-	20	18	20	18	13	18	11	13
7	24	-	23	-	-	-	-	-	21	19	21	18	13	18	12	13
8	25	-	24	-	-	-	-	-	22	20	21	18	14	19	16	14
9	-	-	24	–	-	-	-	-	24	22	23	21	14	20	16	14
10	-	-	25	-	-	-	-	-	24	22	23	21	15	21	16	15
11	-	-	-	-	-	-	-	-	25	22	23	21	15	21	17	15
12	-	-	-	-		-	-	-	-	22	24	23	17	23	19	18
The mean and ± 1 standard deviation of captured larvae after1 hour of exposure	14.5 ± 2.4				22 ± 1.4				8.5 ± 2.6				5.5 ± 1.3			
The mean and ± 1 standard deviation of captured larvae after12 hours of exposure	25 ± 0				25 ± 0				23.5 ± 1.29				19.25 ± 2.63			
*R1-R4 - Quadruplicates																

Table 2
Stage-wise capture of *An. stephensi* larvae by *Utricularia aurea* during the first 12 hours of exposure.

Hours	1st instar				2nd instar				3rd instar				4th instar			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
1	10	11	13	11	9	13	14	13	18	17	19	20	4	9	9	6
2	12	11	13	11	9	14	15	14	20	17	19	20	6	12	12	7
3	12	13	13	12	9	19	16	16	20	18	20	21	14	17	13	9
4	14	13	14	13	10	20	20	17	21	18	21	23	16	18	15	10
5	16	14	15	13	13	21	21	19	21	20	23	23	18	19	15	12
6	17	15	15	15	15	24	22	21	21	23	25	24	19	21	17	13
7	18	16	15	15	16	24	22	22	22	23	-	24	19	21	17	13
8	18	18	16	17	17	25	22	22	22	23	-	25	21	21	17	13
9	19	18	18	22	18	-	23	23	24	24	-	-	21	22	21	13
10	19	19	19	23	20	-	23	23	24	24	-	-	21	22	21	13
11	20	21	20	23	22	-	24	24	25	25	-	-	22	22	21	13
12	24	22	20	23	23	-	24	24	-	-	-	-	22	22	21	14
The mean and ± 1 standard deviation of captured larvae after1 hour of exposure	11.25 ± 1.3				12.25 ± 2.2				18.5 ± 1.3				7 ± 2.4			
The mean and ± 1 standard deviation of captured larvae after12 hours of exposure	22.25 ± 1.7				24 ± 0.81				25 ± 0				19.75 ± 3.86			
*R1-R4 – Quadruplicates																

Table 3
Stage-wise capture of *Cx. quinquefasciatus* larvae by *U. aurea* during the first 12 hours of exposure.

Hours	1st instar				2nd instar				3rd instar				4th instar			
	*R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
1	20	22	13	6	10	11	16	7	14	11	4	7	5	1	0	1
2	23	23	18	18	13	12	16	11	15	15	7	13	6	3	0	2
3	23	24	21	19	15	15	21	15	15	15	12	14	6	3	0	3
4	24	25	22	21	17	20	23	17	16	15	13	16	7	5	0	4
5	24	-	23	21	18	20	23	19	19	17	14	18	7	6	2	4
6	24	-	25	21	18	21	25	23	20	17	22	21	8	6	2	4
7	24	-	-	21	19	21	-	23	20	17	22	21	9	7	3	4
8	24	-	-	21	19	22	-	23	21	17	22	21	10	8	3	4
9	24	-	-	21	19	22	-	24	21	19	22	23	11	10	5	5
10	25	-	-	22	19	23	-	24	21	20	23	23	12	11	6	6
11	-	-	-	22	20	23	-	25	22	22	25	23	13	12	7	8
12	-	-	-	23	20	24	-	-	23	23	-	24	15	14	9	10
The mean and ± 1 standard deviation of captured larvae after1 hour of exposure	15.25 ± 7.3				11 ± 3.7				9 ± 4.39				1.75 ± 2.2			
The mean and ± 1 standard deviation of captured larvae after12 hours of exposure	24.5 ± 1				23.5 ± 2.38				23.75 ± 0.95				12 ± 2.94			
*R1-R4 - Quadruplicates																

During the first hour of the exposure, *U. aurea* predated 52–72% (13–18 of 25) of the 1st instars of *Ae. aegypti*; the mean and standard deviation of the predated larvae were 14.5 and 2.4, respectively (Table 1). At the end of 5 to 10 hours of exposure, *U. aurea* predated 100% of 1st instars of *Ae. aegypti*. During the first hour of the

exposure, *U. aurea* predated 80–92% (20–23 of 25) of the 2nd instars of *Ae. aegypti*; the mean and standard deviation of the predated larvae were 22 and 1.4, respectively. At the end of 2 to 4 hours of exposure, *U. aurea* predated 100% (25 of 25) of the 2nd instar larvae of *Ae. aegypti*. During the first hour of exposure, *U. aurea* predated 20–44% (5–11 of 25) of the 3rd instars of *Ae. aegypti*; the mean and standard deviation of the predated larvae were 8.5 and 2.6, respectively. Within 12 hours, *U. aurea* predated 88–100% (22–25 of 25) of 3rd instars of *Ae. aegypti*; the mean and standard deviation of the predated larvae were 23.5 and 1.29, respectively. During the first hour, *U. aurea* predated 16–28% (4–7 of 25) of the 4th instar larvae of *Ae. aegypti*; the mean and standard deviation of the predated larvae were 5.5 and 1.3, respectively. During the 12 hours of exposure, *U. aurea* predated 68–92% (17–23 of 25) of the 4th instar stage of *Ae. aegypti*; the mean and standard deviation of the predated larvae were 19.25 and 2.63, respectively. The number of predated 1st and 2nd instars of *Ae. aegypti* differed significantly from that of the 3rd and 4th instars (Fig. 4). Interestingly, *U. aurea* predated a significantly higher number of 2nd instars of *Ae. Aegypti* compared to 1st, 3rd, and 4th instars of *Ae. aegypti* (Fig. 4).

During the first hour of the exposure, *U. aurea* predated 40–52% (10–13 of 25) of the 1st instars of *An. stephensi*; the mean and ± 1 standard deviation of the predated larvae were 11.25 and 1.3, respectively (Table 2). At the end of 12 hours, *U. aurea* predated 88–96% (22–24 of 25) of 1st instars of *An. stephensi*; the mean and standard deviation of the predated larvae were 22.25 and 1.7, respectively. During the first hour of the exposure, *U. aurea* predated 36–56% (9–14 of 25) of *An. stephensi*'s 2nd instars; the mean and standard deviation of the captured larvae were 12.25 and 2.2; respectively. At the end of 12 hours, *U. aurea* predated 56–80% (14–20 of 25) of the 2nd instars of *An. stephensi*; the mean and standard deviation of the predated larvae were 24 and 0.81, respectively. During the first hour of the exposure, *U. aurea* predated 72–80% (18–20 of 25) of the 3rd instars of *An. stephensi*; the mean and standard deviation of the predated larvae were 18.5 and 1.3, respectively. At the end of 6 to 11 hours of exposure, *U. aurea* predated 100% (25 of 25) of the 3rd instars of *An. stephensi*. During the first hour of the exposure, *U. aurea* predated 16–36% (4–9 of 25) of the 4th instars of *An. stephensi*; the mean and standard deviation of the predated larvae were 7 and 2.4, respectively. At the end of 12 hours, *U. aurea* predated 36–88% (14–22 of 25) of the 4th instars of *An. stephensi*; the mean and standard deviation of the predated larvae were 19.75 and 3.86, respectively. During the first hour of the exposure, the predatory effect of *U. aurea* on the 4th instars of *An. stephensi* was significantly lower than on the 1st, 2nd, and 3rd stages of *An. stephensi* (Fig. 4). Interestingly, the predatory effect of *U. aurea* on the 3rd instars of *An. stephensi* is significantly higher than that on the 1st, 2nd, and 4th instar stages of *An. stephensi*.

During the first hour of the exposure, *U. aurea* predated 24 to 88% (6 to 22 of 25) of the 1st instars of *Cx. quinquefasciatus*; the mean and ± 1 standard deviation of the predated larvae were 15.25 and 7.3, respectively (Table 3). However, at the end of 2 hours, *U. aurea* predated 72–92% (18 to 23 of 25) of the 1st instars of *Cx. quinquefasciatus*. Interestingly, in the 3 out of 4 replicates, *U. aurea* predated 100% (25 of 25) of the *Cx. quinquefasciatus* 1st instars within 4–10 hours. During the first hour of the exposure, *U. aurea* predated 28–64% (7–16 of 25) of the 2nd instars of *Cx. quinquefasciatus*; the mean and ± 1 standard deviation of the predated larvae were 11 and 3.7, respectively. *U. aurea* predated 100% of the *Cx. quinquefasciatus* 2nd instars within 6–12 hours. During the first hour of the exposure, *U. aurea* predated 16–56% (4–14 of 25) of the 3rd instars of *Cx. quinquefasciatus*; the mean and ± 1 standard deviation of the predated larvae were 9.0 and 4.39, respectively. During the first hour of the exposure, *U. aurea* predated 0–20% (0–5 of 25) of the 4th instars of *Cx. quinquefasciatus*; the mean and ± 1 standard deviation of the predated larvae were 1.75 and 2.2, respectively.

At the end of 12 hours, *U. aurea* predated ~ 96–98% of the 1st, 2nd, and 3rd instars of *Cx. quinquefasciatus* and 36–60% of the 4th instars. Similarly, after the first hour of exposure, the predatory effect of *U. aurea* on the 4th instars of *Cx. quinquefasciatus* was significantly lower than that on the 1st, 2nd, and 3rd instars of *Cx. quinquefasciatus* (Fig. 4; $p < 0.05$).

Subsequently, to test whether *U. aurea* predation varied with the mosquito species (irrespective of instar stage) and the instar stage (irrespective of mosquito species), we analyzed the first hour data using two-way ANOVA along with Tukey post hoc tests for multiple comparisons of means. The predatory effect of *U. aurea* significantly varied among the three mosquito species ($F = 5.4$; $p < 0.05$); while there was no significant difference between the predatory effect of *U. aurea* on *Ae. aegypti* and *An. stephensi* ($p > 0.05$), the predatory effect of *U. aurea* on the *Cx. quinquefasciatus* differed significantly from that on *Ae. aegypti* ($p < 0.05$) and *An. stephensi* ($p < 0.05$). In addition, the predatory effect of *U. aurea* significantly varied with the instar stage of the mosquitoes ($F = 24.967$; $p < 0.05$), The predatory effect of *U. aurea* on the 4th instars was significantly lower than on the 1st, 2nd, and 3rd instar stages ($p < 0.05$), but not among the 1st, 2nd, and 3rd instar stages. Furthermore, there was a significant interaction between the species-type and instar-type ($F = 8.7$ and $p < 0.05$) suggesting the predatory effect of *U. aurea* on the three mosquito species varied significantly with respect to the instar stage.

The predatory effect of *U. aurea* on the immature (larvae and pupae) *Ae. aegypti* and *An. stephensi* in their natural breeding habitat were assessed (Fig. 5). At the end of 16 days, *U. aurea* reduced the density of immature *Ae. aegypti* and *An. stephensi* by 76 and 71%, respectively.

Discussion

We assessed the predatory ability of *U. aurea* on the different larval stages of *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, in laboratory and field settings. The results show *U. aurea* to be a voracious predator of mosquito larvae, and is in line with the earlier larval predatory reports of *U. reflexa* (Africa) [27, 28] and *U. macrorhiza* (North America) [29]. Previous studies have reported the number of larvae predated by *Utricularia* spp. each day over a period of 5 days [35]. Here, we report the hourly larval predatory rates of *U. aurea* over a period of 12 hours. *U. aurea* predated ~ 95% of the 1st, 2nd, and 3rd instars of the three mosquito species and ~ 80% of the 4th instars of *Ae. aegypti* and *An. stephensi* and ~ 60% of 4th instars of *Cx. quinquefasciatus*. The predatory activity of *U. macrorhiza* on 1st to 3rd instars of *Ae. aegypti* and *Ae. albopictus* over 24 hours was 71.5 and 81.5%, respectively [29]. The superior predatory activity of *U. aurea* could be because the experiments were initiated within 24 hours of collecting the plants from their natural environment, while experiments with *U. macrorhiza* were carried out after 30 days of acclimatization in the laboratory [29]. Laboratory acclimatization (reduces the trap size) and nutrient availability are key determinants of *Utricularia*'s predatory ability [24, 36, 37, 38, 39]. For the first time, this study also reports the predation of the 4th instar mosquito larvae by *U. aurea*, although at lower rates, which could be attributed to their lower mobility (reduced interaction with *Utricularia*) and bigger size.

The major breeding habitats of *Ae. aegypti* and *An. stephensi* are water-containing cisterns, wells, overhead and underground tanks, curing pits at construction sites, gutters in the roof, fountains, drums, and ornamental tanks, which are rarely cleaned [40]. *U. aurea* exhibited tremendous potential in controlling the larvae of *Ae.*

aegypti and *An. stephensi*, in curing water pits at construction sites. The predatory potential of *U. aurea* in the field depends on the ratio between the number of traps and the density of mosquito larvae. Future studies should evaluate the utility of *Utricularia* in the settings mentioned above in addition to curing water pits.

The advantages of using *Utricularia* as a bio-larval control agent are detailed below. *Utricularia* has been shown to selectively target mosquito species, and not affect non-target organisms in man-made water storage containers [29]. Furthermore, water bodies with *Utricularia* have been shown to be preferred oviposition sites for odonates, the predators of mosquitoes [41]. *Utricularia* is highly resistant to insecticides, herbicides, and pesticides [42, 43, 44], therefore, can be used in combination with other chemical control agents [29]. The distribution of diverse *Utricularia* species across different geographical regions [14, 27, 45] negates the need to introduce new non-native species for larval control [29].

Even though *Utricularia* holds a lot of promise in the control of mosquito larvae in both natural and manmade breeding habitats, robust ecological studies on *U. aurea* must be carried out to evaluate its invasiveness in natural waters, seasonal distribution, and effect on the food chain. In the present study, we observed *U. aurea* traps to be higher and well-developed in the months of December, January, and February. However, the peak malaria season in southwest India is from June to September, and the peak disease transmission season may not coincide with the predatory period of the *U. aurea*. In order to effectively control the transmission of mosquito-borne diseases using *U. aurea* as a biological agent, the plants must grow abundantly in the waters during the transmission seasons of malaria and dengue. Overall *U. area* holds a lot of promise as a bio-larval agent, and future studies should explore its efficacy in different natural transmission settings.

Conclusion

U. aurea, a widely distributed aquatic carnivorous plant in India, exhibits high predatory activity on the larval stages of the mosquito vectors of public health importance. Overall *U. area* holds a lot of promise as a bio-larval agent, and future studies should explore its predatory efficacy in different natural transmission settings.

Declarations

Acknowledgement

The authors are thankful to Mr. Prathamesh, Mr. Shamu, Mr. Jagadishwaran, Mr. Bhalchandra, and Mr. Pritesh for their help in the collection of plant material and maintaining the larval population. We are thankful to ICMR-National Institute of Malaria Research for the research facilities.

Author's contributions

AG, CS, and AM performed the experiment and manuscript preparation. AKM, RV, PBN, and MKJ designed the study, analyzed data, and reviewed and edited the manuscript. All authors read and approved the final manuscript.

Competing interests

All authors declare no conflict of interest.

References

1. Robinson ML, Kadam D, Khadse S, Balasubramanian U, Raichur P, Valvi C, et al. Vector-Borne Disease is a Common Cause of Hospitalized Febrile Illness in India. The American journal of tropical medicine and hygiene. 2018;98 5:1526-33; doi: 10.4269/ajtmh.17-0571. https://www.ncbi.nlm.nih.gov/pubmed/29582731.
2. Subbarao SK, Nanda N, Rahi M, Raghavendra K. Biology and bionomics of malaria vectors in India: existing information and what more needs to be known for strategizing elimination of malaria. Malaria journal. 2019;18 1:396; doi: 10.1186/s12936-019-3011-8. https://www.ncbi.nlm.nih.gov/pubmed/31796010.
3. Mutheneni SR, Morse AP, Caminade C, Upadhyayula SM. Dengue burden in India: recent trends and importance of climatic parameters. Emerging microbes & infections. 2017;6 8:e70; doi: 10.1038/emi.2017.57. https://www.ncbi.nlm.nih.gov/pubmed/28790459.
4. Nagpal B, Srivastava A, Saxena R, Ansari M, Dash A, Das S. Pictorial identification key for Indian anophelines. Delhi: Malaria Research Centre. 2005;40:34-8.
5. Barraud PJ. The fauna of British India, including Ceylon and Burma. Diptera. Volume V. Family Culicidae. Tribes Megarhinini and Culicini. London: Taylor and Francis; 1934.
6. FW M: Manual on integrated vector management India https://nvbdcp.gov.in/WriteReadData/l892s/IVM-Manual-Draft-2015.pdf (2015).
7. Bharati M, Saha D. Assessment of insecticide resistance in primary dengue vector, Aedes aegypti (Linn.) from Northern Districts of West Bengal, India. Acta tropica. 2018;187:78-86; doi: 10.1016/j.actatropica.2018.07.004. https://www.ncbi.nlm.nih.gov/pubmed/30026024.
8. Kumar A, Sharma VP, Sumodan PK, Thavaselvam D, Kamat RH. Malaria control utilizing Bacillus sphaericus against Anopheles stephensi in Panaji, Goa. J Am Mosq Control Assoc. 1994;10 4:534-9. https://www.ncbi.nlm.nih.gov/pubmed/7707060.
9. Raghavendra K, Velamuri PS, Verma V, Elamathi N, Barik TK, Bhatt RM, et al. Temporo-spatial distribution of insecticide-resistance in Indian malaria vectors in the last quarter-century: Need for regular resistance monitoring and management. Journal of vector borne diseases. 2017;54 2:111-30. https://www.ncbi.nlm.nih.gov/pubmed/28748832.
10. Rai P, Bharati M, Subba A, Saha D. Insecticide resistance mapping in the vector of lymphatic filariasis, Culex quinquefasciatus Say from northern region of West Bengal, India. PloS one. 2019;14 5:e0217706; doi: 10.1371/journal.pone.0217706. https://www.ncbi.nlm.nih.gov/pubmed/31141548.
11. Kumar A, Sharma VP, Sumodan PK, Thavaselvam D. Anopheles stephensi build-up and accelerated malaria transmission in the Post Bio-control intervention phase in Candolim PHC of Goa, India. Journal of parasitic diseases. 1999;23:41-4.
12. Kumar A, Sharma V, Thavaselvam D, Sumodan PK, Kamat R, Audi S, et al. Control of Culex quinquefasciatus with Bacillus sphaericus in Vasco City, Goa. Journal of the American Mosquito Control Association. 1996;12:409-13.
13. Imbahale SS, Mweresa CK, Takken W, Mukabana WR. Development of environmental tools for anopheline larval control. Parasites & vectors. 2011;4:130; doi: 10.1186/1756-3305-4-130.

https://www.ncbi.nlm.nih.gov/pubmed/21733150.

14. Poppinga S, Weisskopf C, Westermeier AS, Masselter T, Speck T. Fastest predators in the plant kingdom: functional morphology and biomechanics of suction traps found in the largest genus of carnivorous plants. AoB Plants. 2015;8; doi: 10.1093/aobpla/plv140. https://www.ncbi.nlm.nih.gov/pubmed/26602984.
15. Harms S. The effect of bladderwort (Utricularia) predation on microcrustacean prey. 2002;47 9:1608-17; doi: https://doi.org/10.1046/j.1365-2427.2002.00897.x. https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2427.2002.00897.x.
16. Kurbatova SA, Yershov IY. Crustaceans and rotifers in the predatory feeding of Utricularia. Inland Water Biology. 2009;2 3:271-5; doi: 10.1134/S1995082909030122. https://doi.org/10.1134/S1995082909030122.
17. Nikolai Mette NW, Wilhelm Barthlott. Food composition of aquatic bladderworts (Utricularia, Lentibulariaceae) in various habitats. Beitr Biol Pfl. 2000;72:1-13.
18. Matheson R. The Utilization of Aquatic Plants as Aids in Mosquito Control. The American Naturalist. 1930;64 690:56-86. http://www.jstor.org/stable/2456799.
19. Sanabria - Aranda L, Gonzalez - Bermudez A, Torres NN, Guisande C, Manjarres -Hernandez A-V, Vanessa, Diaz-Olarte J, et al. Predation by the tropical plant Utricularia foliosa. 2006;51 11:1999-2008; doi: https://doi.org/10.1111/j.1365-2427.2006.01638.x. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2427.2006.01638.x.
20. Andrikovics S, Forro L, Zsunics E. The zoogenic food composition of Utricularia vulgaris in the Lake Fertő. Opuscula Zoologica. 1988;23:65-70.
21. Darwin C, Darwin F. Insectivorous plants. London: J. Murray; 1888.
22. Alkhalaf IA, Hübener T, Porembski S. Prey spectra of aquatic Utricularia species (Lentibulariaceae) in northeastern Germany: The role of planktonic algae. Flora - Morphology, Distribution, Functional Ecology of Plants. 2009;204 9:700-8; doi: https://doi.org/10.1016/j.flora.2008.09.008. https://www.sciencedirect.com/science/article/pii/S0367253009000310.
23. Gordon E, Pacheco S. Prey composition in the carnivorous plants Utricularia inflata and U. gibba (Lentibulariaceae) from Paria Peninsula, Venezuela. Rev Biol Trop. 2007;55 3-4:795-803; doi: 10.15517/rbt.v55i3-4.5956. https://www.ncbi.nlm.nih.gov/pubmed/19086385.
24. Guiral D, Rougier C. Trap size and prey selection of two coexisting bladderwort (Utricularia) species in a pristine tropical pond (French Guiana) at different trophic levels. Ann Limnol - Int J Lim. 2007;43 3:147-59. https://doi.org/10.1051/limn:2007009.
25. Vincent O, Weisskopf C, Poppinga S, Masselter T, Speck T, Joyeux M, et al. Ultra-fast underwater suction traps. Proceedings Biological sciences. 2011;278 1720:2909-14; doi: 10.1098/rspb.2010.2292. https://www.ncbi.nlm.nih.gov/pubmed/21325323.
26. Adamec L, Sirová D, Vrba J, Rejmánková E. Enzyme production in the traps of aquatic Utricularia species. Biologia. 2010;65 2:273-8; doi: 10.2478/s11756-010-0002-1. https://doi.org/10.2478/s11756-010-0002-1.
27. Ogwal-okeng J, Namaganda M, Bbosa G, Kalema J. Using carnivorous plants to control malaria-transmitting mosquitoes. MalariaWorld Journal. 2013;4 10.

28. Ogwal-Okeng J, Kalema J, Namaganda M, Lubega A, Zziwa M, Bbosa G. Larvicidal activity of Aquatic carnivorous plant on Anopheles Mosquito larval stages. Research Journal of Biological Sciences. 2011;6 9:436-9.
29. Couret J, Notarangelo M, Veera S, LeClaire-Conway N, Ginsberg HS, LeBrun RL. Biological control of Aedes mosquito larvae with carnivorous aquatic plant, Utricularia macrorhiza. Parasites & vectors. 2020;13 1:208; doi: 10.1186/s13071-020-04084-4. https://www.ncbi.nlm.nih.gov/pubmed/32317006.
30. Janarthanam M, Henry AN. Bladderworts of India. 1992:30-4.
31. Sanjeet Kumar SST, Gopinath Mondal, PD Singh, RK Labala, LA Singh, BG Somkuwar, B Thongam. Morphological characterization and ecology of Urticularia aurea Lour. Adv Plants Agric Res. 2019;9:61-3; doi: 10.15406/apar.2019.09.00412.
32. Rutishauser R. The developmental plasticity of Utricularia aurea (Lentibulariaceae) and its floats. Aquatic Botany. 1993;45 2:119-43; doi: https://doi.org/10.1016/0304-3770(93)90018-R. https://www.sciencedirect.com/science/article/pii/030437709390018R.
33. Taylor P. The genus Utricularia : a taxonomic monograph. London: H.M.S.O.; 1989.
34. Mulla MS, Norland LR, Fanara DM, Darwazeh HA, McKean DW. Control of Chironomid Midges in Recreational Lakes13. Journal of Economic Entomology. 1971;64 1:300-7; doi: 10.1093/jee/64.1.300 %J Journal of Economic Entomology. https://doi.org/10.1093/jee/64.1.300.
35. Ogwal-okeng J, Namaganda M, Bbosa GS, Kalema J. Using carnivorous plants to control malaria-transmitting mosquitoes. MWJ. 2013;4 10:1-3.
36. Knight SE. Costs of carnivory in the common bladderwort, Utricularia macrorhiza. Oecologia. 1992;89 3:348-55; doi: 10.1007/BF00317412. https://www.ncbi.nlm.nih.gov/pubmed/28313083.
37. Twinn CR. Observations on some aquatic animal and plant enemies of mosquitoes. The Canadian Entomologist. 1931;63 3:51-61; doi: 10.4039/Ent6351-3. https://www.cambridge.org/core/article/observations-on-some-aquatic-animal-and-plant-enemies-of-mosquitoes/A5EA6A0FB947EF5047DE7D73C94B02C9.
38. Friday LE. The Size and Shape of Traps of Utricularia vulgaris L. Functional Ecology. 1991;5 5:602-7; doi: 10.2307/2389478. http://www.jstor.org/stable/2389478.
39. Knight SE, Frost TM. Bladder Control in Utricularia Macrorhiza: Lake-Specific Variation in Plant Investment in Carnivory. 1991;72 2:728-34; doi: https://doi.org/10.2307/2937212. https://esajournals.onlinelibrary.wiley.com/doi/abs/10.2307/2937212.
40. Thomas S, Ravishankaran S, Justin JA, Asokan A, Mathai MT, Valecha N, et al. Overhead tank is the potential breeding habitat of Anopheles stephensi in an urban transmission setting of Chennai, India. Malaria journal. 2016;15 1:274; doi: 10.1186/s12936-016-1321-7. https://www.ncbi.nlm.nih.gov/pubmed/27169513.
41. Sawchyn WW, Gillott C. The Biology of two related species of Coenagrionid dragonflies (ODONATA: ZYGOPTERA) in western Canada. The Canadian Entomologist. 1975;107 2:119-28; doi: 10.4039/Ent107119-2. https://www.cambridge.org/core/article/biology-of-two-related-species-of-coenagrionid-dragonflies-odonata-zygoptera-in-western-canada1/9288625EB51BA70507AE4DBD0A2042E2.

42. Hanson WR. Effects of Some Herbicides and Insecticides on Biota of North Dakota Marshes. The Journal of Wildlife Management. 1952;16 3:299-308; doi: 10.2307/3796642. http://www.jstor.org/stable/3796642.
43. Smith CS, Pullman GD. Experiences Using Sonar® A.S. Aquatic Herbicide in Michigan. Lake and Reservoir Management. 1997;13 4:338-46; doi: 10.1080/07438149709354324. https://doi.org/10.1080/07438149709354324.
44. Brewer JS. Effects of competition, litter, and disturbance on an annual carnivorous plant (Utricularia juncea). Plant Ecology. 1999;140 2:159-65; doi: 10.1023/A:1009796818877. https://doi.org/10.1023/A:1009796818877.
45. Gordeev M, Sibataev A. Influence of predatory plant bladderwort (Utricularia vulgaris) on the process of selection in malaria mosquito larvae. Russian Journal of Ecology. 1995;26 3:216-20.

Figures

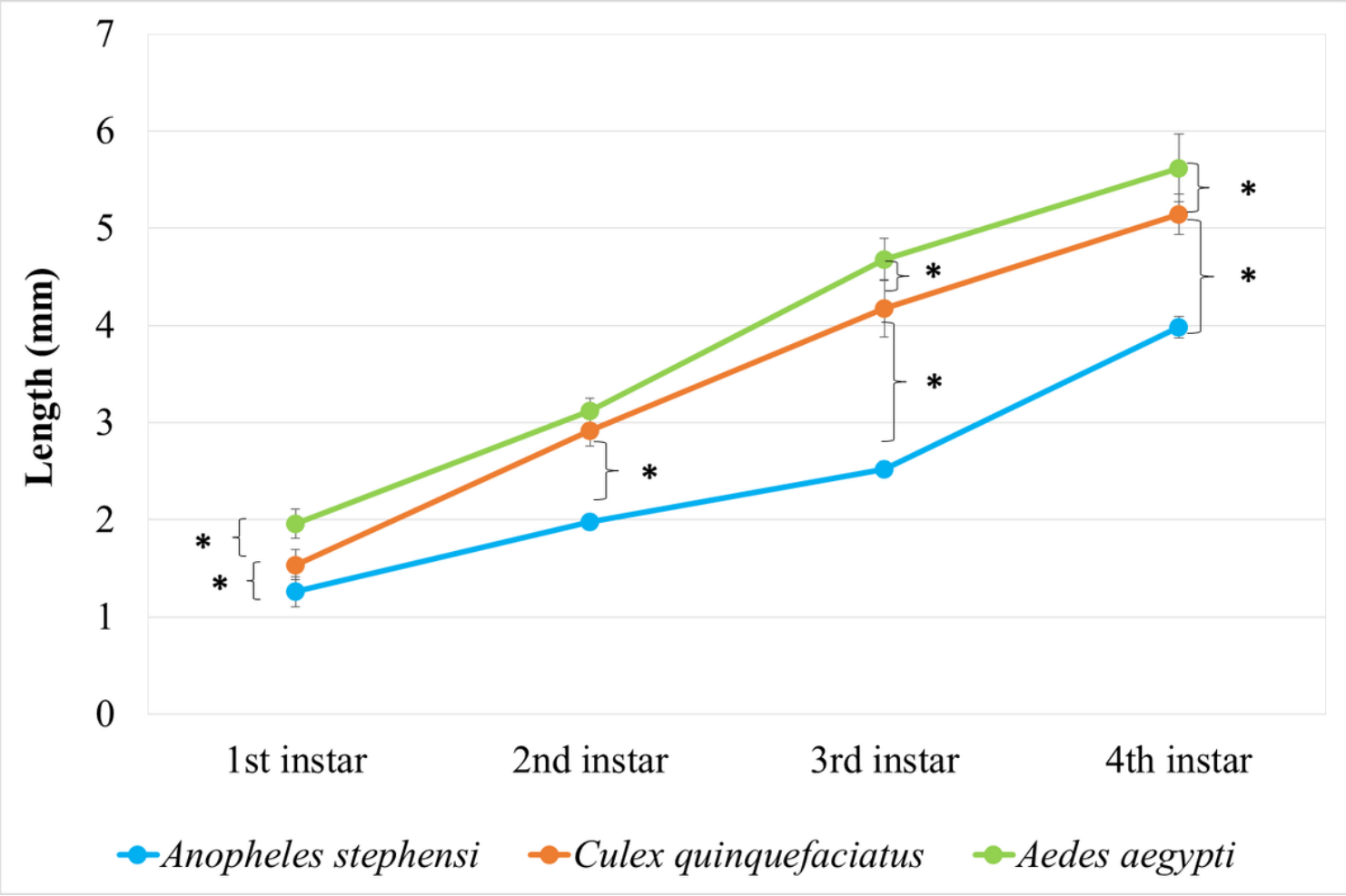

Figure 1

Length of the instars (or larval stage) of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus.* Data are means along with their ±1 standard deviation (n=5). For each instar stage, the mean lengths of larvae of the three mosquito species were compared using one-way ANOVA along with Tukey's post hoc tests. * indicates the means are significantly different ($p < 0.05$).

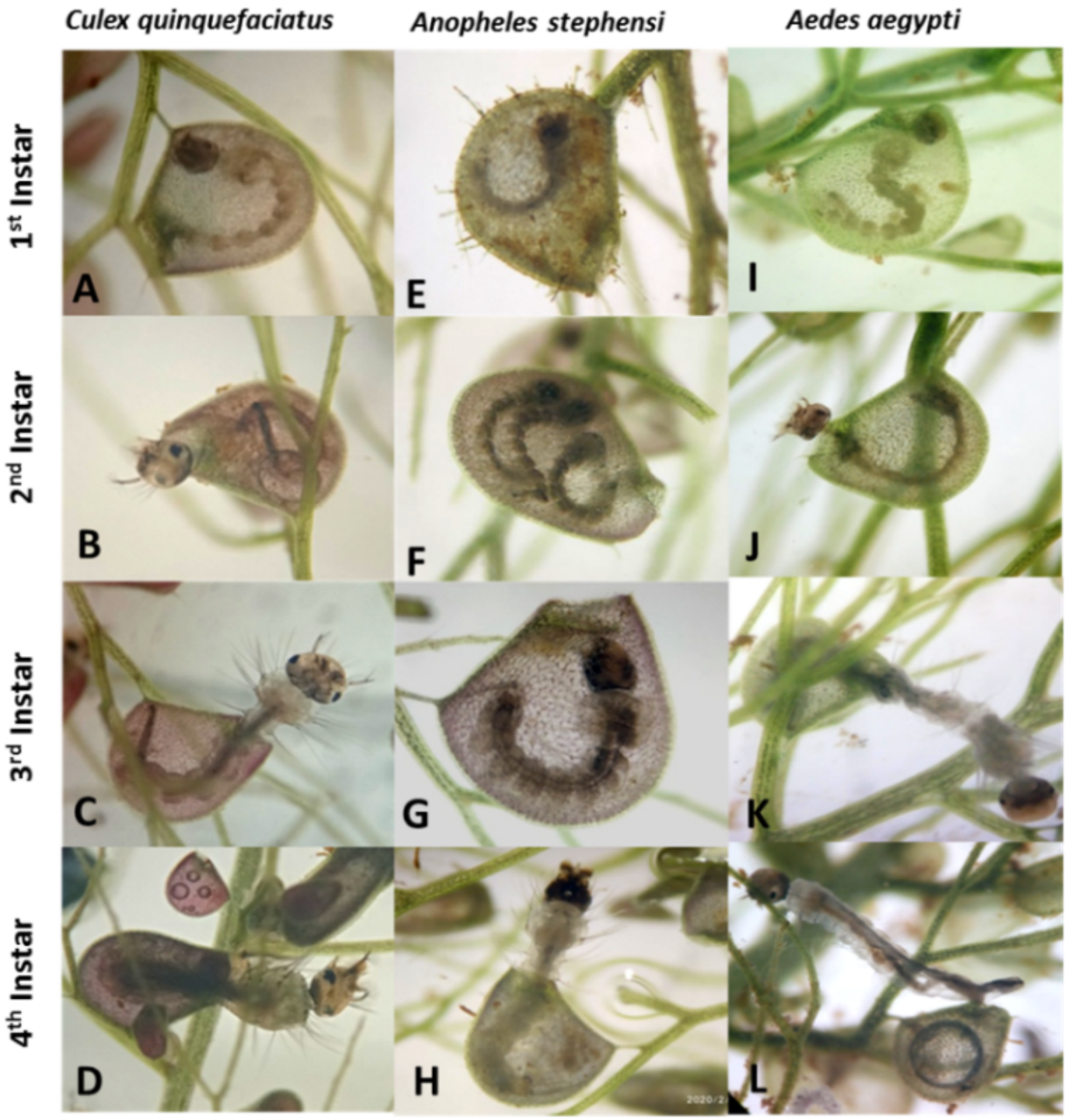

Figure 2

Photographic images of *U. aurea* along with captured instars of *Cx. quinquefasciatus* (A-D), *An. stephensi* (E-H) and *Ae. aegypti* (I-L).

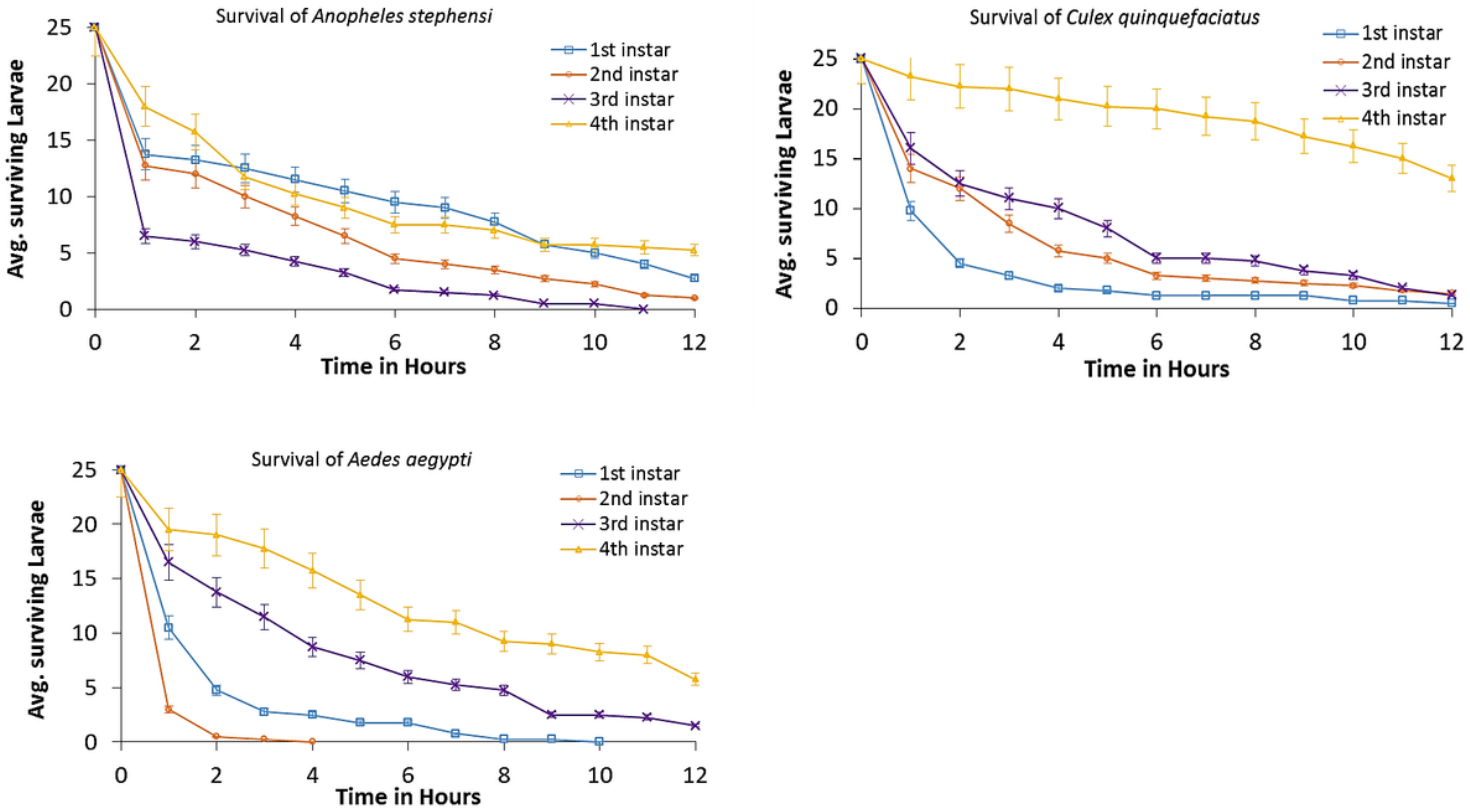

Figure 3

The rate of predation of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* larvae by *U. aurea*. Data are means of surviving larvae along with their ±1 standard deviation (n=4).

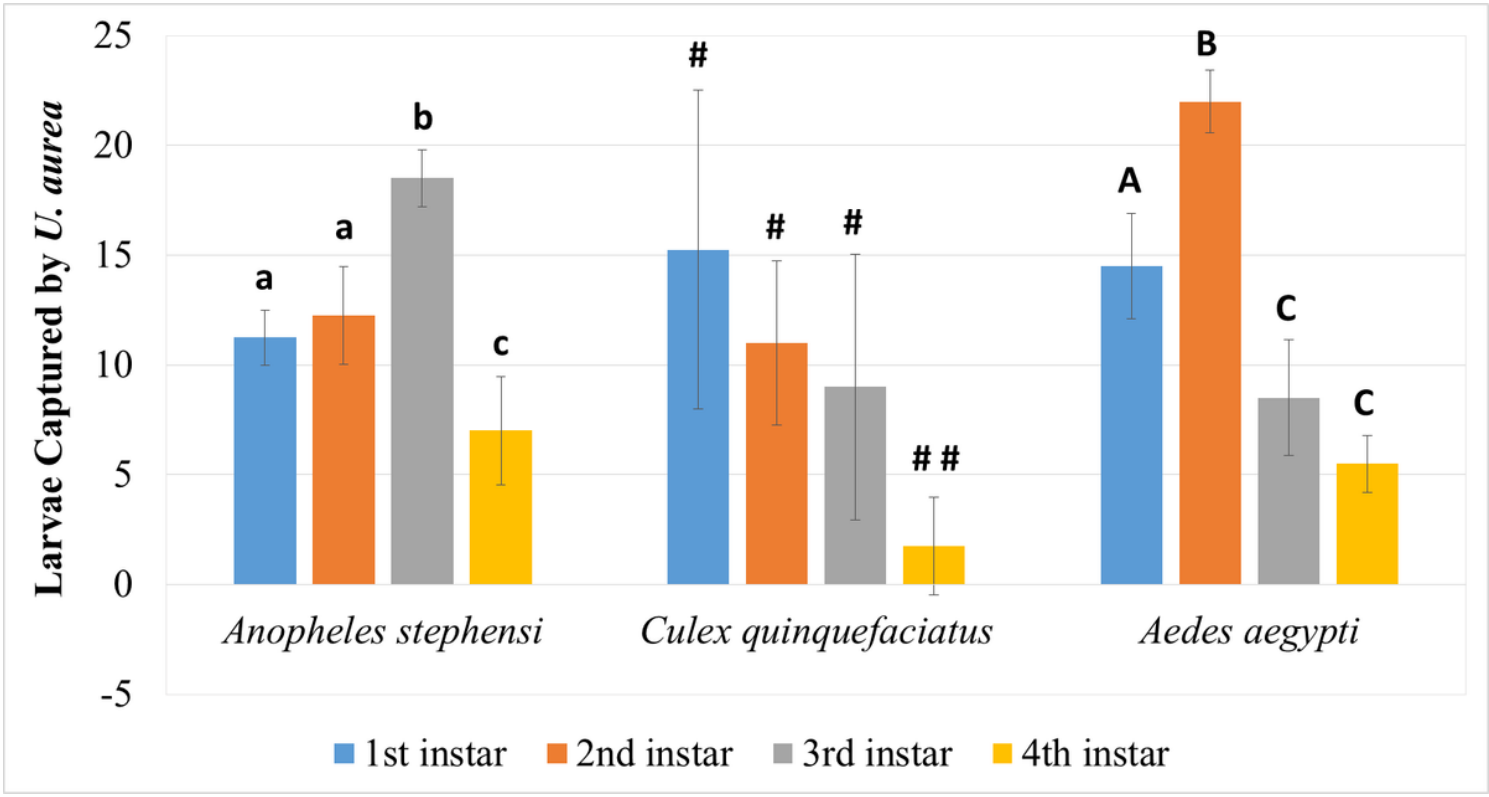

Figure 4

Number of larvae predated by *Utricularia aurea* during the first hour of predation experiment in the laboratory mesocosms. Each bar represents the mean of the larvae captured by *U. aurea*and the error bars are ±1 standard deviation of the mean. The mean number of captured larvae was compared among the four instar stages of each mosquito species using one-way ANOVA followed by Tukey's post hoc tests. The bars connected with the different letters or symbols are significantly different from each other ($p < 0.05$).

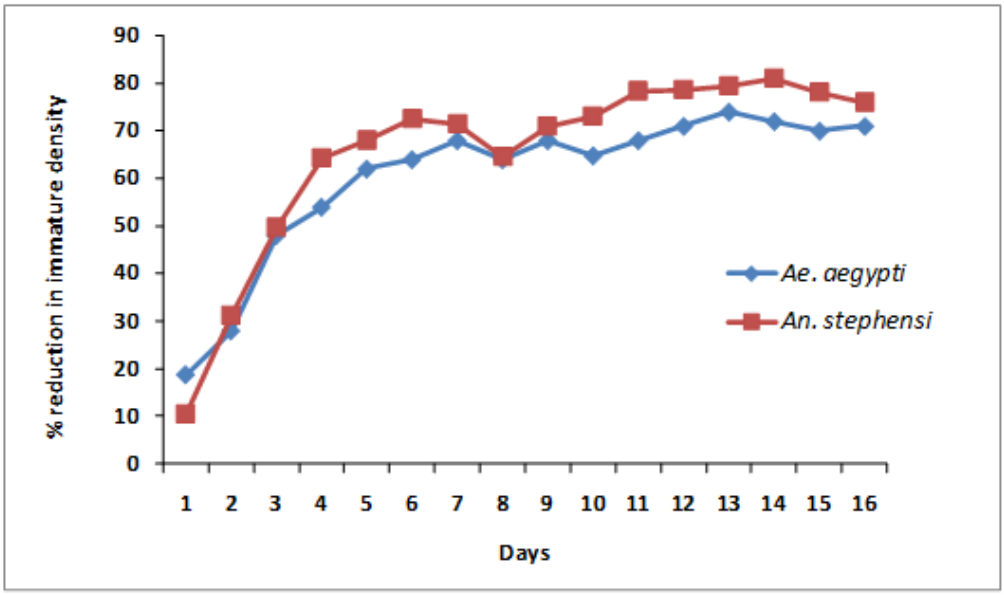

Figure 5

Predation of immature stages of *Ae. aegypti* and *An. stephensi* by *U. aurea* in the field macrocosms (specifically, stagnant curing water at construction sites).