Biological control of mosquito larvae using aquatic insect, *Diplonychus sp*

Joydeep Das[1] and Joydev Maity[1]

[1] Department of Fishery Sciences, Vidyasagar University, Midnapore, West Bengal, India

Correspondence Author: Joydeep Das

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Abstract

For dipteran larvae, water bugs are voracious feeders. The predatory activities of such aquatic insects, as well as those of their vertebrate and invertebrate predators, may have a strong influence on the evolution of aquatic insect communities. Predator – prey relations also impact on reproduction, feeding, abiotic adaptation and defense, which are the four basic survival and sustainability requirements of animals. *Diplonychus rusticus* is an insect native to the India, Australia, and Philippines, commonly known as "water bug." It lives in shallow waters and feeds on aquatic insects, including mosquito larvae. Water bugs could be an effective biological control agent for dengue-carrying mosquitoes in a study conducted by Dr. Pio Javier of the University of the Philippines in Los Baños Laguna, Philippines. He found that water bug can consume 86-99 full-grown mosquito larvae per day, is tolerable for chlorinated water, and can reproduce easily. As a result, water bugs can be distributed to all mosquito habitats, such as used tires, water containers and other mosquito breeding sites.

Keywords: *Diplonychus species*, dipteran larvae, biological control, aquatic insects

Introduction

According to Javier carried out a study entitled, Water bug *Diplonychus rusticus*: Promising predator for *Aedes aegypti* (Dengue mosquito). This study explored the possibility that the dengue-carrying mosquito population in the Philippines might use a water bug. Dr. Javier said that in a study conducted in India, the water bug against mosquito larvae has already been demonstrated to be successful. But the water bug which we are currently researching in the Philippines is native and endemic. The water bug examined in India is smaller than the Diplonychus indicus." Dr. Javier's water bug species are commonly found in rice fields and are regarded as a voracious predator, like mosquitoes, of aquatic insects. Compared with other predators, the main advantage of the water bug is its availability in many of the aquatic environments, its chlorination tolerance and its reproductive ability, Dr. Javier explained. Early study findings showed that 86-99 total cultivated mosquito larvae can be eaten per day by water bug. The depredator immediately catches the larvae with his forelegs in a matter of seconds, when inserting mosquito larvae into the water bug in a water container, said Dr. Javier. The water bug can spread to all mosquito habitats, including used pneumatically modified tires, water containers and others. While the trap attracts the laying of women's eggs, the water bug will feed on eggs that have been hatched. This stops dengue-bearing mosquitoes from occurring [9, 10].

Diplonychus rusticus (Fabricius) predatory efficiency was recorded at various prey densities of different salinity. The rate of predation of the bug decreased when the salinity level (ppt) was increased. In the fifth nymphal stage of the exposure, the level of 2, 4 and 6 ppt was higher in prey densities 50, 100, 150 and 200 both for 1 hour and 24 hours. In a prey density of 150, in 2 ppt salinity both in l hr and 24-hour therapies, more adult bugs killed prey (Chandramohan *et al*., 2008) [2, 12]. The effect of the light and habitat structure of the common heteropteran water bug, *Diplonychus annulatus*, *Diplonychus rusticus*, and *Anisops bouvieri* on the predation of *Culex quinquefasciatus* larvae was evaluated in the laboratory. Water bugs have been found to be more present than in dark environments in the presence of light. In organized conditions, Anisops bouvieri ate more prey; In open conditions, *Diplonychus annulatus* and *Diplonychus rusticus* ate more prey. The choice of prey size and the respective numbers differed greatly between predators and therapies. An indicator of predatory performance, prey vulnerability (PV) was highest for *Diplonychus annulatus*, moderate for *Diplonychus rusticus* and low for Anisops bouvieri. Prey consumption and PV values under different treatment conditions suggest that the order of prey consumption under different environments and light/dark combinations for Belostomatidae water bugs *Diplonychus annulatus* and *Diplonychus rusticus* is light open > dark open > light vegetated > dark vegetated. In the case of the backswimmer, *Anisops bouvieri*, light structured > dark structured > light open > dark open tended to be the order of prey intake. These results were consistent with resource-partitioning by the same guild sharing water bug organisms. The efficacy of these predators in managing mosquito populations would differ according to the structural complexity of environments and the intensity of light if the reported findings are applied to natural settings (Gautam *et al*., 2007: 2008) [8, 13].

Belostomatidae, generally known as the giant water bug, belongs to the Nepomorpha infraorder. Three species of the genus Diplonychus were recorded from India by Thirumalai (2007) [16]. These predatory insects are nourished by aquatic crustaceans, fish, amphibians, and larvae of mosquitoes. The findings of Saha (2007) show that 11-87 fourth instar larvae of Culex quinquefasciatus Say could be eaten per day by a single adult of Diplonychus rusticus. Depending on the prey and predator densities, *Diplonychus annulatus* consumes 33-122 fourth instar larvae per day. These insects are used as water quality bioindicators and methods of biocontrol. The Ion concentration of AI, Cd, Cr, Cu, Zn, Fe and Mn in adult Belostomatidae was analysed by Corbi *et al*. (2010) [3]. In recent studied, Diversity, Dominance status, and species richness of *Diplonychus species* were studied in Kangsabati River. They were subdominant species in river kangsabati, Midnapore, West Bengal. *Diplonychus rusticus* was found in river kangsabati (Das, J and Maity, J. 2019; 2020) [4, 5].

Materials and methods

Maintenance of the predator sample

Collected sample: Sample were taken to the Aquaculture Research Laboratory with vegetation to avoid mortality.

Aquarium Size: 24"× 12"×12"

Maintained aquarium: Aquarium maintained at (28 °C ± 2°C) room temperature with vegetation *Pistia sp, Hydrilla sp, Eichornia sp.* to make the favourable condition to live in the aquarium.

Feeding of predator

Frog Tadpole & Dipteran Larvae.

Collection of sample prey

Sample Culex larvae were collected from Standing water body of surrounding local areas of Midnapore Town, West Bengal, India, with hand net.

Maintenance of sample prey

Sample were taken to Aquaculture Research Laboratory and transferred to a plastic Jar containing clean water. The Jar was covered with the mosquito mesh to avoid oviposition of mosquitoes in the maintained one.

Culex quinquefasciatus larvae were taken as prey during the investigation. A sample of Culex larvae was collected from standing water from the surrounding local areas of Midnapore city using a hand net. Samples were taken to an aquaculture research laboratory and transferred to a plastic container containing clean water. The container was covered with a mosquito net to prevent mosquitoes from settling in the maintained. Larvae were categorized based on their size as large and small, rather than going through molting stages, and kept in enamel dishes covered with nylon mesh. Large Culex larvae measured about 0.67 mm and small Culex larvae measured about 3.6 mm in length.

The average body length of the Belostomatids, *Diplonychus rusticus*, used as experimental species, was 15.26 mm (range 14.6–16.2 mm). Small and large culex larvae were placed separately in plastic troughs.

Results and discussions

Bedbugs were exposed to large and small Culex quinquefasciatus and prey density was set at 50,100,150, 200 for each predator. Bed bug predation was observed at the end of 24 h in each aquarium after the exposure period. The plastic container was covered with a mosquito net to prevent mosquitoes from settling in the maintained eggs. Adults of the mosquito population that emerged in the experimental chambers were killed by smearing the nylon cover net with cotton soaked in chloroform. After the introduction of culex larvae, preys-number killed was recorded in 24 hours.

Experiment 1 and experiment 2 (Graph 1 to Graph 4) were each conducted for 14 days with small prey and large prey and prey density (50). Another Experiment 1 and Experiment 2 (Graph 5 to Graph 8) were conducted for 14 days each with small prey and large prey and prey density (100). Additional experiments were conducted with prey densities of 150 (Graph 9 to Graph 12) and densities of 200 (Graph 13 to Graph 16). In the experiments, 8 trials of each treatment were performed for each set at each prey density. Performance of predation of different size groups of *Diplonychus rusticus* was evaluated in relation to selected parameters to determine the significance of differences in predation performance (dependent variable) for revealing main and interaction effects of categorical independent variables (called "factors"), e.g., prey density, prey size and exposure time. The results show that prey-number killed by the predator was low at a low prey density of 50. But with other prey densities, the number of preys killed was high regardless of exposure time.

At high prey density 200 than prey density 50,100,150, the number of preys killed by the predator increased slightly from prey density 50 to prey density 200. The number of preys killed by predator at different prey-density was directly proportional to prey size. The number of preys killed by the predator was low at a prey density of 50 with a phenomenal increase from a prey density of 100, 150, 200. Large prey was noted to be killed by the predator more than any other size group.

Identifying characters of three species of *Diplonychus* of India (Chandra, K. and Jehamalar, E.E. 2012) [1]

Diplonychus rusticus **(Fabricius, 1781) [7]**

1. Posterior pronotal angles less acute. 2. Respiratory straps with cluster of setae.

Diplonychus annulatus **(Fabricius, 1781) [7]**

1. Hemelytra without spiny patch on corium. 2. Fore tarsus 2 jointed 3. Apex of head acute 4. Respiratory straps without cluster of setae. 5. large species.

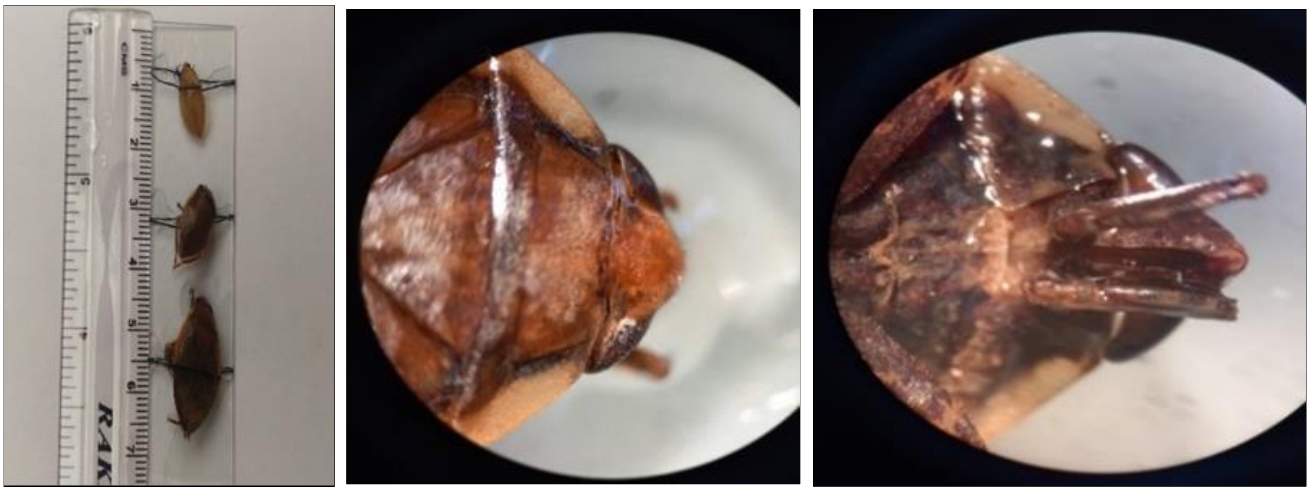
Diplonychus molestus **(Dufour, 1863) [6]**

1. Respiratory straps without cluster of setae. 2. Posterior pronotal angles more obtuse. 3. Hemelytra with spiny patch on corium. 4. Fore tarsus 1 jointed. 5. small species.

In Townsville, Queensland, Australia, which is situated in the arid tropics, a twelve-month survey of mosquito predators was carried out. The survey showed that five predatory insects were present, but only *Anisops species* (backswimmers) and species of *Diplonychus* were common. Predatorial ability and factors affecting this capacity were then evaluated against *Culex annulirostris* mosquito immaturity under laboratory conditions for adult *Anisops species* and adult and nymph stage *Diplonychus species*. Predatorial ability bioassays showed that both larval and pupal stages of *Culex annulirostris* were successfully preyed upon by adult *Diplonychus species*. With smaller prey immatures, *Diplonychus species* nymphs proved to be more competitive, and adults of *Anisops species* did not successfully prey on any prey pupae. The increase in the area of foraging and the introduction of aquatic vegetation greatly decreased the predatory ability of *Diplonychus species*, nymphs, while only the vegetation and not the area of foraging had a substantial impact on the predation capacity of adult *Diplonychus species*. Overall, adult *Diplonychus species* have proven to be more successful predators than *Anisops species*, and field trials are now suggested to further evaluate the ability of *Diplonychus species* as a biocontrol agent (Shaalan, *et al*., 2007) [15].

Biological control of dipteran larvae - eco-friendly approach

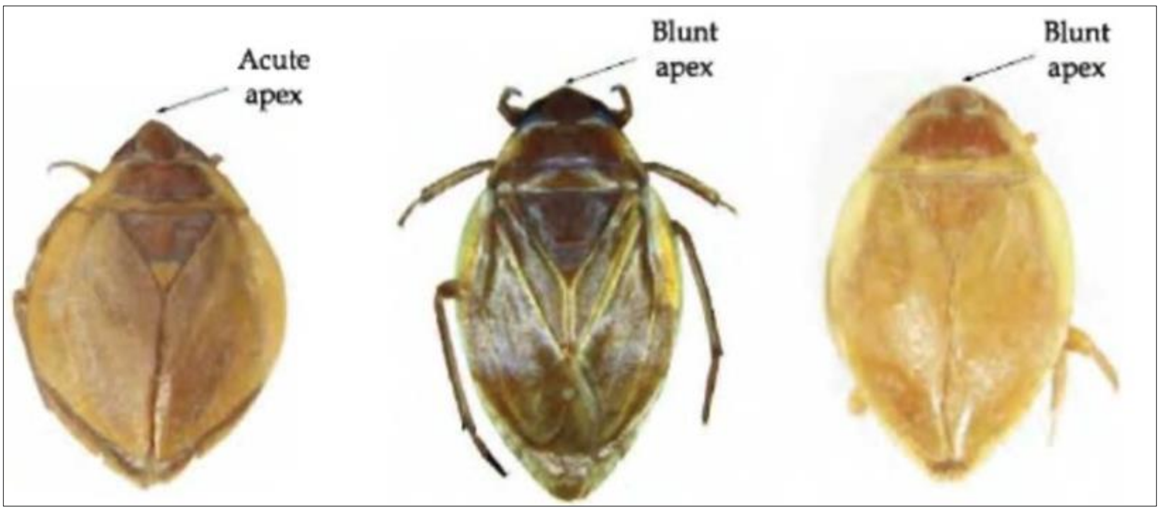
Since a range of diseases such as dengue fever, malaria, Japanese encephalitis, yellow fever etc. can be transmitted by mosquitoes, attempts have been made to monitor them. Control steps could be targeted at various stages of the life cycle of an insect. Biological, environmental (physical) and chemical monitoring can be defined as measures. To achieve successful and efficient control of mosquitoes with minimal control, an integrated mosquito management strategy should be adopted. Since biological control does not cause chemical contamination, it is regarded by many individuals as a better method for mosquito control. There are drawbacks to the use of biological agents for mosquito control, however. To give a desirable effect, the added agent typically must be significant in number and it is ideally unique as a mosquito predator (Centre for Health Protection, 2004) [8]. Extreme biting pests and mandatory vectors of several vertebrate pathogens is mosquitoes. In most tropical and many temperate water bodies, their immature larval and pupal life stages are a common feature and sometimes form a substantial proportion of the biomass. During disease transmission cycles, control strategies depend mainly on the use of larvicides and environmental change to minimize recruitment and adulticides. Larvicides are generally chemical, although biological toxins, agents or species may be involved. In mosquito control, the use of insect predators has been exploited in a limited way and there is a great deal of space for further investigation and implementation. Mosquito control was introduced by the World Health Organization as the only tool to prevent or control certain diseases. While interest in the biological control agents of mosquitoes was strong at the beginning of the 20th century, it has stopped after the insecticidal properties of DDT were discovered in 1939. Insecticides have been used widely for mosquito control since that time. The quest for environmentally friendly insecticide alternatives has become increasingly important because of their deleterious health and environmental impacts (Shaalan, E.A.S. and Canyon, D.V. 2009) [14].



A-1 *Diplonychus sp* A-2 (DV)-H-E A-3 (VV)-M-FL

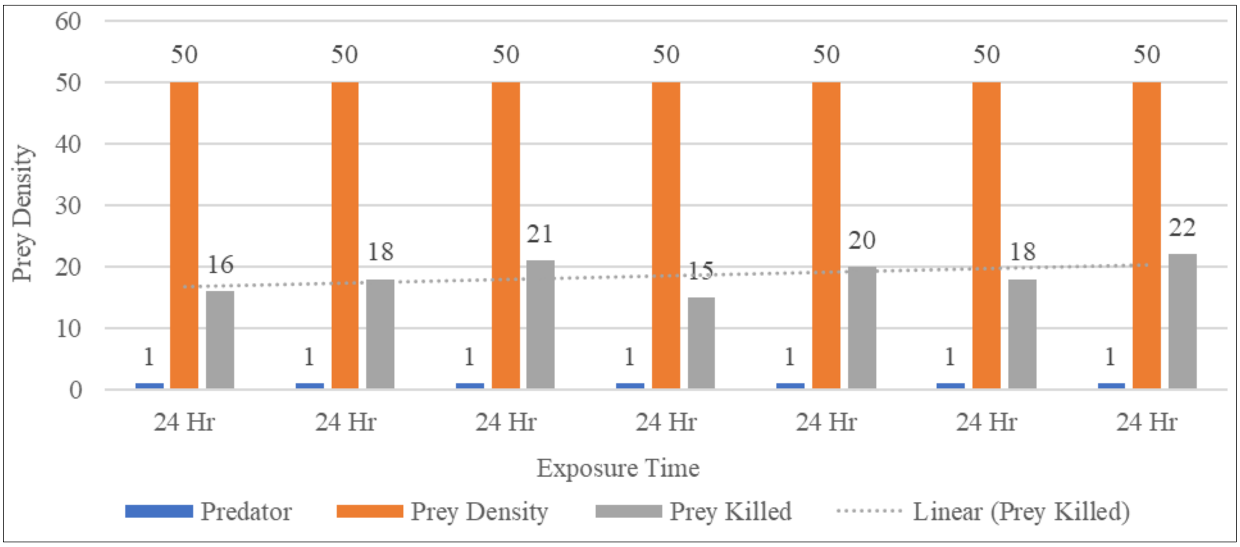
DV=Dorsal View, VV= Ventral View, H=Head, E=Eye, M=Mouth, FL=Fore Legs

Fig 1: Some microscopic images of aquatic entomofauna by dissecting stereo microscope

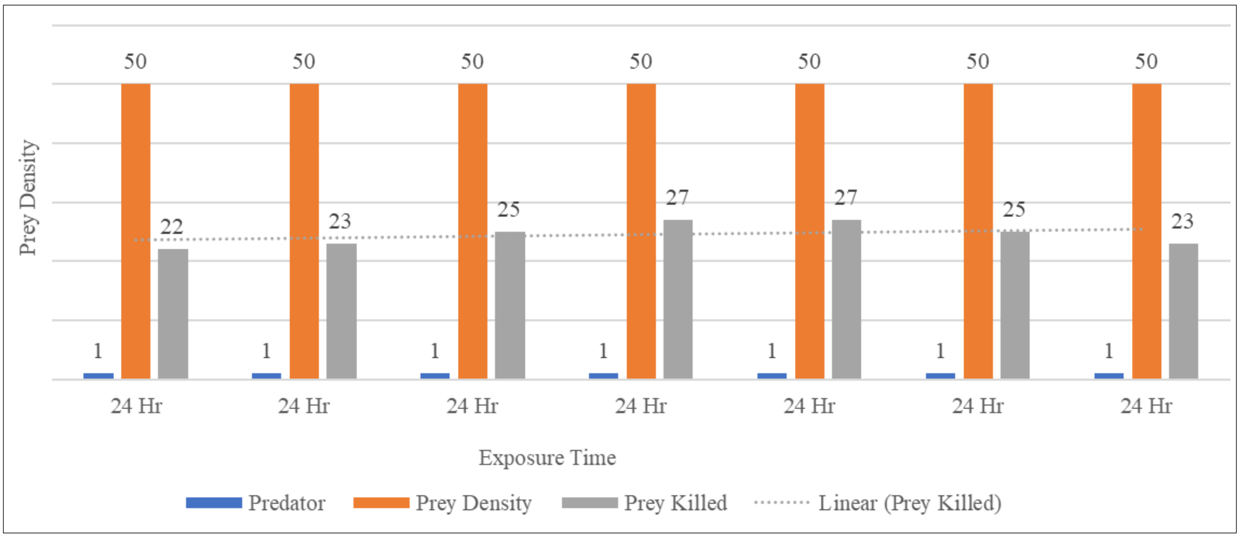


A-4 Diplonychus annulatus A-5 Diplonychus molestus A-6 Diplonychus rusticus

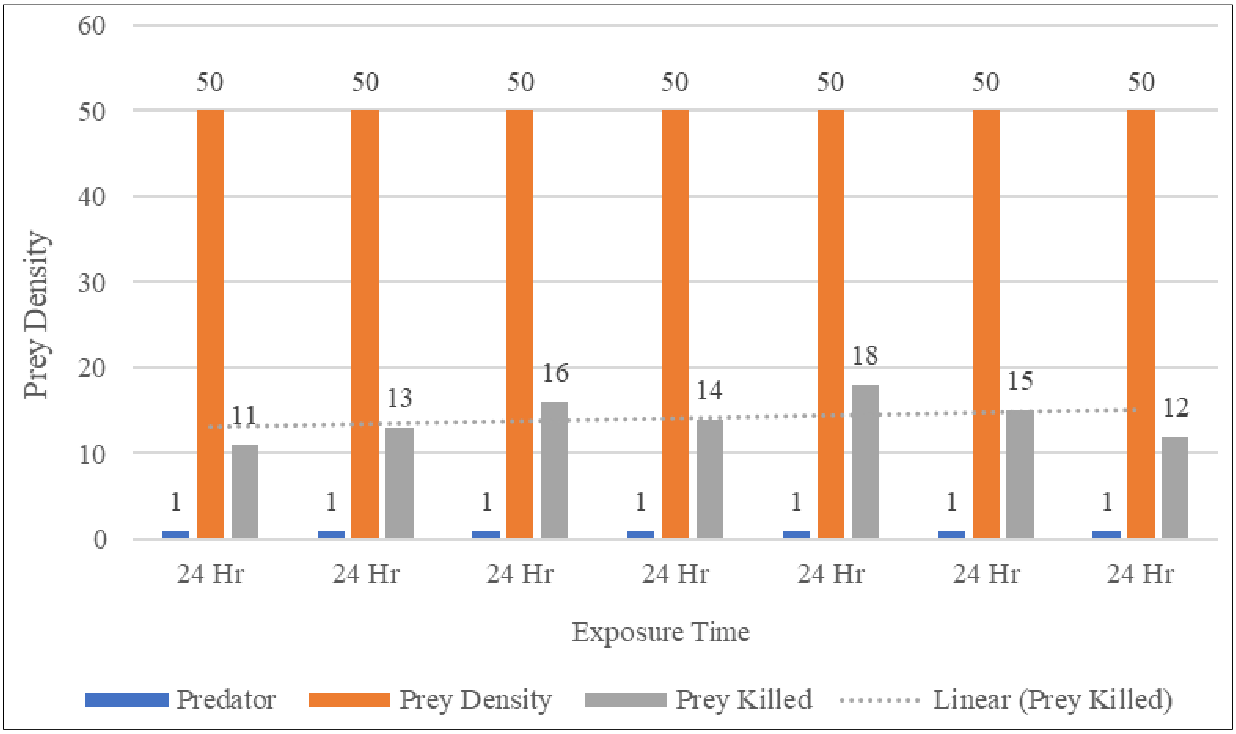
Fig 2: Chandra, K., Jehamalar, E.E. 2012. Morphological differences in three species of the genus Diplonychus (Hemiptera: Belostomatidae) known from India, Rec. Zoo. Surv. India 112(Part-2): 97



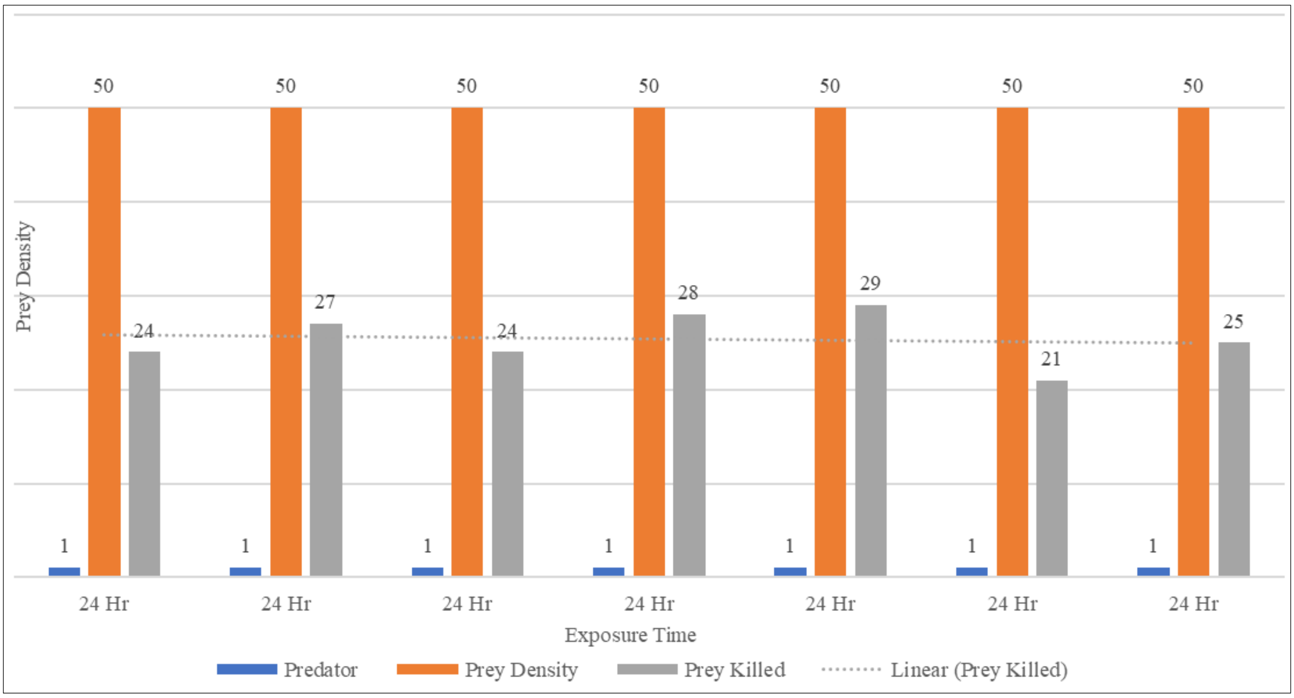
Graph 1: Experiment 1 Prey (Small Size) Killed & Prey Density (50)



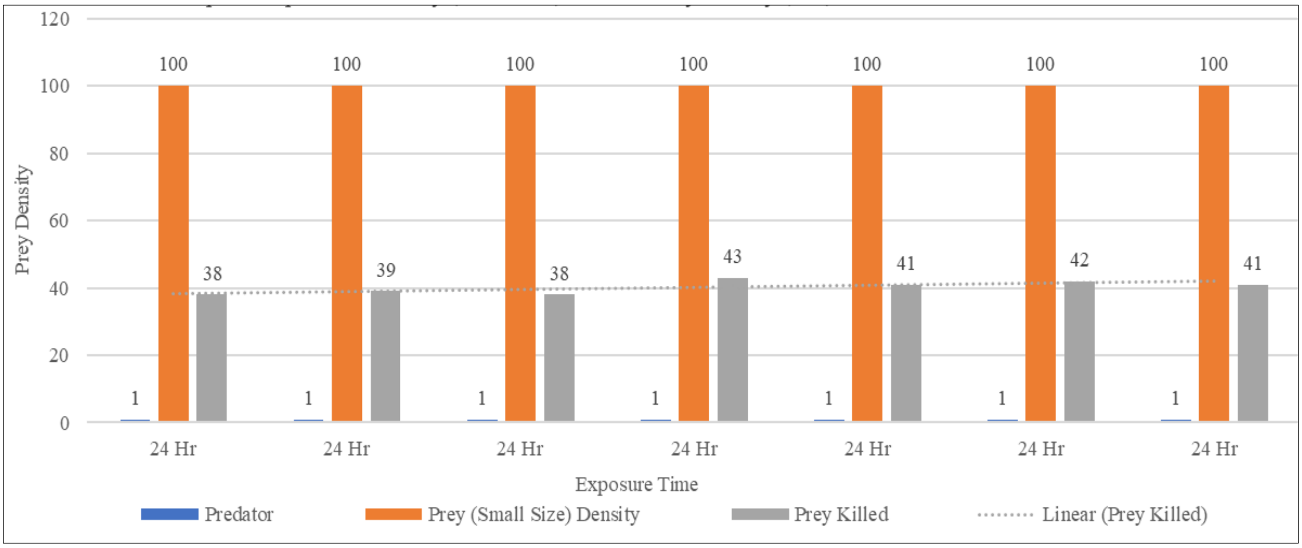
Graph 2: Experiment 1 Prey (Large Size) Killed & Prey Density (50)



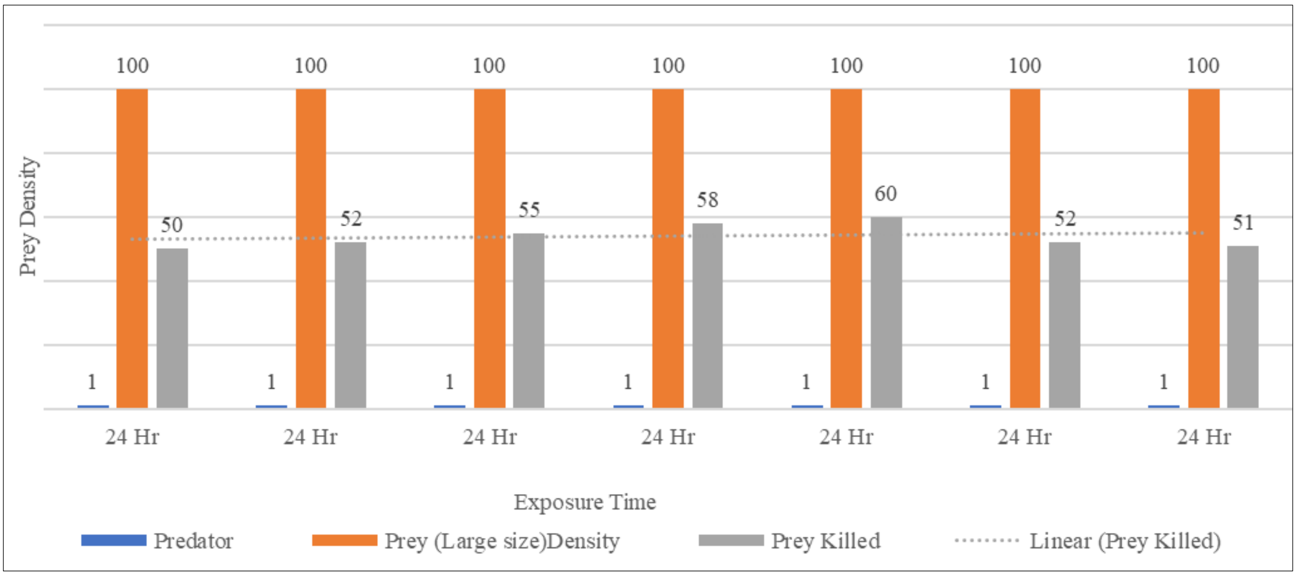
Graph 3: Experiment 2 Prey (Small Size) Killed & Prey Density (50)



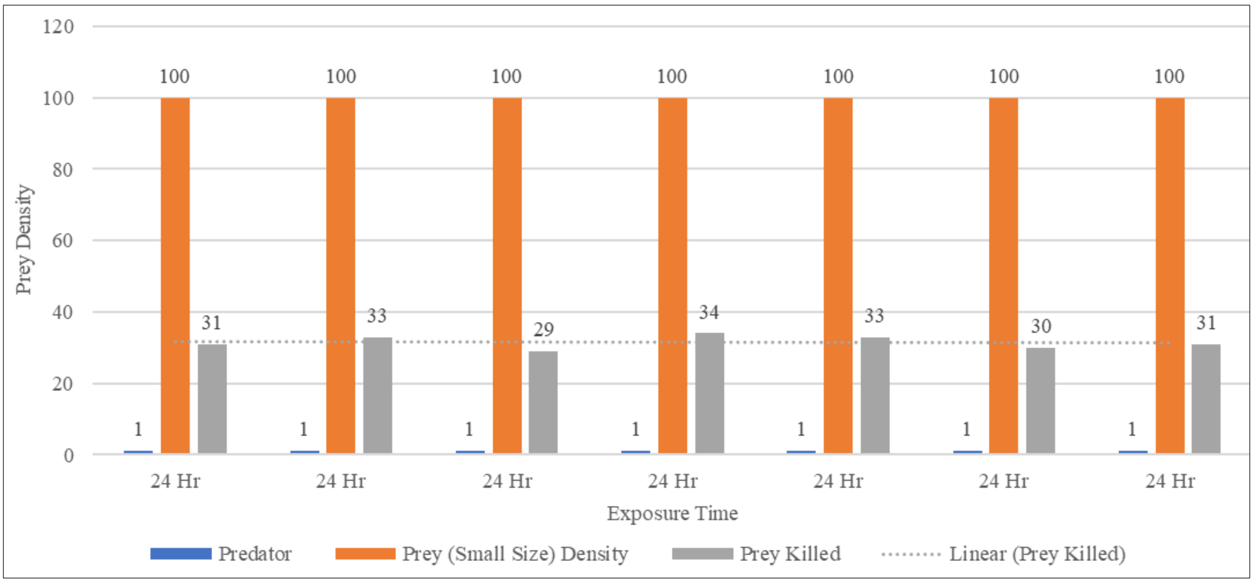
Graph 4: Experiment 2 Prey (Large size) Killed & Prey Density (50)



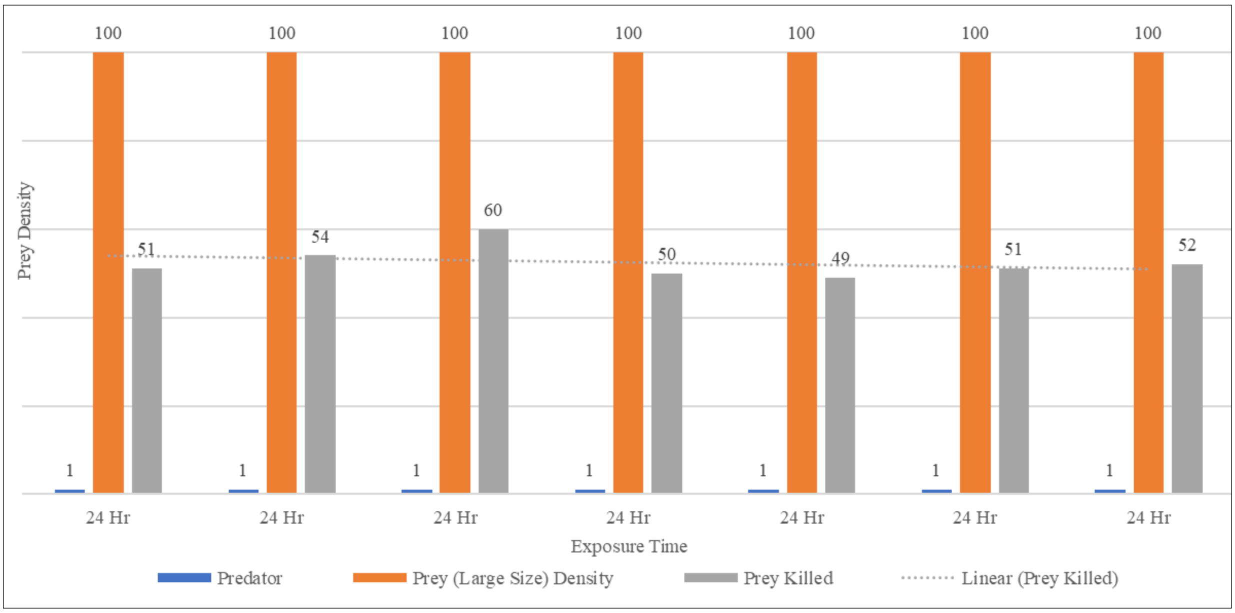
Graph 5: Experiment 1 Prey (Small size) killed & Prey Density (100)



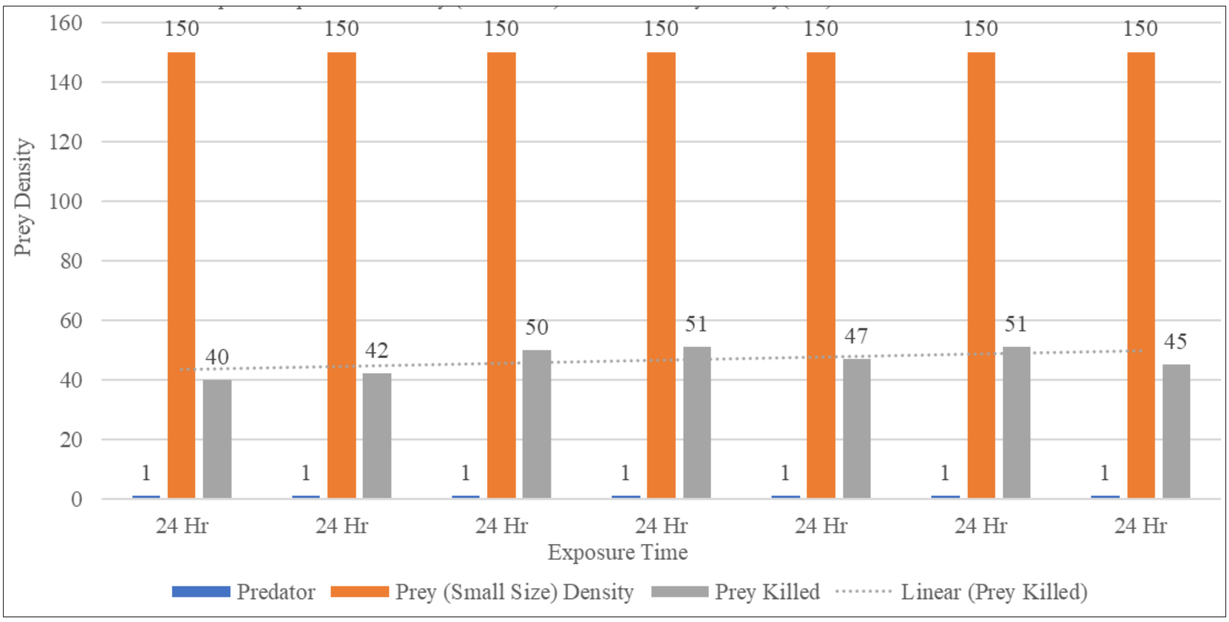
Graph 6: Experiment 1 Prey (Large Size) killed & Prey Density (100)



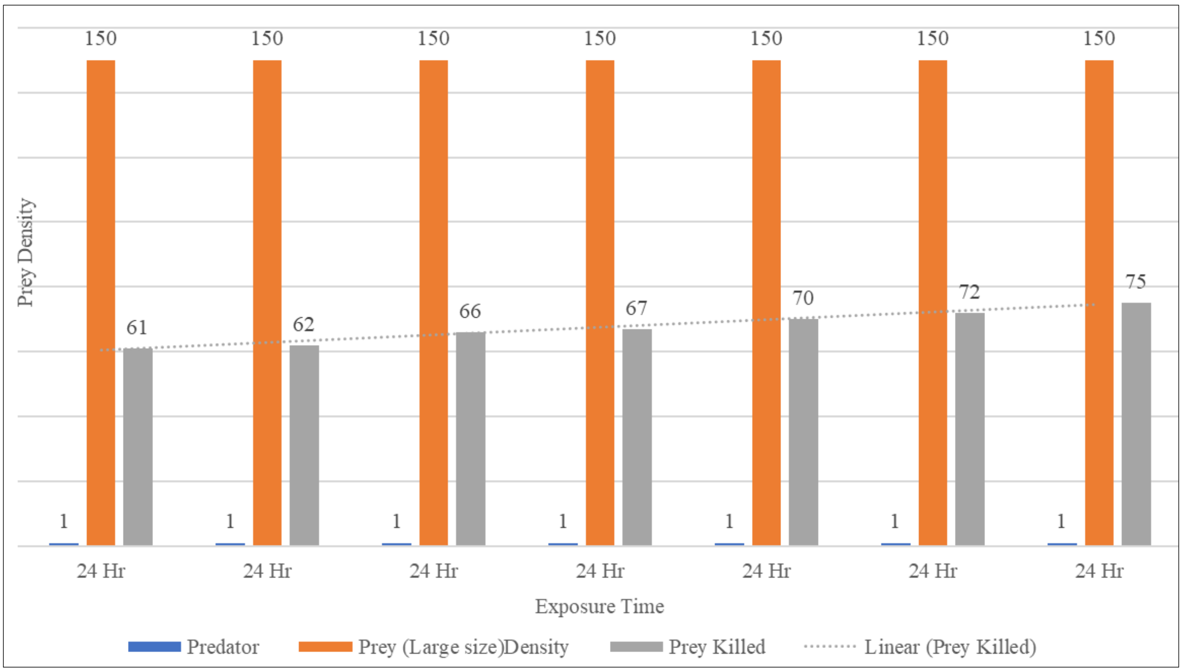
Graph 7: Experiment 2 Prey (Small size) and Prey Density (100)



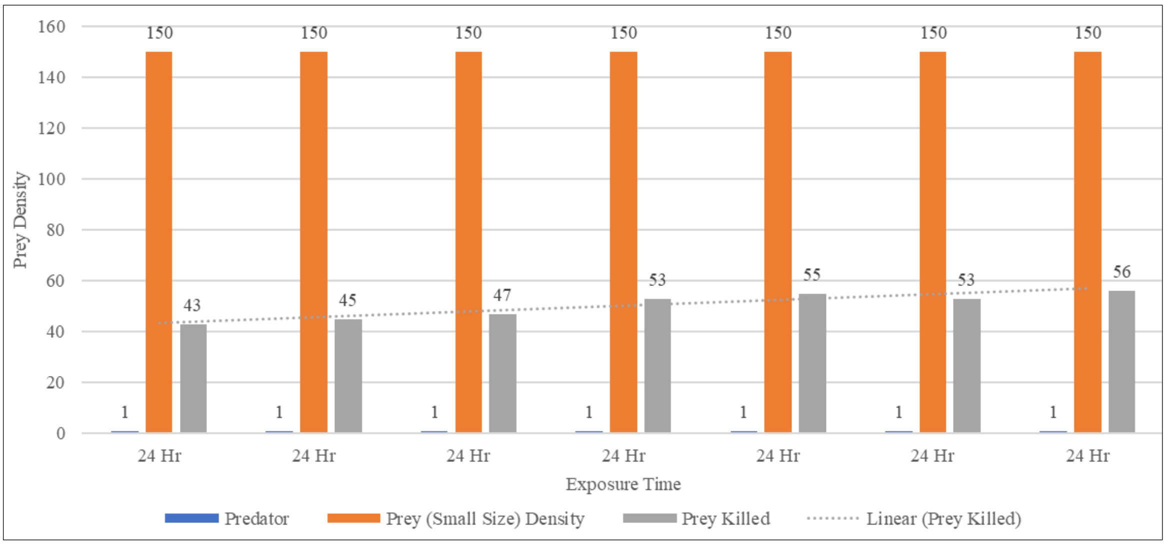
Graph 8: Experiment 2 Prey (Large size) killed & Prey Density (100)



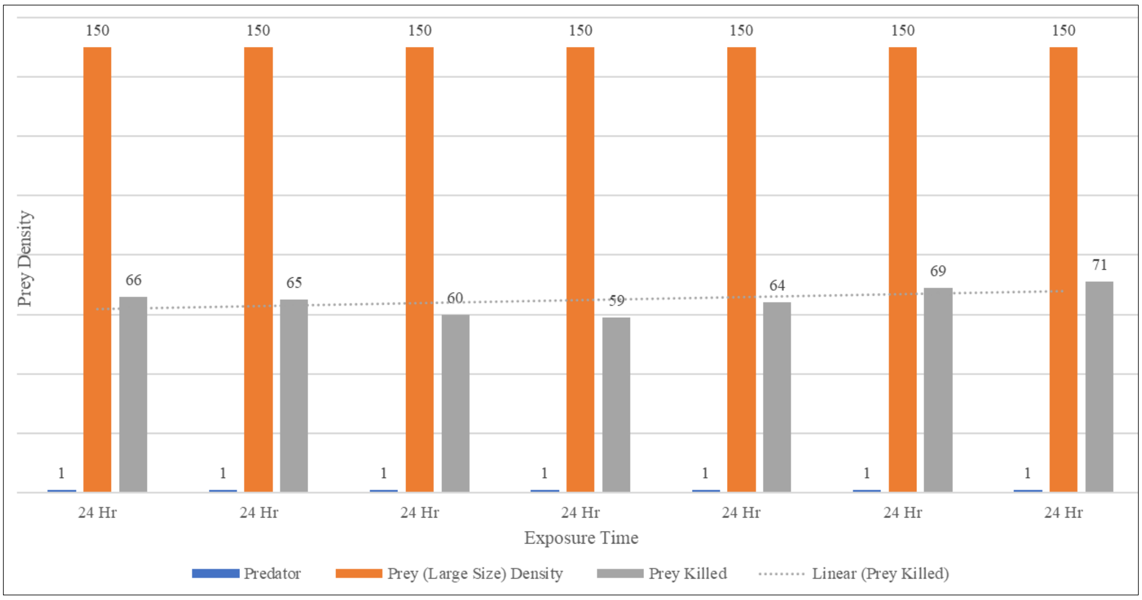
Graph 9: Experiment 1 Prey (small size) killed and Prey Density (150)



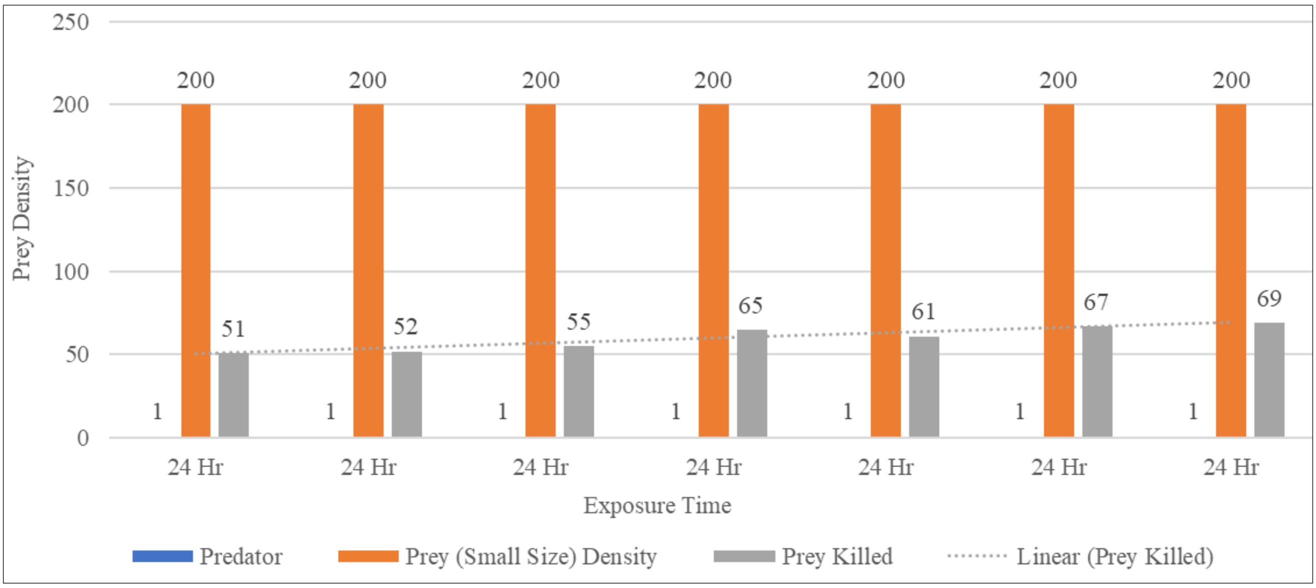
Graph 10: Experiment 1 Prey (Large size) killed & Prey Density (150)



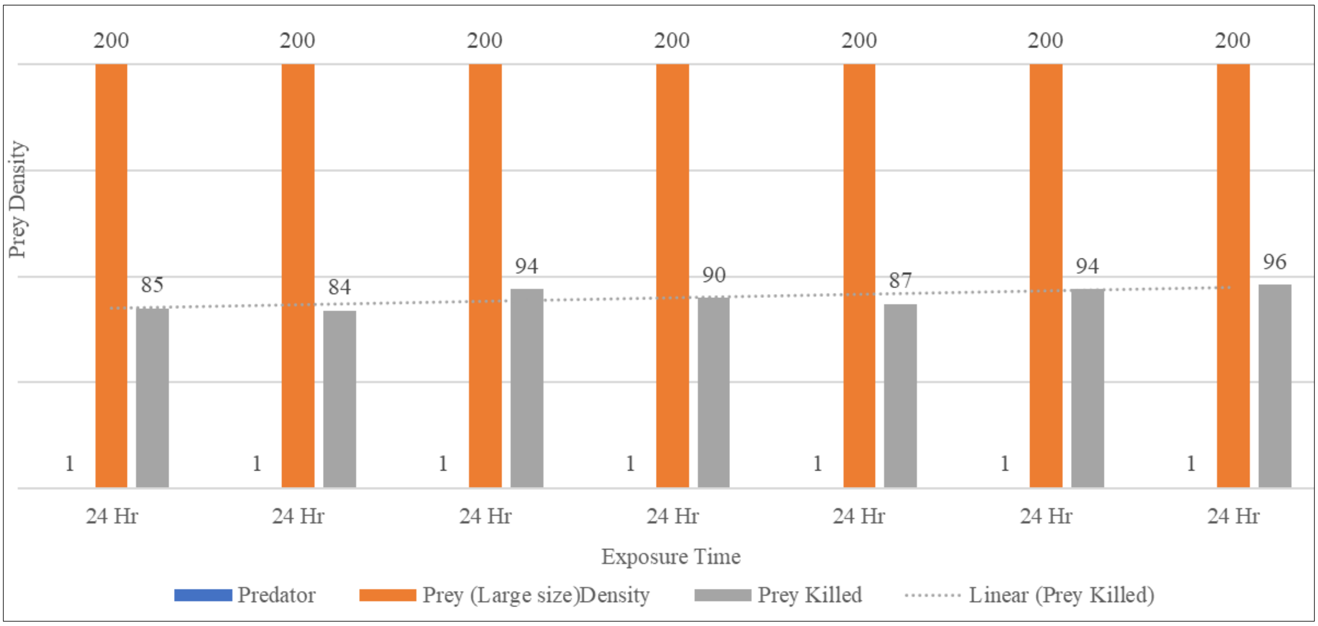
Graph 11: Experiment 2 Prey (Small size) killed & Prey density (150)



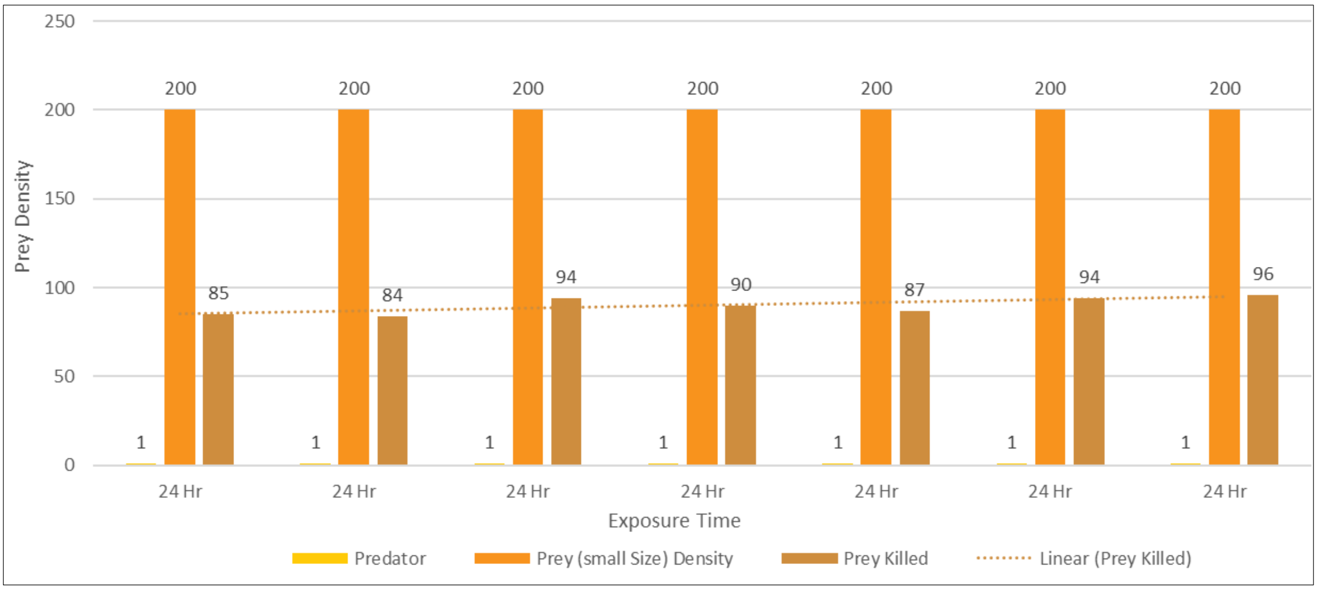
Graph 12: Experiment 2 Prey (Large size) killed & Prey Density (150)



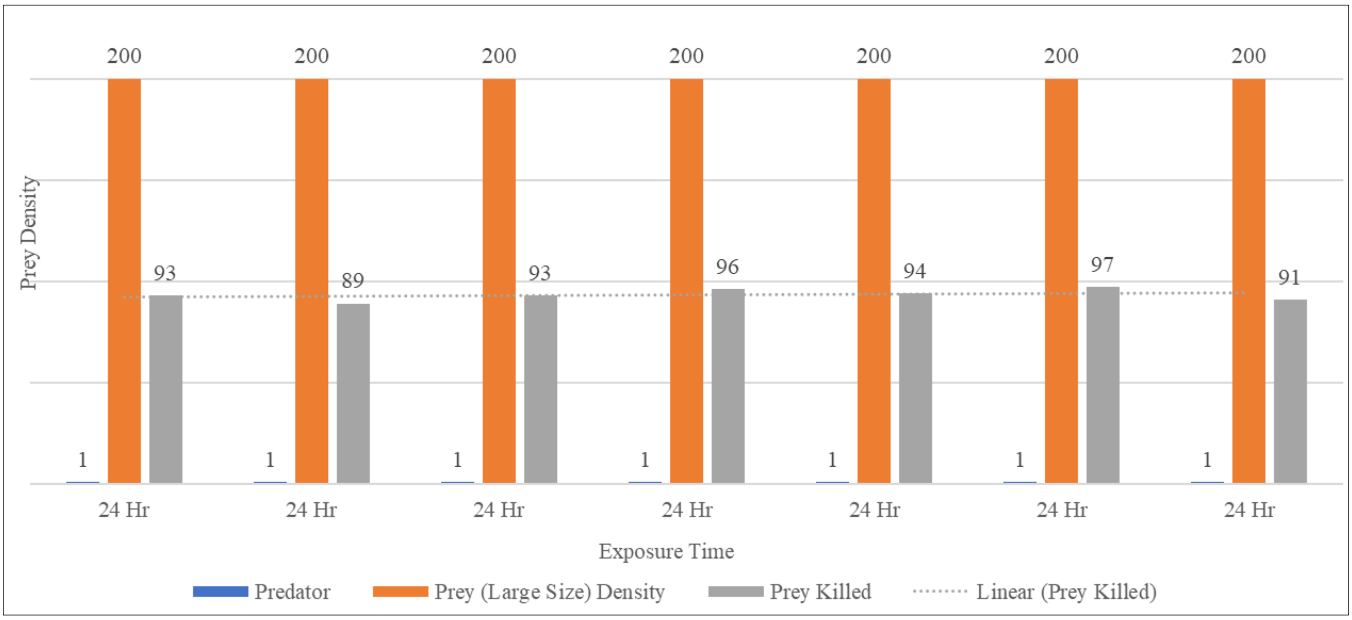
Graph 13: Experiment 1 Prey (Small Size) killed & Prey Density (200)



Graph 14: Experiment 1: Prey (Large size) killed & Prey Density (200)



Graph 15: Experiment 2 Prey (Small size) killed & Prey Density (200)



Graph 16: Experiment 2 Prey (Large size) killed & Prey Density (200)

Conclusions

To help minimise the use of insecticides, which are currently the primary method for mosquito control, biocontrol strategies for mosquito-borne diseases are required. Methods that are environmentally friendly, healthy, and long-lasting should be established to target a variety of mosquito species.

Acknowledgements

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