Studies on the efficacy of *Toxorhynchites* larvae and three larvivorous fish species for the control of *Aedes* larval populations in water-storage tanks in the Matale district of Sri Lanka

W.M.G.S. Wijesinghe[a], M.B. Wickramasinghe[b], P.H.D. Kusumawathie[c], G.A.J.S.K. Jayasooriya[c] and B.G.D.N.K. De Silva[a#]

[a]*Department of Zoology, Faculty of Applied Science, University of Sri Jayewardenepura, Sri Lanka*

[b]*Retired Entomologist, Anti Malaria Campaign, Colombo 05, Sri Lanka*

[c]*Regional Office, Anti Malaria Campaign, Dutugemunu Mawatha, Watapuluwa, Kandy, Sri Lanka*

Abstract

A study was conducted to compare the feeding efficacy of *Toxorhynchites* larvae (L3 & L4) and three larvivorous fish species on *Aedes* larvae. Ground-level cement water-storage tanks (20%–80%) and water-storing barrels (8.33%–54.55%) formed the majority of *Aedes*-positive outdoor containers. *Ae. albopictus, Ae. macdougali* and *Ae. vittatus* were recorded in water-storage tanks, with *Ae. macdougali* being dominant.

In the laboratory, the consumption rate (time to devour 10 *Ae. albopictus* L3 larvae in a vessel of 78.57 cm^2 of surface area) for *Toxorhynchites* was significantly lower (mean time of 330 minutes) than for any of the tested fish species, *Poecilia reticulata* (*Guppy*), *Puntius bimaculatus* (*Ipilli Kadaya*) and *Rasbora caveri* (*Dandiya*), which needed 16.67, 27.33 and 24 minutes respectively. There were no significant differences ($P=0.062$) between the consumption rates of the three fish species.

A field study was carried out to determine the feeding efficacy *of Toxorhynchites* larvae*, P. reticulata, P. bimaculatus and R. caveri* on *Aedes* larval populations in outdoor cement tanks by noting the percentage reduction of *Aedes* larvae per 100 cm^2 surface area after one week. *Toxorhynchites* larvae caused a 20%–83.33% reduction with 1–8 larvae per tank. A complete reduction (100%) was achieved with *P. bimaculatus* and *R. caveri* with 1–3 fish per tank. *P. reticulata* showed similar results, but with 90% reduction being achieved once with two fish per tank. There was a higher possibility of losing *Tx*. larvae than the fish species during the removal of water by the householders. The efficiency of the three fish species for consuming *Aedes* larvae was greater than that with *Tx*. larvae. It appears feasible to use *Puntius bimaculatus*, *Rasbora caveri* and *Poecilia reticulata* for controlling *Aedes* breeding in outdoor cement water-storage tanks in Sri Lanka.

Keywords: *Toxorhynchites* larvae; larvivorous fish; *Aedes* control; Sri Lanka.

[#]*E-mail: nissankakolitha@gmail.com*

Introduction

Dengue fever (DF) and dengue haemorrhagic fever (DHF) is caused by DENV-1 to 4 serotypes of *Flavivirus* belonging to the family *Flaviviridae*. This virus is transmitted by the female mosquitoes of the genus *Aedes*. *Ae. aegypti* is the most important epidemic vector while *Ae. albopictus*, *Ae. polynesiensis* and *Ae. niveus* have been incriminated as secondary vectors in some parts of the world.[1]

In Sri Lanka, the first epidemic outbreak of dengue occurred in 1965–1966 during which a few cases of DHF were reported. An outbreak in 1989 caused 203 reported DHF cases with 20 deaths. In recent outbreaks, dengue occurred over a bigger geographical area of the country with multiple serotypes of the virus in circulation. At present, dengue is endemic and an important public health problem in Sri Lanka.[2-3]

Two peaks of dengue fever occur in Sri Lanka annually in conjunction with the south-west monsoon in June-July and the north-east monsoonal rains during October-December. Water collected in man-made containers in domestic and peridomestic environments are important oviposition sites of *Ae. aegypti*. Ground-level cement water-storage tanks and barrels are reported to be the major larval habitats of *Ae. aegypti* and *Ae. albopictus* in both Kandy[4] and Matale districts.[5]

In the absence of a vaccine for the prevention of dengue infection and of a specific treatment for DF/DHF, control of dengue is primarily dependent on the control of *Ae. aegypti*, the most important vector species, and *Ae. albopictus,* the secondary vector. Chemical larvicides suitable for use against *Aedes* breeding in domestic, water-storage tanks, barrels, etc. are extremely limited. Furthermore, frequent use of these chemicals has the potential of developing vector resistance to insecticides. Thus, greater attention has been paid to the use of biological agents for controlling *Aedes* breeding in such breeding habitats. Larvivorous fish offer considerable potential for the control of mosquito larvae.[6] The possibility of using *Toxorhynchites* larvae as a biological agent has also been identified in studies conducted by several investigators.[7] The present study was carried out to compare the efficacy of *Toxorhynchites* larvae (L3 and L4 stages) and three species of larvivorous fish for the control of *Aedes* breeding in peridomestic, cement water-storage tanks.

Materials and methods

Study site

The study was conducted in Highlevel gardens, Kaudupelella, in Walliwela Grama Niladhari (GN) division of the Matale district (7° 20′ – 8° 15′ N; 80° 25′ – 81° 00′ W). This area receives piped water originating from a natural water spill. Water is supplied by the Matale Pradeshiya Sabha (urban council). There were frequent interruptions, sometimes for several days, in the supply of unchlorinated water. This encouraged residents to store water for household use in domestic and peridomestic water-storage tanks for several weeks. These storage tanks have been identified as major breeding sites of *Ae. aegypti* and *Ae. albopictus* in this area.[8]

Production of *Toxorhynchites* larvae

Three tyres standing in an upright position and filled to two-thirds of their volume with tap water were kept in vegetated areas. Tyres were inspected after a week for *Toxorhynchites* larvae. Larvae were identified to species using a standard key.[9]

Determination of feeding efficacy of *Toxorhynchites* larvae and fish in the laboratory

Feeding efficacy tests were carried out under laboratory conditions with three replicates. In each replicate, one L4 *Toxorhynchites splendens* larva and one *Poecilia reticulata, Puntius bimaculatus* or *Rasbora caveri* fish (Figure 1) were placed in separate plastic containers (11 cm height x 10 cm diameter) with well water up to 6 cm deep. They were acclimatized for two hours without food. Then 10 *Ae. albopictus* larvae were added to each container and the time taken to consume the introduced larvae was noted. Data were analysed by One-Way ANOVA using Minitab.

Figure 1: Fish species used in feeding efficacy tests

Puntius bimaculatus

Rasbora caveri

Poecilia reticulata

Aedes larval surveys to determine larval density in water-storage tanks

Weekly field observations were made from May 2007 to February 2008 in 34 selected houses in the study area to investigate larval breeding in outdoor water-storage containers. During observations, 10 *Aedes* larvae were collected randomly from each *Aedes*-positive container using a standard ladle or dropper and placed in separate small plastic bottles. Ten larvae attributed for 100% sensitivity of *Aedes* surveys[10] were collected. If a particular container had less than 10 larvae, all larvae were collected. Larval identification was carried out using standard keys[9] and larval density was expressed by calculating the House Index (HI) (percentage of houses positive for *Ae. aegypti*/*Ae. albopictus*), Container Index (CI) (percentage of containers positive for *Ae. aegypti*/*Ae. albopictus*) and Breteau Index (BI) (number of containers positive for *Ae. aegypti*/*Ae. albopictus* per 100 houses).[11]

Application of *Toxorhynchites* larvae and fish species to the water-storage tanks

Aedes larval density per 100 cm^2 of surface area of each tank was noted before application of *Toxorhynchites* larvae or fish (*P. reticulata, P. bimaculatus and R. caveri*). *Toxorhynchites splendens* and *Tx. minimus* L3 and L4 larvae were added to *Aedes*-positive tanks at the rate of 1–4, 6 and 8 larvae per tank, with 1–3 fish per tank. *Aedes* larval density per 100 cm^2 of surface area of each tank was noted one week after application. Three replicates were carried out for each test.

Results

The mean time taken by *Toxorhynchites* larvae to consume 10 *Aedes albopictus* larvae was 330.0 minutes. Mean times for fish to consume 10 *Ae. albopictus* larvae were: *Poecilia reticulate* – 16.66 minutes, *Puntius bimaculatus* – 27.33 minutes, and *Rasbora caveri* – 24 minutes (Table 1). There was no significant difference (P=0.062) between the mean time taken to consume 10 larvae by the three fish species.

Table 1: Time taken to consume 10 *Aedes albopictus* larvae by each species of *Toxorhynchites* larvae and three species of fish

Species	Time taken to consume 10 *Ae. albopictus* larvae (minutes)			
	1	2	3	Mean (minutes)
Toxorhynchites splendens larvae	360	300	330	330.00
Poecilia reticulata	15	14	21	16.67
Puntius bimaculatus	25	25	32	27.33
Rasbora caveri	22	20	30	24.00

Potential outdoor breeding habitats of *Aedes* species included ground-level cement water-storage tanks, barrels (plastic and metal), plastic buckets, plastic cans, aluminium pots, clay pots, metal pots and coconut shells. Ground-level cement water-storage tanks (20.00%–80.00%) and barrels (8.33%–54.55%) were the major contributors to *Aedes*-positive outdoor containers in the study area. Plastic buckets (0.00%–33.33%), plastic cans (0.00%–33.33%), aluminium pots (0.00%–12.50%), clay pots (0.00%–16.67%) and metal pots (0.00%–9.52%) were the other important breeding habitats.

Three *Aedes* species (*Ae. macdougali, Ae. albopictus* and *Ae. vittatus)* were found breeding in cement tanks. *Ae. macdougali* was the dominant species with a contribution of 61.61% of the total collection. *Ae. albopictus* contributed 37.79% while *Ae. vittatus* contributed 0.59% to the total collection (Table 2). The selected study area was primarily semi-urban in nature, with more potential breeding sites suitable for *Ae. albopictus*. All *Ae. aegypti* breeding sites were treated with Abate by government authorities. These factors contributed to the absence of *Ae. aegypti*.

Table 2: Species composition of *Aedes* larvae in water-storage cement tanks in the study area

Aedes species	Number of larvae	Percentage of larvae
Ae. macdougali	724	61.61
Ae. albopictus	444	37.79
Ae. vittatus	7	0.59
Total	1175	100

A House Index (HI) greater than 5% was obtained for *Aedes* species, including *Ae. albopictus,* in all 29 field visits. A Container Index (CI) greater than 20% was recorded for *Ae. albopictus* on seven occasions, and for all *Aedes* species on 18 occasions.

There was no relationship between the number of *Toxorhynchites* larvae applied and the reduction of *Aedes* larvae per 100 cm^2 of surface area. In tanks with *Toxorhynchites* added at the rate of 1, 2, 3, 4, 6 and 8 larvae per tank, 20.00%–83.33% reduction of *Aedes* larval density was observed. Although a considerable reduction of larvae per tank was obtained, 100% reduction was not achieved even with eight *Toxorhynchites* larvae (Figures 2 and 4). In the *P. reticulata* tanks at

Figure 2: Reduction of *Aedes* larval populations using *Toxorhynchites* larvae

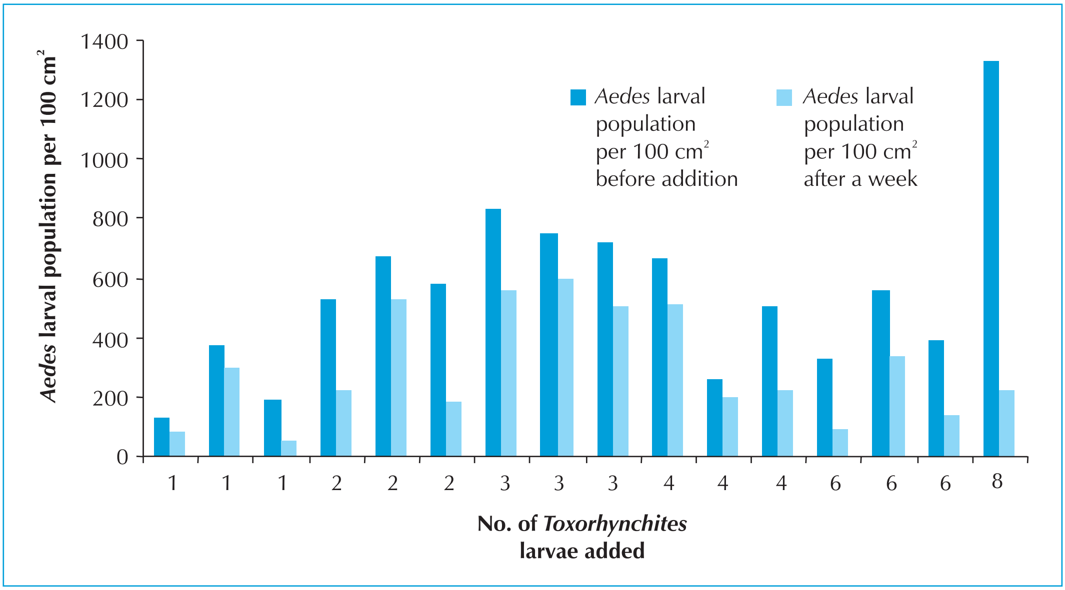


Figure 3: Reduction of *Aedes* larval populations using three fish species

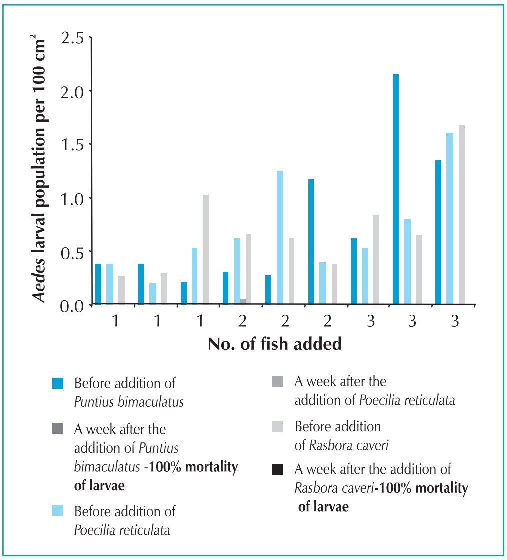
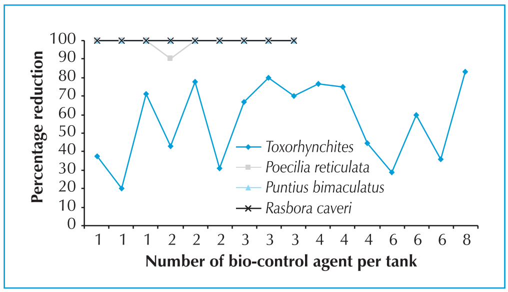


Figure 4: Percentage reduction of *Aedes* larval population by each bio-control agent



the rate of 1, 2 and 3 fish per tank, there was a 100% reduction of *Aedes* larvae, except on one occasion in tanks with two fish, when it was 90% (Figures 3 and 4). A 100% reduction of *Aedes* larvae was also observed with the application of 1, 2 or 3 *Puntius bimaculatus* (*Ipilli Kadaya*) and *Rasbora caveri* (*Dandiya*) (Figures 3 and 4).

Discussion

Water storage is a common household practice in areas with irregular water supply. In our study area, residents store water in cement tanks and barrels for domestic and peridomestic use due to uncertainty of water supply. These tanks were rarely cleaned at weekly intervals, thus providing ideal developmental sites for both *Aedes* and other container-breeding mosquitoes. During the present study, a high percentage of ground-level cement water-storage tanks (20%–80%) contained *Aedes* larvae. Cement water-storage tanks also have been reported as important breeding habitats of *Ae. aegypti* and *Ae. albopictus* in dengue transmission areas in Matale district,[5] Kandy and Nuwara Eliya districts[4,10,12,13,14] and in Tangalle.[15]

The presence of *Ae. macdougali* was reported in the Suduganga area of Matale district[8] and we found *Ae. macdougali* in cement tanks in high density (61.61% of the total collection) and sharing the habitat with *Ae. albopictus* and *Ae. vittatus*. The sharing of water-storage tanks by *Ae. macdougali*, *Ae. aegypti*, *Ae. albopictus* and other non-*Aedes* species was reported in other studies.[16]

Our data suggest that cement water-storage tanks are important breeding sites of dengue vectors (*Ae. aegypti* and *Ae. albopictus*) and other nuisance mosquitoes. Thus, mosquito control in these tanks would help to prevent/control both dengue and mosquito nuisance. The development and presence of other mosquito species along with *Ae. aegypti* and *Ae. albopictus* habitats require greater care to be taken during larval collections, and identification and calculation of relevant larval indices. The collection of 10 randomly selected *Aedes* larvae (or all larvae if the container had <10 larvae) has been adopted for 100% sensitivity of dengue vector surveillance.[10]

Toma and Miyagi reported the consumption of 20–26 larvae per day by 1st and 2nd instar *Tx. splendens* with an average consumption of 389±26 larvae during their entire larval lifespan.[17] However, the success of using *Toxorhynchites* in *Aedes* larval control in field settings is controversial.[7] In Malaysia, 2nd instar larvae of *Tx. splendens* have been used successfully to control *Ae. aegypti* and *Ae. albopictus* breeding in domestic water containers,[18] although in Java, Indonesia, introduction of *Tx. amboinensis* larvae to control *Ae. aegypti* and *Ae. albopictus* in the same type of habitats was less successful.[19] We observed that *Toxorhynchites* larvae only gave 20%–80% reduction of *Aedes* larval density in the water-storage tanks.

Complete reduction of *Aedes* larvae was obtained with 1–3 fish of *P. reticulata*, *P. bimaculatus* and *R. caveri* except on one occasion with two *P. reticulata* per tank in which case it was 90%. *Puntius bimaculatus* (an endemic species) and *Rasbora caveri* (a common fresh-water fish in Sri Lanka) are reported to have shown larvivorous activity.[20] Thus, the use of *Puntius bimaculatus, Rasbora caveri* and *Poecilia* is recommended for *Aedes* mosquito control in water-storage tanks in this study area. *Danio malabaricus*, *Oreochromis mossambicus*, *O. niloticus* and *Poecilia reticulata* are reported to be good larvivorous fish species for anopheline mosquito control.[16] Thus, further studies on the effectiveness of different fish species for *Aedes* larval control in water-storage tanks in different eco-epidemiological areas are warranted. Such studies would help to identify the most suitable species of fish for a particular eco-epidemiological area as some fish species do not survive well in some areas. *Poecilia reticulata* is now considered an invasive species and thus great care should be taken when it is applied to natural waters[21] considering the ecology and the epidemiological conditions.

Two species of *Toxorhynchites* (*splendens* and *minimus)* have been recorded from Sri Lanka[8,22] and *Tx. minimus* in the Suduganga area in Matale.[8] During the present study, natural breeding of *Tx. splendens* was observed in a water-filled barrel in the study area. This indicates the possibility of establishing *Tx. splendens* and *Tx. minimus* as part of an integrated vector control programme.

Acknowledgements

The authors wish to thank Dr Ajantha de Alwis, Department of Zoology, University of Sri Jayewardenepura, for helping in the identification of fish species, and Mr P. Dias, Department of Mathematics, University of Sri Jayewardenepura, for his support in the statistical analysis of data.

References

[1] *Prevention and control of dengue and dengue haemorrhagic fever - comprehensive guidelines*. New Delhi: WHO Regional Office for the South-East Asia, 1999. pp. 29; 134.

[2] Kulatilaka TA, Jayakuru WS. Control of dengue/dengue haemorrhagic fever in Sri Lanka. *Dengue Bull* 1998;22:53-59.

[3] *External in-depth review of DF/DHF control programme in Sri Lanka*. New Delhi: WHO Regional Office for South-East Asia, 2005. pp. 75.

[4] Kusumawathie PHD, Wickremasinghe AR, Karunaweera ND, Wijeyaratne MJS. Larvivorous potential of fish species found in river bed pools below the major dams in Sri Lanka: *J Med Entomol* 2006;43(1):79-82.

[5] Yapabandara AMGM, Bandara DMLS. *Entomological and sociological investigations in dengue transmission areas in the Matale District. Sri Lanka Association for the Advancement of Science, Proceedings of 62nd annual session, Sri Lanka*, 2006. pp.1.

[6] Wickramasinghe MB, Costa HH. Mosquito control with larvivorous fish. *Parasitology Today* 1986;2(8):228-230.

[7] Collins LE, Blackwell A. The biology of *Toxorhynchites* mosquitoes and their potential as biocontrol agents. *Biocontr News Info* 2000;21:105N-116N.

[8] Yapabandara AMGM, Abeykoon AR. *Aedes aegypti and Aedes albopictus breeding sites in the Matale Municipal council area. Sri Lanka Association for the Advancement of Science, proceedings of 58th annual sessions, Sri Lanka,* 2002. pp. 20.

[9] Sewell RBS, Edward FW. *Fauna of British India, including Ceylon & Burma, order: diptera*. 1st edition, New Delhi: Today & Tomorrows Printers & Publishers, 1934.

[10] Kusumawathie PHD, Jayasooriya GAJSK, Wickremasinghe R. *Sensitivity of different larval collection methods in dengue vector surveillance in the Kandy and Nuwara Eliya districts. Sri Lanka Association for the Advancement of Science: proceedings of the 62nd annual sessions*. 2006. pp. 1-2.

[11] Pant CP, Self LS. *Vector ecology and bionomics. Monograph on dengue/dengue haemorrhagic fever*. WHO Regional Publication, SEARO No 22. New Delhi: WHO-SEARO, 1993. pp. 121-138.

[12] Kusumawathie PHD, Jayasooriya GAJSK, Wickremasinghe AR. *Most productive and prevalent breeding habitats of Aedes aegypti and Ae. albopictus (pupae) in the Kandy and Nuwara Eliya districts of Sri Lanka. Sri Lanka Association for the Advancement of Science, Proceedings of the 63 rd Annual Sessions, Sri Lanka* 2007. pp. 7-8.

[13] Kusumawathie PHD, Wickremasinghe AR, Jayasooriya GAJSK. *Disease transmitting mosquito species breeding in water storage tanks in Kandy, Sri Lanka. Sri Lanka Association for the Advancement of Science, Proceedings of the 61st Annual Sessions* 2005. pp. 8.

[14] Kusumawathie PHD, Siyambalagoda RRMLR. Distribution and breeding sites of potential dengue vectors in Kandy and Nuwara Eliya districts of Sri Lanka: *Ceylon Med J* 2005;48(2):43-52.

[15] Epidemic of dengue fever/dengue haemorrhagic fever in Tangalle. *Weekly Epidemiological Report Sri Lanka Ministry of Health* 2001;29(50):1-4.

[16] Kusumawathie PHD, Wickramasinghe AR, Karunaweera ND, Wijeyaratne MJS. Use of Sri Lankan larvivorous fish species in mosquito control. In: Karunaratne SHP. Eds. *Programme & proceedings national symposium on mosquito control*. Colombo: Post Graduate Institute of Science, University of Peradeniya and National Science Foundation, 2005. pp. 58-69.

[17] Toma T, Miyagi I. Laboratory evaluation of *Toxorhynchites splendens* (Diptera: Culicidae) for predation of *Aedes albopictus* mosquito larvae. *Med Vet Entomol* 1992:6;281-289.

[18] Chuah MLK, Yap HH. Studies on biological control potentials of *Toxorhynchites splendens* (Diptera: Culicidae). *Trop Biomed* 1984;1:145-150.

[19] Annis B, Nalim S, Widiarthi H, Boewono DT. *Toxorhynchites amboinensis* larvae released in domestic containers fail to control dengue vectors in a rural village in central Java. *J Am Mosq Contr Assoc* 1990;6:75-78.

[20] Pethiyagoda R. *Fresh water fishes of Sri Lanka*. Colombo: Wildlife Heritage Trust of Sri Lanka, Sarvodaya Vishva Lekha, 1991.

[21] Shirantha RRAR, Amarathunga AAD, Weerasekara KAWS. Do feral populations of *Poecilia reticulata* ready to bring its overall efficacy as a mosquito bio-control agent or pose threats to aquatic biodiversity in Sri Lanka. In: *The proceeding of National Symposium on Invasive Alien Species* (*IAS 2008*), Sri Lanka Association for the Advancement of Science (Section D), National Science Foundation and Biodiversity Secretariat, Colombo 7, 2008. pp. 8.

[22] Jayasekera N, Chelliah RV. *An annotated checklist of mosquitoes of Sri Lanka UNESCO: man and the biosphere national committee of Sri Lanka*. Publication No. 8, 1981. pp. 16.