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Pre- and Post-Intervention Study on *Aedes* Larvae in Water Storage Containers Adding of Native Larvivorous Fish *Aplocheilus panchax* in Hpa-an Township, Kayin State

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Effective mosquito control strategies in temporary water storage containers in community are urgently needed to reduce dengue fever and dengue hemorrhagic fever through mosquito larvae reduction without harmful to environment, ecosystems and community. For biological control of *Aedes aegypti* larvae, native larvivorus fish as *Aplocheilus panchax* were collected from Hpa-an Township, Kayin State. Mingalar Ywar Thit and Taung Nar Village were selected as test and control villages and 51 households each were randomly selected to recruit the study. Laboratory and field study were done from February 2016 to January 2017. In the laboratory, a series of laboratory experiments compared *Aedes* larva consuming rates of *Aplocheilus panchax* and *Trichogaster trichopterus* in different water volumes, in order to determine their potential as larva control agent in water storage containers. In the field, *Aedes* larvae and pupae positivity in containers were recorded in both villages. During intervention, two *Aplocheilus panchax* were put into all major, minor and miscellaneous containers monthly for 3 months in test area of Mingalar Ywar Thit Village. Larval and pupal indices were determined before and after intervention. Laboratory result found that one gram weight of *Aplocheilus panchax* consumed 463.04 3rd and 4th instar *Aedes* larvae within 24 hours. It was 3.727 fold higher in consuming rate than one gram of *Trichogaster trichopterus* against 124.24 *Aedes aegypti* larvae. After intervention, House Index (HI), Container Index (CI) and Breteau Index (BI) were significantly reduced from 86.27%, 59.86% and 172.55 to 1.96%, 1.36% and 3.92, respectively ($P<0.05$). Hundred percent reduction was found in key containers and 90% reduction was found in key premises in test area. Pupal indices were also significantly reduced. Native larvivorus fish *Aplocheilux panchax* is an efficient biological agent for the control of DF and DHF vector *Aedes* immature stages in Kayin State. The biological method is simple and cost-effective method for control of DF/DHF in community.

Keywords: Larvivorous fish, *Aplocheilus panchax*, Key container, Key premises, Container

INTRODUCTION

Aedes aegypti is one of the world's most widely distributed mosquitoes and is of considerable medical importance as a vector of dengue, Zika, yellow fever, and Chikungunya.[1] A severe outbreak of dengue hemorrhage fever (DHF) occurred for the first time in Yangon in 1970. The highest number of cases and deaths recorded were 9, 149 DHF cases and 55 deaths recorded across Myanmar.[2] *Aplocheilus panchax* is a widespread species in Southern and Southeast region reported from Pakistan, India, Bangladesh, Myanmar, Sri Lanka, Nepal and Indonesia. Fishes are surface feeder and used as potent biocontrol agent in several aquatic habitats.[3] Rapid, poorly planned urbanization in association with weak regulatory policies for discharge of solid waste has resulted in the accumulation of discarded containers in most developing countries. These accumulations have favored the establishment and geographic spread

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of this mosquito. The strategies to control dengue transmission used by the public vector control programs have not been adequate in most countries. The emergence of insecticide resistance, the difficulty of eliminating larval population through environmental sanitation and lack of efficacy of ultralow-volume insectcide spraying to control adults are factors which have limited the effectiveness of vector control programmes.[4] Synthetic chemicals and insectcides used for control of vectors are causing irreversible damage to the ecosystem, as some of them are non-degradable in nature. Some repellents of synthetic origin may cause skin irritation and affect the dermis.[5] The indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insectcide resistance, and toxic hazards to humans. Globally, there have been conscientious efforts to overcome these problems, and great emphasis has been placed recently on environment friendly and economically viable methodologies for mosquito control.[6]

Dengue fever (DF) and dengue hemorrhage fever (DHF) are increasingly becoming serious public health problems in Myanmar, especially among the 5-10 and 11-15 years old age groups and now noted 15 years above, a vast majority of the cases occur in 5-8 years old age group.[7, 8] Three hundred and seventy-nine DHF cases were recorded in Kayin State.[9] Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air.[10] Therefore, there is a need to find out the alternative ways for environmental safety, bio-friendly, cost-effective and indigenous methods which are more potential natural products as native larvivorous fish for vector control. The study was under-taken to assess the larvivorus effect of native larvivorous fish to control of DHF vector *Aedes aegypti* larvae in domestic water storage containers, and to determine the larval and pupal indices of before and after intervention using larvivorous fish in test and control villages of Pa-an Township Kayin State.

MATERIALS AND METHODS

Study design

The study was focused in laboratory to determine the potential native larvivorous fish and field-based pre- and post-intervention experimental study on water storage container-bred *Aedes* larvae. The study was conducted in Mingalar Ywar Thit (intervention village) and Taung Nar (non-intervention village) in Hpa-an Township, Kayin State. Fifty-one houses each were randomly selected from both villages.

Study population

Larvivorous fishes were collected from creeks, river beds, rice fields and water ponds by fish nets which were called *yin quin* and *yin thet* in Myanmar name in Hpa-an Township. *Aedes* larvae were counted from major (metal drums, plastic drums, concrete tanks, concrete jars, big Bago jars etc.), minor (small Bago jars, buckets, plastic bowls, earthen pots spirit bowls, flower pots etc.) and miscellaneous containers (coconut shells, tin cans, car tire, discarded earthen pots, broken Bago jars, plastic bottles, broken flower pots etc.) by visual methods[11] in both villages. Before intervention, larval and pupal positivity were measured in all major, minor and miscellaneous containers in both villages in July.

Larva consuming tests

Twenty-four hours predatory rates of two native larvivorous fish namely *Aplocheilus panchax* and *Trichogaster trichopterus* were tested against laboratory-reared *Aedes* larvae. Larva consuming rate of both fishes were measured using laboratory-reared 300 larvae for one fish, in different amount of water volumes (one, two and three liters). Before testing, fish's weight and length were measured by HP 300 digital balance and ruler.[11]

Intervention study

Before intervention, larval and pupal indices were recorded from each randomly selected 51 house holds in both villages. During inter-vention, two *Aplocheilus panchax* each were put into every water storage containers (major, minor and miscellaneous containers) from randomly selected 51 households of Mingalar Ywar Thit Village as test village for 3 months. Water storage container from randomly selected 51 household of Taung Nar Village as control village were not introduced any larvivorus fish. After intervention, larval and pupal indices were recorded.

Data analysis

Data entry, processing, larva consuming rate, larval and pupal indices and percent reduction

were measured using Microsoft Excel software. Larval and pupal indices were analyzed by following methods.

Aedes indices

Household Index (HI) = Percentage of house positive for *Ae. aegypti* larvae
Container Index (CI) = Percentage of water holding container infested with *Ae. aegypti* larvae
Breteau Index (BI) = Number of container with *Ae. aegypti* breeding per 100 house inspected
Key container = Those containers with >500 larvae
Key premises = Households with 3 and above positive water container for *Ae. aegypti* larvae
Pupae/house = No. of pupae per house
Pupae/container = No. of pupae per container
Pupae/person = No. of pupae per person
Pupae/child = No. of pupae per child under 12 years.

Ethical consideration

Ethical clearance was obtained from DMR Ethical Review Committee (7816/2016 July).

RESULTS

In comparison of one gram weight of both fishes, *Aplocheilus panchax* consumed more larvae than *Trichogaster trichopterus*i. ie, 463.04 and 124.24 3rd and 4th instar *Aedes* larvae in 3 liters of water volume within 24 hours. It was 3.727 fold higher in consuming rate than one gram of *Trichogaster trichopterus* against *Aedes aegypti* larvae in 3 liters of water volume in the laboratory (Fig.1).

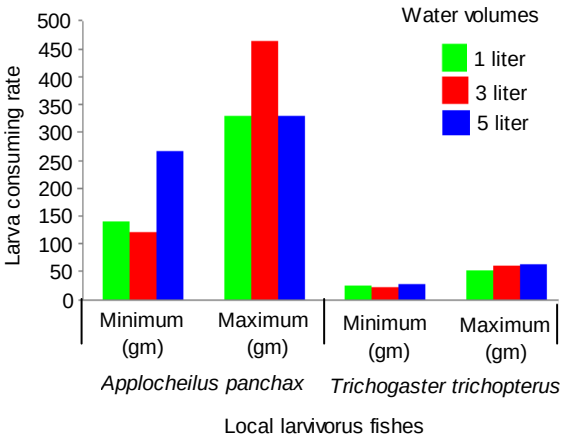

Fig.1. Comparison of minimum and maximum larva consuming rate of one gram of *Aplocheilus panchax* and *Trichogaster trichopterus* within 24 hours in 3 different water volumes.

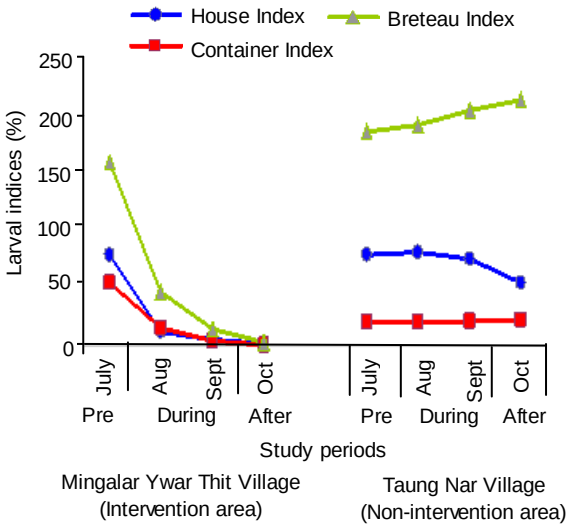

Fig. 2. Larval indices of intervention and non-intervention areas

Fig. 2 shows that House Index Container Index and Breteau Index were significantly reduced from 86.27%, 59.86% and 172.55 to 1.96%, 1.36% and 3.92, respectively ($p<0.05$) in intervenetion area. In non-intervention village, larval indices were gradually increased during the study periods.

Table 1. Pupal indices of before and after releasing of *Aplocheilus panchax* in test and control villages areas

Pupal indices	Mingalar Ywar Thit Village (test area)		Taung Nar Village (control area)	
	Pre-Intervention (July)	After-Intervention (Oct)	Non-Intervention (July)	Non-Intervention (Oct)
Pupae/house	14.02	0.25	22.96	26.82
Pupae/person	3.59	0.07	8.36	9.77
Pupae/child	12.54	0.23	37.77	44.12
Pupae/container	4.86	0.90	2.50	2.93

X^2 =11.3895, p=0.00979, highly significant

Table 1 shows that pupae/house, pupae/person, pupae/child and pupae/container were also significantly reduced from 14.02, 3.59 12.54 and 4.86 to 0.25, 0.07, 0.23 and 0.90, respectively.

Table 2. Comparison of percentage reduction of key containers in test and control areas using *Aplocheilus panchax* before and after intervention

Areas	Pre-intervention	Post-intervention	Percentage reduction
	K C (n)	K C (n)	
Mingalar Ywar Thit Village (Test area)	7	0	100% reduction
Taung Nar Village (Control area)	6	13	53.85% increase

X^2=9.5789, p=0.00196, highly significant, KC=Key container

Table 2 shows that after intervention in Mingalar Ywar Thit village, 100% reduction was found in key containers (500 & above larvae positive/container), although 53.85% of key containers were increased in non-intervention area of Taung Nar Village.

Table 3. Percentage reduction of key premises in intervention and non-intervention areas of Hpa-an Township, Kayin State

Areas	Before intervention	After intervention	Percentage reduction
	K P (n)	K P (n)	
Mingalar Ywar Thit Village (Test area)	10	1	90% reduction
Taung Nar Village (Control area)	22	28	27.27% increase

X^2=7.9553, p=00479, KP=Key premises

Table 3 shows that after intervention 90% reduction was found in key premises (3 & above larval positive container/house) in test area of Mingalar Ywar Thit Village, although 27.27% of key premises were found increased in control area of Taung Nar Village (highly significant).

DISCUSSION

Small fish such as *Claris fuscus*, *Tilapia nilotica*, and *Macropodus sp* have been used in many regions to eliminate larvae in domestic water containers with considerable success. The use of catfish appears to be particularly effective.[12] These larvivorous fishes have been widely used as biological control agents of mosquito larvae, but they have their own limitations, for instance, such fish are expensive to rear and do not survive for long in small places (like containers, etc.). For several decades, different species of fish have been used in biological control of mosquito larvae, especially in natural breeding sites.[13]

Fish bred in artificial containers, such as large domestic tanks [14] and rain water vessels, have already been used as an alternative to biological control in various parts of the world, including Nicaragua and Mexico.[15] *Aplocheilus panchax* is a potential native larvivorus fish used widely as biological control of mosquitoes larvae.[16] There are many biological control methods available. However, suitable cost-effective and long-term indigenous control method is required. In control measure, some larvivorous fish species can be used as biological control agents.[17] The dragonfly nymphs *Bradinopyga germinate rambur* were also found to be highly larvivorous.[18]

Among two larvivorous fishes namely *Aplocheilus panchax* and *Trichogaster trichopterus*, *Aplocheilus panchax* species is native to southern Asia from Pakistan to Indonesia including Myanmar. It was discovered in two hot springs in Singapore. *Aplocheilus panchax* and *Trichogaster trichopterus* are found abundantly in ponds and creeks in many parts of Myanmar and also abundantly found in rivers, creeks and rice fields in Hpa-an Township. Both species were tested against third and fourth instars *Ae. aegypti* larvae in different water volumes in the laboratory at 27ºC, 70-80% Rh and pH 6.5-7.

One gram weight of *Aplocheilus panchax* consumed highest 463.04 larvae within 24 hours, and it was 3.727 fold higher consuming rate than one gram of *Trichogaster trichopterus* against *Ae. aegypti* larvae in the laboratory. *Aplocheilus panchax* consumed over 100 *Anopheles* larvae within 24 hours.[19] One study mentioned that the number of larvae consumed varied from 188 larvae/day/g by *Trichogaster trichopterus* to 523 larvae/day/g by male *Poecilia sphenops* and female *Betta splendens.*[20] Jayasree and Panicker [21] reported that *Trichogaster trichopterus* is capable of consuming up to 47 *Culex quinquefasciatus* larvae/day/gm. This study observed that *Trichogaster trichopterus* consumed 124 larvae/ day/g of *Aedes* larvae; this may be the nature of two different species of larvae. Studies by Gene *et al*.,[22] showed that *Astyanax bimaculatus* has a very high consumption capacity, managing to consume between 342 and 1000 larvae in 24 hours (at an average of 655 larvae). The present study revealed that one gram weight of *Aplocheilus panchax* consumed very high number of 3rd and 4th instar *Aedes* larvae than *Trichogaster trichopterus*.

Aplocheilus panchax was found to be highly useful as an effective biological control of well-breeding *An. dirus* larvae to control malaria in Mon State.[23] In the present study *Aplocheilus panchax* has been used to control container-bred *Aedes* larvae in water storage containers of Mingalar Ywar Thit Village. In this study, after intervention House Index (HI), Container Index (CI) and Breteau Index (BI) were significantly reduced from 86.27%, 59.86% and 172.55 to 1.96%, 1.36% and 3.92, respectively ($p<0.05$) in

Mingalar Ywar Thit Village. Hundred percent (100%) reduction was found in key containers (500 & above larvae positive/container) and 90% reduction was found in key premises (3 & above larval positive container/house) in test area. The result agreed with a study using dragonfly nymph in Thakayta Township, Yangon Region which revealed that larval indices of HI, CI, BI, key container and key premises were significantly reduced in post-intervention period in test area but in control area larval indices were gradually rising in post-intervention months.[18]

Field study demonstrated that *Ae. aegypti* immature can be removed rapidly and efficiently from water storage containers (100% reduction) in key containers and 90% reduction in key premises after using *Aplocheilus panchax*. The results are the same with those in the study of Thakayta[18] and Shwepyi tha Townships.[24] Pupae/house, pupae/person and pupae/child were also significantly reduced from 14.02, 3.59 and 12.54 to 0.25, 0.07 and 0.23, respectively. Pupal indices were also significantly reduced in post-intervention months. Distribution of *Ae. aegypti* pupae/container is strongly clumped to DHF endemicity and *Aedes* adult density.[18] Pe Than Htun *et al.,*[19] revealed that *Aplochilus panchax* consumed over hundred *Anopheles* larvae within 24 hours in laboratory condition. Due to the high larvivorous potential, *Gambusia affinis* was introduced from Southern USA to various countries in the world.[25] Gambusia is an effective predator of mosquitoes and had positive impact on controlling mosquitoes in many countries for mosquito control and other purposes.[26]

Most *Colisa fasciata* are habited in large amount of water in lakes and rivers and larva consume rate is high as it can consuming over 300 larvae within one hour.[19] Ritchie and Laidlaw-Bell [27] found that ovipositing *Aedes taeniorhynchus* strongly avoided sites with high densities of *Gambusia holbrooki* and shifted to adjoining habitats with few or no predatory fish. A vector control programme in northern Viet Nam using copepods in large water-storage tanks, combined with source reduction, successfully eliminated *Ae. aegypti* in many communes and has prevented dengue transmission for a number of years.[3] *Aplocheilus panchax, A. melastigma, A. lineatus, A. mbassisnama, A. ranga, Badis badis, Barbus sp*. and *Anbas testudineus* are the most important larvicidal fishes, occurring in fresh waters of Bengal, Bihar and Orissa.[28] As *Aplocheilus panchax* is a typical surface feeder and, thus, it mainly prefers to take food from surface and subsurface environment.[3] Larval predatory rate of *Aplocheilus panchax* was found to be highest against *Aedes* larvae but it was not significantly different in terms of consuming rate between other *Anopheles* and *Culex* mosquito's larvae in laboratory.[29] Based on the knowledge, effective control strategies should emphasize on larval source reduction which will be an important achievement for long-term vector control. Native larvivorous fish *Aplocheilus panchax* is a very effective and suitable to control *Aedes* larvae in water storage container in Hpa-an Township. Effective mosquito control strategies in temporary water storage containers in community are urgently needed to reduce dengue fever and dengue hemorrhagic fever through mosquito larvae reduction without harmful to environment, ecosystems and community. *Aplocheilus panchax* acts as potential larvivirous effect of *Aedes* larvae and an effective biological control agent, eco-friendly and cost-effective to be considered as potent natural larvicidal agent.

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Competing interests

The authors declare that they have no competing interests.

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