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Biological Control of Malaria Vector (*Anopheles sinensis* Wied.) by Combined Use of Larvivorous Fish (*Aplocheilus latipes*) and Herbivorous Hybrid (*Tilapia mossambicus niloticus*) in Rice Paddies of Korea*

천적포식어 (*Aplocheilus latipes*)와 식식성 어류 (*Tilapia mossambicus niloticus*) 의 병합적 처리에 의한 논에 서식하는 말라리아 매개모기 (*Anopheles sinensis* Wied.) 의 생물학적 방제

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ABSTRACT A combined field release of indigenous larvivorous fish (*Aplocheilus latipes*) and hybrid herbivore (*Tilapia mossambicus niloticus*) was conducted to determine the effectiveness of biological control, in particular, against malaria vector (*anopheles sinensis*), breeding in weedy habitat of rice fields at the University rice paddies at Suwon during the period of June through September in 1988. A combined fish introduction at the release rate of 2.0 fish per M^2 for *Aplocheilus* and 1-pair per $10M^2$ water surface for *Tilapia* resulted in 70.8 % *Anopheles* larval reducton in a week period, the rates of control increased to 73.5% and 80.2% in 4th and 5th week respectively after the fish introduction, maintaining mosquito suppression in range of 80~82% control after the 5th week against *Anopheles* and *Culex* spp. combined. In a single fish treatment with *Aplocheilus* at 2.0 fish/M^2 release rate, *Anopheles* larval reduction ranged on the average 51.4~56.5% in 4 week period which was later integrated with *B.t.i.* (R153.78) treatment at 1.0 kg/ha dosage rate to suppress vector mosquito population down and the the results was compared with that of combined fish introduction.

KEY WORDS *Anopheles sinensis*, *Aplocheilus latipes*, *Bacillus thringiensis israelensis* (*B.t.i.*), biological control, *Tilapia mossambicus niloticus*

抄 錄 1988년 6월부터 9월까지 4개월간 수초자생 논에서 서식하는 말라리아 매개모기(*Anopheles sinensis*)를 대상으로 포식천적 송사리(*Aplocheilus latipes*)와 식식성 어류(*Tilapia mossambicus niloticus*)의 병합적 처리에 의한 생물학적 방제효과를 경기도 수원소재 대학 부속농장 실험포장에서 야외 조사하였다. 포식 천적어 송사리는 논 수면에 2.0 fish/M^2 방사율로, 식식성 어류 *Tilapia*는 1-pair/$10M^2$ 비율로 병합적 처리한 결과, 처리 일주일후에 말라리아 매개모기는 대조군에 비해 70.8%의 방제효과를 나타내었고 4주와 5주후에 방제율은 점차 증가하여 각각 73.5%와 80.2%에 달하였으며, 5주후에는 말라리아 매개모기와 집모기(*Culex pipiens*)를 합하여 80~82%의 모기개체군 밀도의 억제효과를 계속 유지하였다. 송사리만의 2.0 fish/M^2 비율로 단일처리한 논에서는, 4주후 말라리아 매개모기의 방제효과는 평균 51.4~56.5%로 비교적 저조하게 나타났으며, 모기 유충의 개체군 밀도를 억제하기 위한 미생물제제 *B.t.i.*(R153.78)을 1 kg/ha의 농도로 병합처리하여 그 방제효과를 비교 고찰하였다.

檢 索 語 송사리, 틸라피아(*Tilapia mossambicus niloticus*), 말라리아 매개모기(*Anopheles sinensis*), 미생물제제(*B.t.i*), 병합적 방제

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Recent research interest has been directed to the release experiment of the fish in the rice paddy condition, particularly aiming at control effectiveness against malaria vector, *Anopheles sinensis* Wiedmann which bred in higher density throughout summer in situation where aquatic algae and weeds growing rice fields.

During the past years, a series of rice paddy trials of native fish predators, *Aplocheilus latipes* and *Aphyocypris chinensis*, had been conducted, indicated that a single fish treatment rarely yield a continuous successful mosquito larval suppression presumably due to other environmental factor, particularly aquatic algae and weeds (Yu 1986).

An increase of temperature above 25°C near the end of July used to influence on the speedy growth and spread of aquatic weeds which led gradual covering of algal mat over the most of the rice paddy water throughout the months of late-August to September; the algal phenomenon probably hampered significantly the predation activities of introduced fish predators (Shim & Self 1971, Yu 1981 WHO Working Paper).

The successful use of herbivorous fish, *Tilapia* in biological control of mosquitoes comes about through their feeding activities on emergent vegetation and algal mats, thereby eliminating protective niches for mosquitoes to be predated by other larvivorous fishes(Legner & Medeved 1972).

Thus, this report discusses the synergistic effect of herbivorous fish, *Tilapia mossambicus niloticus* in getting rid of aquatic weeds for native fish predator, *Aplocheilus latipes* to prey on malaria vecter mosquito larvae in comparison with the results of single fish treatment and the control paddies.

MATERIALS AND METHODS

A confined field plot of ca. 1,000M^2 University rice paddy located outskirt of Suwon City in College of Agricultural Experiment Station, Kyonggi Province was used for the combined fish release where *Anopheles sinensis* Wied. the malaria and also inland filariasis vector was predominantly breeding with steady growth of aquatic weeds(Fig. 1-A & B).

The rice paddy was composed of 6 similar sized(10×15×0.3 m) plots divided by fiberglass slats and were controlled by filling with irrigation water to the depth of ca. 10 cm, with a constant water flow adjusted at the rate of 800~1,000 liters per day per plot and usually the rice transplantation was accomplished two months before the fish introduction.

Of six paddies available, 2 random selection of paddies was made for each group of (i) combined fish release at the rates of 2.0 fish (*Aplocheilus latipes*)/M^2 and 1-pair *Tilapia mossambicus niloticus*/10M^2, (ii) single species of *Aplocheilus* introduction at the same rate above, and (iii) control without fish introduction. The rice paddy of a single fish introduction was later treated with *B.t.i.* (R153.78) at 1.0 kg/ha dosage rate after the comparison for 4 consecutive weeks with that of combined fish treatment in mosquito larval reduction.

An indigenous fish stock (*Aplocheilus*) was originated from holding ponds at Ansan rice paddies ca. 2.5 km northwest to the University, while the herbivorous hybrid (*Tilapia mossambicus niloticus*) were from fishfarm located in Jin-Dong of Masan City, South Kyungsang Province, and both stocks were reared in the laboratory aquaria of 90 × 30 ×

30 cm with proper aeration and control of temperature until the releases (Fig. 1-C&D).

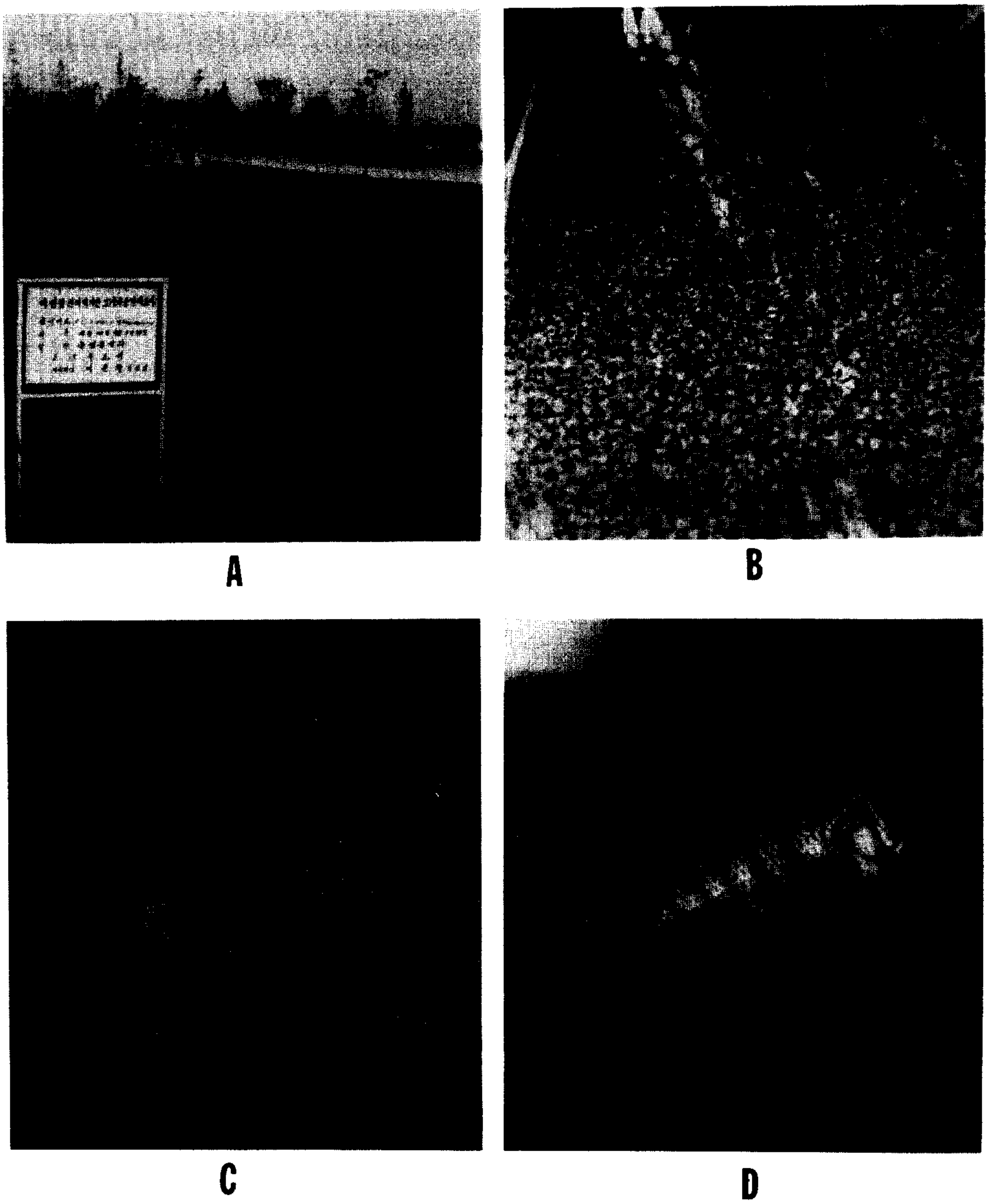

Fig. 1. University Experiment Station in Suwon, Kyonggi Province (A) with aguatic weeds growing on water surface (B). A combined release of larvivorous fish, *Aplocheilus latipes* (C), and herbivorous *Tilapia mossambicus niloticus* (D) was made against malaria vector mosquito, *Anopheles sinensis* Wied., June-October, 1988.

Mosquito larval density before and after the fish introduction was estimated by taking random samplings of mosquitoes by means of 500 ml dipper with 2～4 replicates per rice paddy usually consisted of two dips were pooled. In the similar manner, aquatic samples were also taken by the use of aguatic nylon net of 25 cm diameter with 120 cm wooden handle attached in such that the net-sweepings of 8～10 times per sample from the submerged portion of the rice water. Indentification was made according to the character described in each of the specific key of the corresponding taxa (Usinger 1971, Tsuda 1962, Lacasse & Yamaguti 1955, Chyung 1977).

The statistical analysis was performed to obtain mean number of mosquito larvae per sample in the treatment, and then the mosquito reduction rate in percent was calculated in compliance with the following formula:

$$\% \text{ Reduction} = \frac{(A \times B'/A' - B)}{A \times B'/A'} \times 100$$

Whereas, A = No. mosquito larvae sampled from Control of Post-Treatment.
B = No. mosquito larvae sampled from Treatm. of Post-treatment.
A′ = No. mosquito larvae sampled from Control of Pre-Treatment.
B′ = No. mosquito larvae sampled from Treatm. of Pre-Treatment.

RESULTS AND DISCUSSION

Combined fish release rice paddy

In the first treatment group of the rice paddies where the malaria vector population of *Anopheles sinensis* started to increase in mid-August from an average number of 1.5 larvae per sample to 8.0 according to our pre-treatment sample data, and a combined fish release of *Aplocheilus latipes* and *Tilapia mossambicus niloticus* at 1-pair/M^2 and 1-pair/10M^2 respectively *pipiens* 70.8% mosquito larval reduction against *Anopheles* and 100% against *Cx.* pipiens in a week period (Table 1). Subsequently, the reduction rates increase to 73.5%, 80.2% and 81.6% in 4th, 5th and 6th week respectively in comparison with that of control: while encephalitis vector, *Cx. tritaeniorhynchus* were completely suppressed from the 5th week and thereafter (Table 1 and Fig. 2). When a total mosquito population including *Anopheles* and *Culex* species combined, an overall biological control yielded over 80% after the 5th week by combined fish introduction, maintaining vector population under the minimum average of 2～5 larvae per sample compared with 11.3～27.0 larvae in control (Table 1 and Fig. 2).

Single fish release rice paddy

In the second treatment group of rice paddies where a single fish introduction of *Aplocheilus* was made, vector mosquito larvae (*An. sinensis*) were reduced by 51.4% in a week period, and subsequently, the reduction rates increased to 55.0% and 56.5% in 2nd and 3rd week respectively maintaining the mosquito suppression in range of 50.8～56.5 % in further 4th week, marking only substantial control (Table 2 and Fig. 2). The foregoing result showing rather lower biological control by the introduced larvivorous fish was most probable due to the presence of heavy aguatic weeds (*Lemna. Spirodela* and *Azolla* spp.) covering over the rice water surface which might affect adversely on the penetration and predation of mosquito larvae as was reported in simulated weed-cover habitat, in particular, against malaria vector, *An. sinensis* at NIH, Korea (Yu 1986). An augmentary treatment with an available

Table 1. Average number of mespuito larvae and % reduction before and after the combined release of larvivorous fish *Aplocheilus latipes* at 1-pair/M² water surface together with hervivorous *Tilapia mossambicus* x *niloticus* at 1-pair fish/10M² in University rice paddies in Suwon, Kyunggi Province, June-September, 1988

Sample Date (Time)	*Anopheles sinensis*		*Culex pipiens*		*Culex tritaeniorhynchus*		Total No. Mosquitoes	
	Con.	Treat.	Con.	Treat	Con.	Treat.	Con.	Treat.
PRE-TREATMENT								
Jun. 14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jun. 21	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0
Jun. 28	6.5	4.0	0.0	0.0	0.0	0.0	6.5	4.0
Jul. 6	9.0	10.0	0.0	0.0	0.0	0.0	9.0	10.0
Jul. 12	1.5	1.5	0.0	0.0	0.0	0.0	1.5	1.5
Jul. 21	3.0	2.0	0.0	0.0	0.0	0.0	3.0	2.0
Jul. 26	2.0	1.5	0.0	0.0	0.0	0.0	2.0	1.5
Aug. 2	1.5	1.0	0.0	0.0	0.0	0.0	1.5	1.0
Aug. 10	4.5	5.0	0.0	0.0	0.0	0.0	4.5	5.0
Aug. 12	8.0	11.0	8.5	7.5	0.0	0.0	16.5	18.5
Total	36.5	36.0	8.5	7.5	0.0	0.0	45.0	43.5
Average	3.65	3.60	0.85	0.75	0.00	0.00	4.50	4.35
POST-TREATMENT								
Aug. 19	19.0	5.5	3.5	0.0	0.0	0.0	22.5	5.5
% Reduction*	70.8%			100.0				74.8
Aug. 25	14.5	6.0	0.0	0.0	0.0	0.0	14.5	6.0
% Reduction*	58.2%							57.4%
Sep. 1	10.0	3.0	0.0	0.0	0.0	0.0	10.0	3.0
% Reduction*	69.7%							69.1%
Sep. 8	21.0	5.0	0.0	0.0	1.0	1.0	22.0	6.5
% Reduction*	73.5%					0.0%		69.5%
Sep. 15	25.5	5.0	0.0	0.0	1.5	0.0	27.0	5.0
% Reduction*	80.2%					100.0		80.9%
Sep. 22	11.0	2.0	0.0	0.0	0.25	0.0	11.25	2.0
% Reduction*	81.6					100.0%		82.0
Sep. 29	11.0	2.5	0.0	0.0	2.0	0.0	13.0	2.5
% Reduction*	77.0%					100.0%		80.2%
Total	112.0	29.5	3.5	0.0	4.75	1.0	120.25	30.5
Average	16.0	4.2	0.5	0.0	0.69	0.14	17.18	4.35
% Reduction*	73.5%		100.0%		79.7%			74.4%

* % Reduction=[(A × B'/A'−B)/A × B'/A'] × 100

Whereas, A =No. Larvae survived in Control of Post-Treatment.

B =No. Larvae survivedi n Treatment of Post-Treatment.

A'=No. Larvae survived in Control of Pre-Treatment.

B'=No. Larvae survived in Treatment of Pre-Treatment.

B.t.(H-14) R153.78 formulation at the dosage rate of 1.0 kg/ha one month after the fish introduction made a complete control of *Anopheles* larvae (100%) in 24 hours, the effect of which sustained for a week period (91.5%), however, the control rates decreased in 2nd and 3rd week to 60.5% respectively, demonstrating vector population rebound (Table 2 and Fig. 2) near the end of September, 1988 as the similar phenomenon reported (Mulla et al. 1980, Yu et al. 1982).

The results of combined field release experiment with larvivore, *Aplocheilus* and the herbivore, *Tilapia* indicates that both fishes

Table 2. Average number of mosquito larvae and % reduction before and after the fish introduction of *Aplocheilus latipes* at 1-pair/M^2 water surface and later treated with *Bacillus thuringiensis* (H-14) at 1.0 kg/ha dosage rate in University rice paddies in Suwon, Kyunggi Province, June-September, 1988

Sample Date (Time)	*Anopheles sinensis*		*Culex pipiens*		*Culex tritaeniorhynchus*		Total No. Mosquitoes	
	Con.	Treat.	Con.	Treat.	Con.	Treat.	Con.	Treat.
PRE-TREATMENT								
Jun. 14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jun. 21	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0
Jun. 28	6.5	5.5	0.0	0.0	0.0	0.0	6.5	5.5
Jul. 6	9.0	8.5	0.0	0.0	0.0	0.0	9.0	8.5
Jul. 12	1.5	1.5	0.0	0.0	0.0	0.0	1.5	1.5
Jul. 21	3.0	0.5	0.0	0.0	0.0	0.0	3.0	0.5
Jul. 26	2.0	1.5	0.0	0.0	0.0	0.0	2.0	1.5
Aug. 2	1.5	1.5	0.0	0.0	0.0	0.0	1.5	1.5
Aug. 10	4.5	4.0	0.0	0.0	0.0	0.0	4.5	4.0
Aug. 12	8.0	10.5	8.5	7.5	0.0	0.0	16.5	18.0
Total	36.5	33.5	8.5	7.5	0.0	0.0	45.0	41.0
Average	3.65	3.35	0.85	0.75	0.00	0.00	4.50	4.10
POST-TREATMENT (Fish Introduction)								
Aug. 19	19.0	8.5	3.5	1.0	0.0	0.0	22.5	9.5
% Reduction*		51.4%		67.5%				53.6%
Aug. 25	14.5	6.0	0.0	0.0	0.0	0.0	14.5	6.0
% Reduction*		55.0%						54.5%
Sep. 1	10.0	4.0	0.0	0.0	0.0	0.0	10.0	4.0
% Reduction*		56.5%						56.0%
Sep. 8	21.0	9.5	0.0	0.0	1.0	0.0	22.0	9.5
% Reduction*		50.8%				100.0%		52.5%
B.t.(H-14) TREATMENT (R153.78, 1.0 kg/ha)								
Sep. 9	16.5	0.0	0.0	0.0	0.0	0.0	16.5	0.0
% Reduction*		100.0%						100.0%
Sep. 15	25.5	2.0	0.0	0.0	1.5	0.0	27.0	2.0
% Reduction*		91.5%				100.0%		91.9%
Sep. 22	11.0	4.0	0.0	0.0	0.25	0.0	11.25	4.0
% Reduction*		60.5%				100.0%		60.9%
Sep. 29	11.0	4.5	0.0	0.0	2.0	0.5	13.0	5.0
% Reduction*		55.5%				75.0%		57.5%
Total	128.5	38.5	3.5	1.0	4.75	0.5	136.75	40.0
Average	16.06	4.81	0.44	0.13	0.59	0.06	17.09	5.0
% Reduction*		67.5%		67.5%		89.8%		67.8%

* % Reduction=[(A × B′/A′− B)/A × B′/A′] × 100
Whereas, A =No. Larvae survived in control of Post-Treatment.
B =No. Larvae survived in Treatment of Post-Treatment.
A′=No. Larvae survived in control of Pre-Treatment.
B′=No. Larvae survived in Treatment of Pre-Treatment.

interacted an effective synergistic role in consuming aquatic weeds by *Tilapia* for the larval predation by the larvivorous native fish in sustained larval suppression above 80% after the 5th week period against *Anopheles* and *Culex* species, whereas a single

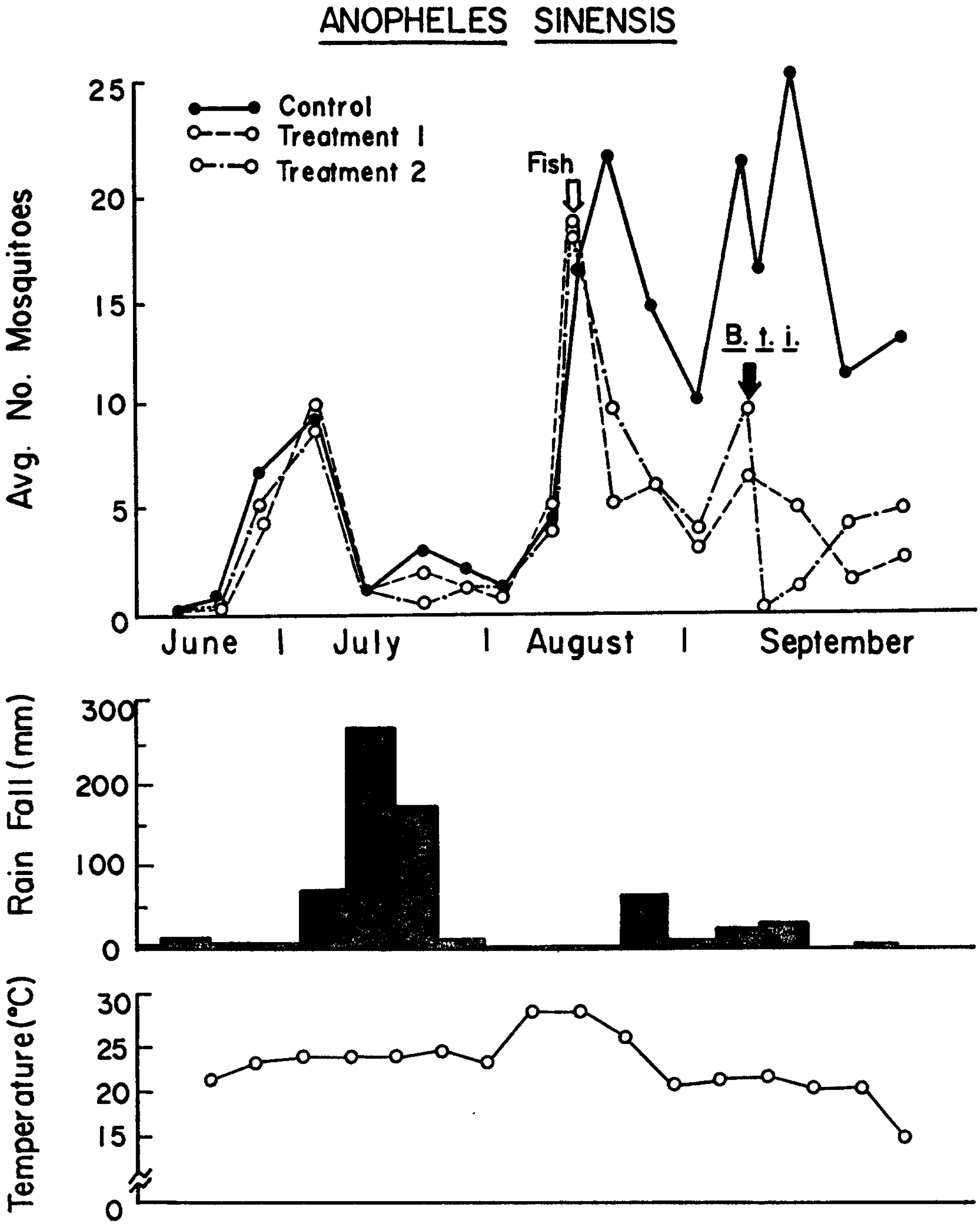

Fig. 2. Populationcurve of ***Anopheles sinensis*** Wiedemann, the vector of malaria and inland filariasis before and after the (i) treatment 1, combined fish release of ***Aplocheilus latipes*** and ***Tilapia mossambicus niloticus***, (ii) treatment 2, a single fish (***Aplocheilus***) release and later integrated with ***B.t.*** (H-14) in weedy rice paddy of University Experiment Station, Suwon, Kyonggi Prov., Korea. June through September, 1988.

fish(*Aplocheilus*) introduction produced only substantial biological control in range of 50 ~56% which required an augmentary and/or integrated with *B.t.* (H-14) to suppress mosquito vector population down, particularly in heavy weed cover habitat of rice paddies in South Korea.

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