STUDIES ON POTENTIAL BIOLOGICAL CONTROL AGENTS OF IMMATURE MOSQUITOES IN SEWAGE WASTEWATER IN SOUTHERN CALIFORNIA[1]

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ABSTRACT. Three biological control agents, a copepod, *Mesocyclops leuckarti pilosa*, and two fish, *Cyprinodon macularius* and *Poecilia reticulata*, were evaluated for their survival in secondary sewage effluent (SSE) and predation potential on mosquito larvae. Results showed that the survival of *M. l. pilosa* was not significantly affected in SSE or SSE diluted (50%) with water. In predation tests, the copepod consumed from 50 to 90% of the 1st-instar larvae of *Culex quinquefasciatus* in 24 to 72 hr and *P. reticulata* fed on almost all stages (egg to pupa) of the test mosquitoes. Survivorship of *P. reticulata* and *C. macularius* in SSE was not significantly affected by SSE under both greenhouse and sewage aquaculture conditions. *Poecilia reticulata* was distributed towards the influent end and *C. macularius* towards the effluent end of the aquaculture ponds, indicating the former species can tolerate higher levels of pollution which exists at the influent end of the pond. However, low water temperature and dissolved oxygen may be detrimental to these fish species in sewage aquacultural systems.

INTRODUCTION

Aquaculture of certain macrophytes provide economical and natural means for the purification of sewage effluents by removing dissolved nutrients, minerals, and by elaborating oxygen into eutrophic sewage effluents (Boyd 1974, Little 1979, Wolverton and McDonald 1979). The use of aquaphytes in sewage wastewater reclamation schemes, however, can result in the production of pest and vector mosquitoes (Dinges 1978, Townzen and Wilson 1983). Mosquito control on a long-term basis in sewage aquacultures may be better achieved through the employment of biological control agents such as fish and invertebrate predators rather than frequent chemical treatments.

To date the most commonly used biological control agent against mosquitoes is the mosquito fish, *Gambusia affinis* Baird and Girard (Hoy and Reed 1971, Hoy et al. 1971, 1972; Legner and Medved 1972, 1974; Meisch 1985). The effectiveness of this fish, however, decreases in sewage aquaculture systems with dense growth of aquaphytes (Townzen and Wilson 1983). In vegetated mosquito breeding milieus, this fish cannot effectively penetrate deep into microhabitats where mosquito larvae prevail. Moreover, *G. affinis* is sensitive to ammonia and certain other pollutants in sewage aquaculture systems (Townzen and Wilson 1983). A close relative of this viviparous fish is the guppy, *Poecilia reticulata* Peters, which can withstand a considerable degree of water pollution and might offer some promise in controlling mosquitoes in polluted waters (Johnson and Soong 1963, Mallers and Fowler 1970, Nakagawa and Ikeda 1968, Sasa et al. 1964, 1965). Likewise, among the oviparous fishes that could be useful in mosquito control is the desert pupfish, *Cyprinodon macularius* Baird and Girard. This fish is an indigenous species found in salty hot springs in the lower deserts of California and Arizona. It is also documented to be an efficient predator of mosquito larvae (Legner and Medved 1974, Legner et al. 1975. Walters 1976,[3] Walters and Legner 1980). Since it is an endangered species, its use in mosquito control in sewage aquaculture systems might enhance its numbers and distribution in California.

Among the invertebrate enemies of mosquito larvae, certain copepods may provide good control of mosquito larvae in sewage aquacultures. One cyclopoid copepod, *Mesocyclops leuckarti pilosa* Keiffer, a good predator of young mosquito larvae (Bonnet and Mukaida 1957), was found effective against *Aedes aegypti* (Linn.) and *Ae. polynesiensis* Marks in ovitraps in Tahiti (Riviere and Thirel 1981) and against container breeding *Aedes albopictus* (Skuse) in Hawaii (Marten 1984).

This paper presents data on the effectiveness of the copepod *M. leuckarti pilosa* and two fish species, *C. macularius* and *P. reticulata*, as mosquito larval predators and their survival and growth in secondary sewage effluent in the

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[3] Walters, L. L. 1976. Comparative effects of the desert pupfish, *Cyprinodon macularius* Baird and Girard, and the mosquito fish *Gambusia affinis affinis* (Baird and Girard) on pond ecosystems; and mass rearing feasibility of *C. macularius*. M. S. Thesis, Univ. of Calif. Riverside, Calif. 249 pp.

laboratory and sewage aquaculture system under field conditions.

MATERIALS AND METHODS

COPEPOD. A colony of the copepod, *P. leukarti pilosa,* originally obtained from Dr. Gerald Martin of the University of Hawaii, was maintained in the laboratory by providing the protozoan, *Paramecium aurelia* Ehrenberg, and the alga *Scenedesmus bijugatus* as food. Laboratory studies were carried out on the survivorship of this copepod in secondary sewage effluent (SSE) and SSE diluted with deionized water (50%). The SSE was obtained from the middle of equalization ponds of the Wastewater Quality Control Plant, Riverside, CA. Two separate tests, each consisting of 4 replications with 5 (2 to 3 males and females) per replication, were conducted at different times for over 12 weeks. The containers used in these tests were 300-ml Pyrex® glass bowls with 200 ml of SSE or deionized water. The copepods were provided with food consisting of *S. bijugatus* (2 ml applied initially) and *P. aurelia* culture (2 ml given twice a week) during the test period.

The predation potential of this copepod on young mosquito larvae was studied in quadruplicate tests under laboratory conditions. Each test consisted of 2 treatments, copepods plus mosquito larvae with larval and copepod food, copepods plus mosquito larvae with larval food, and a blank check of mosquito larvae with larval food only. Each treatment contained 5 unsexed adult copepods and a known density of 1st-instar larvae of *Culex quinquefasciatus* Say in 200 ml water in a glass bowl. The prey density levels in the test consisted of 20, 25, 30 and 50 mosquito larvae/bowl. The consumption of larvae by the copepods was studied at 24-, 48- and 72-hour intervals.

FISH SPECIES. Stocks of both *C. macularius* and *P. reticulata* were maintained in greenhouse aquaria as well as in outdoor experimental ponds at the Aquatic and Vector Control Research Facility, University of California, Riverside. The former species is known to be predaceous on mosquito larvae, the latter species, however, needed further experimental evidence of its predation on immature mosquitoes. Therefore, prior to studies on the survival of these fish in SSE, laboratory tests were conducted to demonstrate the feeding of *P. reticulata* on various developmental stages of *Cx. quinquefasciatus.* In these tests, individual fish—adult male or female, or 4–6 wk old young—were placed in 200 ml of water in 300 ml Pyrex glass bowls. The mean size and weight of female guppies were 30.7 ± 2.6 mm and 325 ± 72 mg; those of males were 21.5 ± 0.6 mm and 205 ± 44 mg and of young fish were 15.4 ± 0.9 mm and 184 ± 19 mg, respectively. Known numbers of immature mosquitoes were added to the bowls with a pipette. Observations were made on the feeding of guppies on various developmental stages of mosquitoes at different time intervals (hr).

The survival of both fish species was studied under greenhouse (27 ± 1°C) and field conditions. In the greenhouse tests, known numbers of each species were placed in 3.7 liter glass jars with deionized water or SSE and each provided with aeration. The aeration was intended to avoid scum formation and low oxygen stress on the activity of fish in each treatment and control jar. The fish in each jar were provided with food (a pinch of Tetramin®) daily. In the case of *C. macularius,* the number of fish used was 5 adults or 5 young fish aged 4 to 6 wk. Each test was replicated 4 times and observations on fish survival were made after 48 hr to 5 wk. In tests on *P. reticulata,* 4 adults were placed per jar and each treatment or control was replicated 3 times. The tests on guppies lasted for 4 weeks.

After successful survival of these fish in greenhouse tests, both species were next tested under actual field conditions in the San Diego Aquaculture Ponds, Wastewater Resources Recovery and Control, San Diego, CA. Each of the two test ponds measured 125.5 × 8.5 × 1.1 m deep and contained SSE. The vegetation cover in each pond consisted of dense growth of water hyacinth (*Eichhornia crassipes*), duck weed (*Lemna minor*), water velvet (*Azolla caroliniana*), and some filamentous algae. Known numbers (mixed age and sex) of *P. reticulata* (245 on June 12, 1985 and 500 on June 19) were stocked in one pond (IV) and *C. macularius* (80 on June 12 and 240 on June 19) in the second pond (V). At each date of fish stocking, 48-hr bioassay tests were conducted to determine the survival of these fish in sewage aquaculture ponds. In the first bioassay test, 6 young *C. macularius* or 10 *P. reticulata* adults were placed in Type Gee minnow traps and 2 such cages per each fish were placed one on each side in the middle of the pond. In the second test conducted 1 wk later, the number of fish per trap remained at 10 for both fish species.

After the initial introduction of fish into each pond, field observations were continued for 10 wk by taking weekly sampling for mosquito abundance and by carrying out biweekly trapping to monitor fish populations. Sampling of the immature stages of mosquitoes was done with a modified dipper, called a plunger, as described by Townzen and Wilson (1983). The plunger, shaped like a four-sided steel pyramid (10 × 10 × 30 cm) was open at the base and mounted point down on a 2.5 m long tubular steel handle. Sampling for mosquitoes was carried out at 10 predetermined sites in each pond. Starting lengthwise from the influent end in each pond, there were 5 sampling sites about 31.3 m apart on both north and south sides. At each site 5 plunges were taken over a distance of about 3 m and composited into one sample in a 2-liter plastic container with a nylon cloth strainer at the top. each composite sample was next transferred to a flat 60 × 45 × 10 cm plastic pan and observations on immature mosquitoes were noted before the pan contents were added back to the pond. Most of the sampling was carried out between 1100 and 1400 hr.

Fish populations were sampled with Type Gee minnow traps at 6 sites in each pond at biweekly intervals. The traps remained in water for 4 hr, generally between 1000 and 1600 hr. Data on water temperature and dissolved oxygen (DO) as collected by San Diego Aquaculture Project personnel, were also compiled. The two study ponds were also routinely treated by the Aquaculture Project personnel with *Bacillus thuringiensis* var. *israelensis* or larvicidal oils (Golden Bear [G.B.] 1111 or 1356) whenever the number of 3rd- and 4th-instar mosquito larvae reached 5 or higher. This was necessary to avoid any complaints by the residents in the vicinity.

All data were statistically analyzed and means were compared by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

COPEPOD. Data generated on the survival of *M. leuckarti pilosa* in SSE indicated that survival of the copepod was not significantly affected either in SSE or SSE diluted with water as compared with the control (Table 1). This was an encouraging sign for the predation capabilities of this copepod, especially if it was to be tested as a mosquito control agent in SSE.

Tests on the predation of *M. leuckarti pilosa* on 1st-instar larvae of *Cx. quinquefasciatus* showed that the copepod consumed from 50 to 90% of mosquito larvae at 24- to 72 hr intervals (Table 2). Neither larval density nor the presence of copepod food markedly affected the level of consumption of mosquito larvae by the copepod. Based on the findings that the copepod showed good predation on mosquito larvae coupled with good survivorship in SSE, *M. leuckarti pilosa* appears to be a good candidate as a biological control agent to be tested against mosquito larvae in SSE under actual field conditions.

FISH SPECIES. Studies on the predation of guppies on immature mosquitoes showed that female guppies fed on almost all developmental stages from eggs to pupae (Table 3). The consumption of immature mosquito stages ranged from 47 to 100% in 10 min to 4 hr. Eighty-three percent of the egg rafts were consumed during 4 hr. Male and young (4–6 wk) guppies fed exclusively on the larvae with feeding preference for younger instars. Moreover, young guppies feeding on 1st-instar larvae consumed over 80% during the first 10 min and over 90% during 30 min of feeding. Feeding by young fish was the highest (40%) during the first 2 min, thereafter gradually falling to as low as 2 larvae/min at the 10th min or so.

Table 1. Survival of *Mesocyclops leuckarti pilosa* adults in secondary sewage effluent (SSE).

Interval (wk)	Mean[a] percent survival in: SSE	SEE ± deionized water (50:50)	Deionized water
0	100 a	100 a	100 a
2	75 a	85 a	78 a
4	65 a	78 a	73 a
6	60 a	70 a	63 a
8	38 a	55 b	40 a
10	33 a	33 a	25 a
12	18 a	13 b	10 b

[a] Mean of 8 replications with 5 adults/cup. Each cup received copepod food consisting of *Scenedesmus bijugatus* (2 ml initially) and *Paramecium aurelia* (2 ml) twice a week during the test period. Means followed by same letter in a row are not significantly different from each other (DMRT, P = 0.05).

Table 2. Consumption of *Culex quinquefasciatus* larvae (1st instar) by the predator *Mesocyclops leuckarti pilosa*.

Larval density[b]	Mean[a] percent kill of larvae at interval (hr): 24			48			72		
	A	B	C	A	B	C	A	B	C
20	44	50	0	59	69	0	—	—	—
25	58	74	0	76	82	0	88	90	0
30	65	58	0	77	65	0	78	70	1
50	51	56	0	72	66	0	80	74	2

[a] Mean of 4 replications with 5 adult copepods/cup.
[b] No./200 ml water in a glass bowl.
A—both mosquito and copepod foods; B—mosquito food only; and C—blank check with mosquito larvae and food and no copepods.

Table 3. Feeding of *Poecilia reticulata* on developmental stages of *Culex quinquefasciatus*.[a]

Stage and no. added		Mean cumulative percent consumption at intervals (min)						
		Adult female[b]			Adult male[c]			Young[d]
		10	120	240	10	120	240	25
Egg rafts	2	33	83	83 a	0	0	0	0
I	20	47	100	100 a	30	68	86 a	96 a
II	10	57	97	97 a	18	50	85 a	100 a
III	5	80	100	100 a	35[e]	73[f]	—	92 a
IV	5	53	93	100 a	0	25	50 a	4 b
Pupae	5	60	67	100 a	0	0	0	0

[a] Mean of 3 for female, 4 for male, and 5 for young ones. I to IV refer to 1st to 4th-instar larvae. Means followed by same letter in a column are not significantly different from each other (DMRT, P = 0.05).
[b] Mean size 30.7 ± 2.6 mm and weight 325 ± 72 mg.
[c] Mean size 21.5 ± 0.6 mm and weight 205 ± 44 mg.
[d] Mean size 15.4 ± 0.9 mm and weight 184 ± 19 mg.
[e] At 5 minutes.
[f] At 60 minutes.

In developing *P. reticulata* and *C. macularius* as control agents against mosquitoes in sewage aquaculture systems, data on the survival potential of both species were generated in greenhouse and field investigations. In the greenhouse, the survival of adult *P. reticulata* was 100% during the first week in both SSE and control (deionized water). The survivorship of fish in SSE was 83 to 75% compared with 67% in the control after wk 2 to wk 4 (Table 4). In tests with *C. macularius,* the mean survival of adult fish after 1 wk was 70% in SSE as against 90% in the control. However, the survivorship of young fish during the first week was 100% in SSE as compared with 95% in the control (Table 4). Fish survival at the end of a 5-wk long period, was 90% in both SSE and control.

In field bioassays, the survival of young *C. macularius* in sewage aquaculture was also slightly higher (100%) than that (92%) of adult fish after 48 hr. In both field bioassay tests, *P. reticulata* showed 100% survival in sewage aquaculture for 48 hr.

Table 4. Survival of two larvivorous fish in secondary sewage effluents (SSE).

Interval (wk)	Mean percent survival			
	P. reticulata[a]		*C. macularius*[b]	
	SSE	Control	SSE	Control
0	100 a	100 a	100 a	100 a
1	100 a	100 a	100 a	95 a
2	83 ab	67 c	95 a	95 a
3	83 ab	67 c	95 a	90 a
4	75 c	67 c	90 a	90 a
5	—	—	90 a	90 a

[a] Mean of 3 replications with 4 fish per replication. Means followed by same letter(s) in a column are not significantly different from each other (DMRT, P = 0.05).
[b] Mean of 4 replications using 5 young fish per replication.

During the 10-wk field sampling, mosquito fauna breeding in sewage aquaculture ponds consisted of 0.2% *Anopheles franciscanus* McCracken, 0.4% *An. freeborni* Aitken, 0.1% *Culex erythrothorax* Dyar, 19.0% *Cx. peus* Speiser, 63.0% *Cx. quinquefasciatus* Say, 16.0% *Cx. tarsalis* Coquillett and 1.3% *Cx. thriambus* Dyar. Control of mosquito larvae by the two fish in sewage aquaculture ponds could not be studied here because these ponds were frequently treated with the microbial larvicide *B. thuringiensis* var. *israelensis* or larvicidal oil GB-1111 or 1356 whenever the number of 3rd- and 4th-instar larvae reached 5 larvae per dip. This was necessary to keep mosquito populations down in order to avoid any complaints by nearby residents.

This study, however, yielded valuable information on the survival and reproduction of both fish species in sewage aquaculture under field conditions. In pond IV the population of *P. reticulata* rose from zero to as high as 12 fish/trap in about 10 wk after its initial introduction into the pond (Fig. 1). Based on fish capture per trap location this fish was found more active and abundant towards the influent end of the pond where the mosquito population was higher (Table 5). Unlike *P. reticulata* in Pond IV, *C. macularius* was distributed more towards the effluent end of Pond V (Table 5). The *C. macularius* population trapped was smaller than that of the former species, rising from zero to 3 fish/trap in about 10 wk after its initial release into the pond (Fig. 2). Further observations revealed that the population of this fish reached its highest point of 6

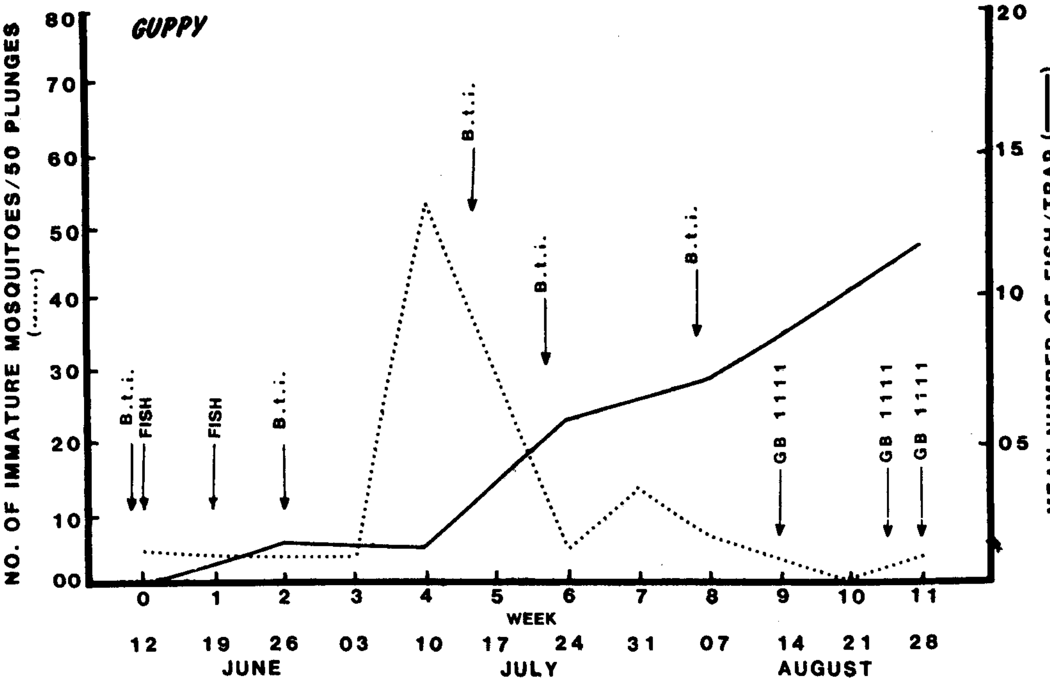

Fig. 1. Pond IV. Population of mosquitoes and guppy, *Poecilia reticulata* in sewage aquaculture, San Diego, CA, during June–August 1985.

fish/trap by October 8 after which it fell to as low as ~ 1 fish/trap by December 1985. It was not ascertained whether this apparent drop in fish population was due to inactivity or death of the fish as result of lower temperatures. However, dead fish were not found near the water surface.

From the data in figures 1 and 2 it appears that larvicidal treatments with *B. thuringiensis* var. *israelensis* or larvicidal oils (GB-1111 or GB-1356) had no adverse effect on the population growth of the two fish species in sewage agriculture.

Table 5. Distribution of mosquito and fish populations in different regions of sewage aquaculture ponds, San Diego, CA, during June–August, 1985.

Pond	Pond region	Population index of mosquitoes[a]	Fish[b] *Poecilia reticulata*	Fish[b] *Cyprinodon macularius*
IV	Influent	6.8	7.5	—[c]
	Middle	1.4	4.8	—
	Effluent	0	4.3	—
V	Influent	48.0	—	0.4
	Middle	1.1	—	0.7
	Effluent	7.1[d]	—	3.6

[a] Mean of 10 weekly samples each consisting of 5 plunges. Each sample consisted of larvae and pupae.
[b] Mean of 2 trap captures at 5 biweekly samplings.
[c] No fish were trapped.
[d] Samples consisted of 50–60% 1st- and 2nd-instar larvae.

The San Diego Aquaculture Project personnel recorded data on water temperatures and dissolved oxygen at 3 sites, each at 30 cm depth, in both ponds for 4 days per week during the entire 10-wk study period. The respective mean temperature and dissolved oxygen for Pond IV were 21.9° ± 0.7°C and 0.9 ± 0.6 ppm and for Pond V were 22.0° ± 0.7°C and 1.0 ± 0.6 ppm. Other water quality parameters measured on July 29, Aug. 3, Aug. 9 and Aug. 29, 1985 for both pond IV and V are summarized in Table 6. Apparently these water parameters at the recorded levels did not have any significant ill effects on the survival of both fish species. After termination of our field experiment by the end of August 1985, however, the dissolved oxygen levels at 30-cm depth in Pond IV decreased to 0.3 ppm or less during the last week of September, resulting in a significant reduction in *P. reticulata* population and/or activity to as low as 1 fish/6 traps by October 23. It was not clear whether the drop in population was due to fish inactivity or death or both as a result of lower DOs coupled with lower water temperatures. In an earlier lab study by Sjogren (1972), the KD_{50} (knock down) oxygen concentrations at 21.1°C for male guppies were 0.3—0.5 ppm and for

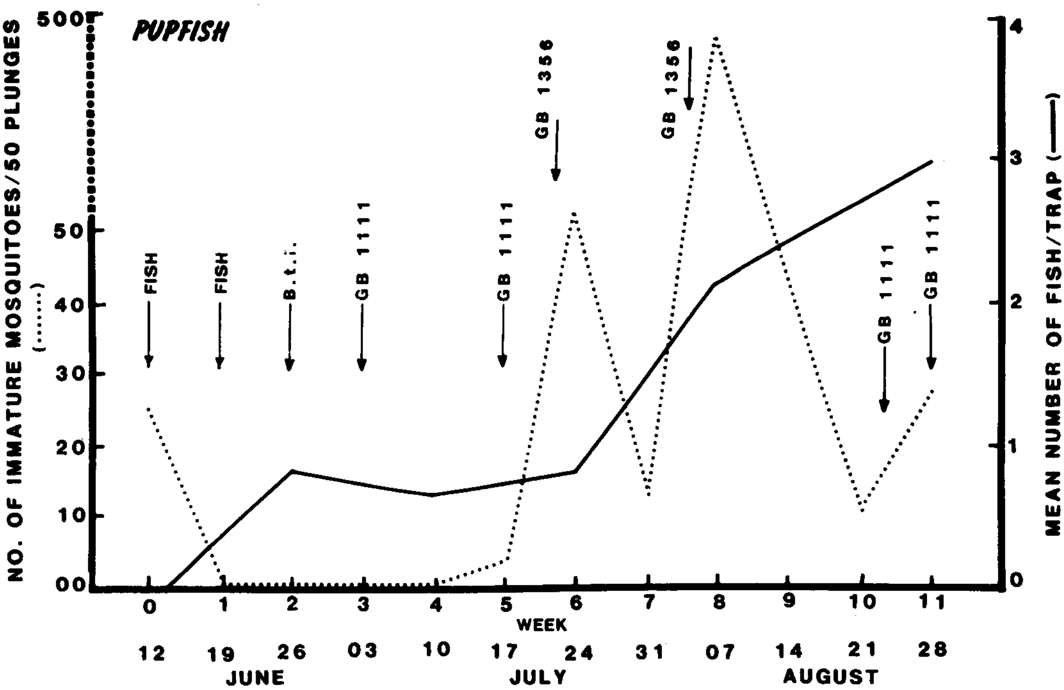

Fig. 2. Pond V. Population of mosquitoes and pupfish, *Cyprinodon macularius* in sewage aquaculture, San Diego, CA, during June–August, 1985.

females 0.20 + 0.30 ppm, indicating that male guppies were more sensitive to low dissolved oxygen concentrations than the female guppies. Apart from the sensitivity of this tropical fish to low dissolved oxygen in water, its population level in sewage aquaculture pond was also subsequently affected by decreasing water temperature, from 20°C in September to as low as 11.9°C in December 1985. The lowest population and/or activity of *C. macularius*, 1 fish/trap by December 1985 could have also been due to a combination of very low levels of both dissolved oxygen and temperature of the water in Pond V.

In conclusion, both fish, being potential predators of mosquito larvae, demonstrated good survival and population growth in sewage aquaculture during the major part (June–August) of the active mosquito breeding season. In winter months, however, the impact of low level of water dissolved oxygen and temperature may exert a tremendous pressure on the survival of these fish. Additional studies are needed to clearly understand the survival dynamics of these fish in sewage aquaculture with water dissolved oxygen concentration of 0.5 ppm or lower during the winter months and also to evaluate both species as effective biocontrol agents against mosquito larvae in diverse sewage aquaculture systems under field conditions. Selection of strains tolerant of low dissolved oxygen and high levels of pollutions should also be attempted.

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Table 6. Water quality parameters of aquaculture ponds, San Diego, CA, during July and August 1985.[a]

Parameter	Concentration[b] in secondary sewage water in			
	Pond IV		Pond V	
	Influent end	Effluent end	Influent end	Effluent end
BOD	45.0	0	42.0	0
TOC (x 0.1)	26.1	18.2	17.8	15.6
COD	117.0	56.0	98.0	44.0
SS	39.0	19.0	23.0	23.0
K^{-6}	906.0	868.0	939.0	894.0
pH	7.0	6.3	7.1	6.5
DO (x 0.01)	1.4	3.9	0.9	4.4
Temp. (°C)	20.4	24.8	25.8	22.9
NH_3-N	21.4	19.4	7.7	2.6
PO_4	11.4	11.1	7.9	6.0
SO_4	125.0	81.0	124.0	118.0

[a] Parameters stand as: BOD—biochemical oxygen demand, TOC—total organic carbon, COD—chemical oxygen demand, SS—suspended solids, K^{-6}—electrical conductivity (μ mhos/cm), pH—hydrogen ion activity, DO—dissolved oxygen, NH_3-N—ammonia nitrogen.

[b] Except for K^{-6}, pH and temperature, the measured concentrations of all other parameters are expressed in mg/L. With the exception of one sample analysis for NH_3-N, PO_4 and SO_4 on Aug. 29, 1985, all other figures are the means of 4 analyzed samples, each taken on a different date i.e., July 29, Aug. 3, Aug. 9 and Aug. 29, 1985.

[c] Influent end samples were taken 30 cm from the influent box discharging secondary sewage water into the pond.

[d] Effluent end samples were taken about 122 m away from the influent end.

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