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PREDATION EFFICACY OF THE FISH MUDDY LOACH, *MISGURNUS MIZOLEPIS*, AGAINST *AEDES* AND *CULEX* MOSQUITOES IN LABORATORY AND SMALL RICE PLOTS

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ABSTRACT. Assessments of the biological control potential of muddy loaches, *Misgurnus mizolepis*, were conducted against *Aedes togoi*, *Culex pipiens pallens*, and *Culex inatomii* larvae both in the laboratory and in small rice plots in Pusan, Korea, from June to September 1997. Medium-sized fish consumed almost all of 500 3rd-stage larvae of *Cx. pipiens pallens* offered daily in the laboratory. Predation by the fish at release rates of 1, 2, 4, 8, and 16 fish per plot resulted in 1,004, 1,197, 1,198, 1,200, and 1,200 larvae of *Ae. togoi* consumed in 12 h, respectively, when each 1,200 3rd-stage larvae were given in the 1-m^2 small rice plots. The average numbers of larvae eaten after introduction of the fish were 1,121.8 larvae in 24 h and 1,195.8 larvae in 36 h. Muddy loaches showed slightly higher predation rates on *Cx. pipiens pallens* and *Culex inatomii* larvae than on larvae of *Ae. togoi*. Therefore, *M. mizolepis* might be an efficient biological control agent against mosquito species in rice fields.

KEY WORDS Predation, mosquitoes, fish, *Misgurnus*

INTRODUCTION

Gerberich and Laird (1968) reported that 253 different fish species possess potential for biocontrol of mosquito larvae. Larvivorous fishes have been used as biological control agents for mosquitoes for nearly 100 years (Meisch 1986). There is renewed interest in the use of larvivorous fishes for mosquito control as an alternative or complement to conventional insecticides in Korea (Yu et al. 1981, 1982; Yu 1986; Kim et al. 1994). Some of the best mosquito predators investigated during recent years were the predatory fishes *Aphyocypris chinensis* Gunther, belonging to the family Cyprinidae (Weiser 1991), and *Aplocheilus latipes* (Temminck et Schlegel), belonging to the family Oryziidae. These fishes were evaluated in collaboration with the World Health Organization in laboratory, semifield, and confined field conditions during the period 1979–89 (Yu et al. 1982, 1983, 1985, 1986; Yu 1986; Yu and Kim 1989). However, the fish population in rice fields has decreased sharply since the advent of conventional cultivation, insecticide usage, and environmental contamination.

In natural habitats and rice fields in Korea, reduced larval populations of *Anopheles sinensis* Wiedemann and *Culex tritaeniorhynchus* Giles were associated with the presence of muddy loaches, *Misgurnus mizolepis* Gunther, belonging to the family Cobitidae (Lee et al. 1997, Lee 1998). *Misgurnus mizolepis* in organically farmed rice fields breeds in close association with mosquito populations, particularly along irrigation ditches and rice fields. Mosquito larval populations in the presence of *M. mizolepis* were investigated at Bulkyo in southwest Korea. Populations appeared suppressed throughout the mosquito breeding season when compared with control rice fields in the same area. The stocking and/or natural breeding of fish species was a common practice in organically farmed rice fields in Korea. This practice was an additional benefit to farmers because the fish are a popular food source.

This is a report of a study of the breeding of local fish in rice fields, particularly the widely distributed common species *M. mizolepis* and its relation to mosquito populations. As a first step, studies were initiated for the predation efficacy of muddy loaches against mosquito larvae in both a laboratory and small rice plots.

MATERIALS AND METHODS

Laboratory study: Muddy loaches, *M. mizolepis*, used in both laboratory and small rice plot experiments, were indoor and outdoor aquaria stocks originally collected from rice fields and irrigation ditches of Milyang, Kyungsangnam-do, South Korea. Collections began 2 wk prior to the beginning of each experimental series. Aquaria were aerated and supplied with unchlorinated underground water. Fish were fed daily with dog chow complete flake diet during acclimation. The laboratory experiment was performed to determine whether *M. mizolepis* preys on *Culex* mosquito eggs and larvae. The experiment used intermediate-sized fish (1-year old, body length avg. 10 cm, body weight avg. 5 g). A single healthy, unsexed fish was placed into each of 4 glass 14.3-liter aquaria, 30.2 cm in diameter. Underground water was maintained at a depth of 20 cm, with continuous aeration. The colonies of *Culex pipiens pallens* Coquillett larvae and eggs that were used in the experiments were from a laboratory stock maintained at Kosin University. Five hundred 3rd-stage larvae, or 20 egg rafts, of *Cx. pipiens pallens* from the mosquito colony were introduced with an adequate supply of dog chow powder for food. The mosquito larvae or egg rafts in each test aquarium were examined subsequently

Table 1. Cumulative average number of 500 3rd-stage larvae or 20 egg rafts of *Culex pipiens pallens* consumed by an intermediate-sized *Misgurnus mizolepis* in an aquarium after 24 and 48 h (average of 4 replicates).

Exposure period (h)	Cumulative number consumed (mean ± SD)[1]			
	Treatment		Control	
	Larvae	Egg raft	Larvae	Egg raft
24	499.5 ± 0.58[a]	20.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
48	500.0 ± 0.00[a]	—	0.0 ± 0.00	—

[1] Means in the same column followed by the same lowercase superscript are not significantly different at the 5% level of probability (Duncan's multiple range test).

to determine the number and condition at a temperature of 25 ± 2°C at 24 and 48 h in comparison with those of the control aquaria. Four treatment and control replicates were tested.

Small rice plot studies: The semifield experiment involved testing whether *M. mizolepis* would prey on larvae of *Aedes togoi* (Theobald), *Cx. pipiens pallens,* and *Culex inatomii* Kamimura and Wada under field conditions. A confined semifield assessment was conducted from June to September 1997 in small plots planted with rice constructed in an open field at Kosin University. The 6 rice plots were made from fiberglass containers (1.2 × 0.8 × 0.5 m) and were filled with silt soil from natural rice fields to a depth of ca. 15 cm and with underground water to a depth of 8 cm above the soil. Rice transplantation was accomplished 1 month before fish introduction in late June. Mosquito larvae of *Ae. togoi* and *Cx. inatomii* were collected from rock pools in Pusan and from a marsh in Woolsan in southeast Korea.

The small rice plots were selected randomly for *M. mizolepis* release at rates of 1, 2, 4, 8, and 16 fishes, with 1 left as a control. After 1 wk, all dragonfly nymphs were removed, and 1,200 3rd-stage larvae of *Ae. togoi* were introduced into the plots. Mosquito larvae present in the rice plots were counted and recorded 12, 24, 36, 48, and 60 h after introduction and compared with those of the control plot. This test was repeated 5 times.

The 2nd experiment focused on determining predation efficacy of *M. mizolepis* against *Cx. pipiens pallens* and *Cx. inatomii* larvae in semifield conditions. For this experiment, intermediate *M. mizolepis* (body length 10 cm) were released in each small rice plot. After 1 wk, 1,200 3rd-stage larvae of both *Cx. pipiens pallens* and *Cx. inatomii* were introduced into the rice plots. Mosquito larvae were counted, recorded, and compared with those of a control plot. The experiment was replicated 5 times. All data were analyzed with Duncan's multiple range test (Ott 1984).

RESULTS AND DISCUSSION

Five hundred *Cx. pipiens pallens* larvae, or 20 egg rafts, exposed to *M. mizolepis* in a glass aquarium in the laboratory experiment were readily preyed upon by muddy loaches. Intermediate-sized *M. mizolepis* consumed an average of 499.5 of the 500 larvae of 3rd-stage *Cx. pipiens pallens.* Predation had reached 100.0% after 48 h (Table 1). The muddy loaches consumed and spat out an average of 45 dead larvae. Muddy loaches remained on the bottom of the aquaria most of the time. The fish moved actively to the water surface in order to search for prey and exchange gases both day and night although this fish is known as a nocturnal species. According to Park et al. (1995), the *Misgurnus* species exchanges gases through its gills and intestines, taking air in from its mouth at the water surface and releasing it through its anus. *Misgurnus mizolepis* consumed all 20 *Cx. pipiens pallens* egg rafts by 24 h, whereas no mortality of larvae or egg rafts was observed in the control glass aquaria.

In Korea, preliminary screening evaluations of 5 species of fish breeding in rice fields had been previously performed (Yu et al. 1982, Yu and Lee 1985). Two species, *A. chinensis* and *A. latipes,* were found to have the most potential for mosquito control. In the laboratory, mature fish of *A. chinensis* and *A. latipes* consumed averages of 175 and 125 3rd-stage *Cx. pipiens pallens* larvae, respectively, after 24 h. Thus, among fish species bred in the rice fields of Korea, the best biocontrol potential for *Cx. pipiens pallens* appears to be by *M. mizolepis.* Moreover, *M. mizolepis* can survive during dry periods when the rice fields are drained. Kim et al. (1994) observed predation by a different species of *Misgurnus, M. anguillicaudatus* (Cantor), and reported that at a later stage in maturity, it consumed a higher number of *Cx. pipiens pallens* larvae than the same species of fish at an earlier stage of development. Two immature *M. anguillicaudatus* consumed 112.7 larvae of 3rd-stage *Cx. pipiens pallens* per day in a 10-liter aquarium, whereas 2 intermediate-sized fish consumed an average of 144 larvae. On the other hand, 2 mature *M. anguillicaudatus* consumed 168.4 larvae per day, which was 49.4% and 16.9% higher than the numbers consumed by immature and intermediate-sized fish, respectively (Kim et al. 1994). Thus, an intermediate-sized *M. mizolepis* appeared to consume at least 2.5- or 2.0-fold more *Cx. pipiens pallens* lar-

Table 2. Cumulative average number of 1,200 3rd-stage larvae of *Aedes togoi* consumed by *Misgurnus mizolepis* in small rice plots after 12, 24, 36, 48, and 60 h of exposure (22–29°C, 5 replicates).

Exposure period, (h)	Cumulative number consumed (mean ± SD)					
	No. *Misgurnus mizolepis* used					
	0 (control)[1]	1	2	4	8	16
12	0.0 ± 0.0	1,004.0 ± 12.0[b]*	1,197.0 ± 0.0[a]	1,198.0 ± 0.0[a]	1,200 ± 0.0[a]	1,200 ± 0.0[a]
24	2.0 ± 1.6	1,121.8 ± 6.4[b]	1,200.0 ± 0.0[a]	1,200.0 ± 0.0[a]	—	—
36	2.0 ± 1.6	1,195.8 ± 1.5	—	—	—	—
48	3.0 ± 1.7	1,197.0 ± 1.0	—	—	—	—
60	5.0 ± 2.2	1,200.0 ± 0.0	—	—	—	—

* Means in the same row followed by the same lowercase superscript are not significantly different at the 5% level of probability (Duncan's multiple range test).

[1] Natural mortality in control plot.

vae than did intermediate-sized and mature stages, respectively, of even 2 *M. anguillicaudatus.*

Under semifield conditions, *M. mizolepis,* at 5 different release rates of 1, 2, 4, 8, and 16 fish per plot, preyed on 1,004, 1,197, 1,198, 1,200, and 1,200 after 12 h, respectively, after 1,200 larvae of 3rd-stage *Ae. togoi* were introduced (Table 2). There was a significant difference ($P < 0.05$) between the predation rates of the fish at release rates of 1 and 2 fish per plot. All of the 1,200 larvae were consumed by 2 individuals of *M. mizolepis* by 24 h. With the muddy loach introduction, the average numbers of larvae consumed were 1,121.8, 1,195.8, 1,197.0, and 1,200.0 larvae, whereas natural mortalities of 2.0, 2.0, 3.0, and 5.0 larvae were observed at the control plot after 24, 36, 48, and 60 h, respectively. The predation efficacies of *M. mizolepis* against *Cx. pipiens pallens* and *Cx. inatomii* larvae are shown in Table 3. The muddy loach showed a slightly higher predation rate for the 2 species of *Culex* than for *Ae. togoi.* An intermediate-sized *Misgurnus* had consumed 975.8 larvae of *Cx. pipiens pallens* after 12 h, 1,192.6 after 24 h, and 1,192.8 after 48 h. Fish consumed 972.2, 1,192.4, and 1,195.8 larvae of *Cx. inatomii* after 12, 24, and 48 h, respectively. After 60 h, all the exposed larvae of both mosquito species in the small rice plots had been consumed by fish. The number of larvae of each mosquito species eaten by the fish after 24 h of exposure may be higher than that of other known mosquito fish in the world.

Misgurnus mizolepis enhanced its value as a mosquito fish by spending a longer duration in rice fields (Lee et al. 1997, Lee 1998). Castleberry and Cech (1990) compared the abilities of pupfish (*Cyprinodon nevadensis amargosae*), mosquitofish (*Gambusia affinis*), and guppies (*Poecilia reticulata*) to control mosquitoes in wastewater marshes. All species of fish reduced mosquito larval populations. Muddy loaches also have potential for mosquito control in wastewater sewages and brackish water because of their ability to survive in those environments as well as in rice fields in Korea. They can tolerate a wide range of temperatures and low dissolved oxygen levels because of their characteristics of overwintering in water or soil (Lee, unpublished data; Park et al. 1995). The attraction of the muddy loach as a protein source could be used as a positive factor to encourage its use in pisciculture. Muddy loaches have not become harmful in Korean rice ecosystems because of their polyphagous feeding habits. Nevertheless, their role in regulating mosquito populations in rice fields appears to be negligible, although this species of fish is widely distributed and abundant in rice fields in Korea, because populations of *M. mizolepis* were almost completely suppressed by an early application of insecticides against rice stem borers in mid-

Table 3. Cumulative average number of 1,200 3rd-stage larvae of *Culex pipiens pallens* and *Culex inatomii* consumed by 1 *Misgurnus mizolepis* in small rice plots after 12, 24, 36, 48, and 60 h of exposure (5 replicates).

Exposure period (h)	Cumulative number consumed (mean ± SD)			
	Cx. pipiens		*Cx. inatomii*	
	Control[1]	Treatment	Control	Treatment
12	0.0 ± 0.0[c]*	975.8 ± 18.8[b]	1.4 ± 1.3[b]	972.2 ± 22.4[b]
24	0.0 ± 0.0[c]	1,192.6 ± 5.8[a]	8.8 ± 1.1[a]	1,192.4 ± 7.3[a]
36	3.8 ± 2.9[b]	1,192.6 ± 5.8[a]	8.8 ± 1.1[a]	1,195.2 ± 4.6[a]
48	3.8 ± 2.9[b]	1,192.8 ± 5.4[a]	10.0 ± 2.5[a]	1,195.8 ± 4.1[a]
60	7.8 ± 3.6[a]	1,200.0 ± 0.0[a]	10.0 ± 2.5[a]	1,200.0 ± 0.0[a]

* Means in the same column followed by the same lowercase superscript are not significantly different at the 5% level of probability (Duncan's multiple range test).

[1] Natural mortality in control plot.

June and never recovered thereafter (Lee et al. 1997, Lee 1998). Therefore, it is necessary to carry out further studies on their regulation of mosquito populations under natural conditions.

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