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Ecological Studies on the Guppy, Lebistes reticulatus PETERS. *II. Experiments on Predation of Mosquito Larvae by guppies**

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Abstract

Predation of mosquito larvae, *Culex pipiens*, by the guppy, *Lebistes reticulatus* PETERS, which has been recently acclimatized in hot springs in Japan, was studied under various experimental conditions.

1. Guppies could not survive in water of 35° C and over, or at 10° C and less.

2. At 25°C guppies showed maximum predation of mosquito larvae. Adult guppies ate 21—91 mg of fourth instar larvae in 24 hours. These amounts correspond to 10—36 larvae in number when the larvae average 2.5 mg in weight per individual. At 30°C the amounts of mosquito larvae eaten by adult guppies decreased from that at 25°C, but the decrease was less than that at 20°C. At 15°C guppies ate few larvae and almost ceased growing. As a rule the quantity of larvae eaten by male guppies was less than that by femals.

3. Guppies could eat mosquito larvae in darkness as well as in light, though some decrease in amount of predation was observed.

4. Guppies ate mosquito larvae even when daphnids were available. There was slightly greater preference for daphnids than for mosquito larvae.

Introduction

Since the end of the nineteenth century various poeciliid fishes, such as the top minnow, *Gambusia affinis* have been introduced into many areas of the world to serve as an important predator of both anopheline and culicine mosquitoes. The guppy, *Lebistes reticulatus* PETERS of the same poeciliid family, however, has been used for mosquito control in only a few southern countries according to

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Gerbrich (1946), though the fish has been widely kept as an aquarium fish. Recently, SASA ET AL. (1965) found that *Lebistes reticulatus*, which was probably introduced from aquaria, was well adapted for life in shallow, polluted pools in Bangkok, and played a notable role in controlling larvae of the filarial mosquito, *Cnlex pipiens fatigans*. In temparate Japan, guppies which were undoubtedly introduced from aquaria have been acclimatized in the waters of some hot springs, such as Togura-Kamiyamada and Rendaiji (OCHIAI ET AL., 1962, YAMAGISHI ET AL., 1966). According to the observation of YAMAGISHI ET AL. (1966) they are not cold-resistant, but live only in water where the temperature is maintained at about 20°C during the winter season through the inflow of hot spring water. In summer time these guppies begin to disperse to the irrigation streams and rice fields, and increase so rapidly in number that they soon fill the summer habitats. In the rice fields they seemed to actively eat larvae of the encephalitis mosquito, *Culex tritaeniorhynchus*. They usually emerge in large number, but very few larvae were discovered in fields inhabited by guppies. These facts suggest that the guppy is a mosquito-eating fish just as are other poeciliid fishes.

The author attempted some experimental studies on the predation of mosquito larvae by guppies. These experiments consisted of i) predation on mosquito larvae at different water temperatures; ii) predation under different levels of illumination; iii) predation of mosquito larvae in the presence of other food organism.

Materials and methods*

The guppies used in the present experiments were laboratory reared offspring of the acclimatized fish in the Togura-Kamiyamada spa, Nagano Prefecture.** Each guppy was placed in a polystyrene glass (5.5 cm in diameter and 8.5 cm in height), the bottom of which was cut away and in its place "salan" net (0.7 mm in mesh) was attached. These glasses were suspended into a glass aquarium ($60 \times 30 \times 30$) cm^3. In addition, one large polystyrene vessel (19 cm in diameter and 14 cm in height) with net bottom was suspended into the aquarium in order to observe the effect of space on predetion. The side of the vessels was covered by a piece of white vinyl cloth so that each fish was completely isolated visually from others. Water in the aquarium was agitated by aeration from the bottom. The water temperature was controlled by eletric heaters and thermoregulator placed in the aquarium. The experimental apparatus was illuminated from the upper side by a fluorescent lamp of 20 W for 12 hours daily regulated timing device.

Laboratory reared larvae of *Culex pipiens* (usually 4th instar) were selected as nearly equal as possible in size and divided into daily portions for each fish. Each of the portion was weighed after absorbing moisture by fillter paper, and

*The present study was carried out at the Department of Parasitology, the Institute for Infectious Diseases, the University of Tokyo from July to September, 1965.

**Four characters of tail figure were observed in the male guppies of the Togura-Kamiyamada spa but in the laboratory guppies were selected so that only the "bottom sword" type of male appeared.

the average weight of individual larvae in each portion was calculated. Larvae were supplied for three days and larvae remaining (including dead ones) were counted every day. The product of the number eaten multiplied by the average weight of larvae was considered as the daily predation amount. The average predation amount per day, however, was calculated by using the data of only the second and third day of the experiment, since the fish were starved for a day before the experiment and on the first day they could consume a greater number of larvae than they would usally eat. At the beginning, and two days after the experiment each fish was weighed and measured under anaesthesia induced with 1 : 7000 MS 222 solution.

Results

1 *Predation of mosquito larvae by guppies at different water temperatures*

Experiments were attempted at 10°, 20°, 25°, 30°, and 35°C, but guppies could not survive at 35° and 10°C for more than six days, though they were warmed or cooled gradually for several days before the experiment*

In **Table 1** the numbers and weights of mosquito larvae eaten by guppies in 24 hours during three days intervals is presented. **Fig. 1** shows the relationships between average predation amounts and weights of fish. At 25°C the predation amounts were largest but differed little from those of 30°C. On the other hand, the difference from 20°C was considerable. In every case males ate fewer mos-

Table 1, a Predation of mosquito larvae by guppies at 15°C

Guppies			Mosquito larvae (4th instar)						
			Eaten number/Supplied number			Predation amount in mg			
Sex	No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day
F	1	102	2/20	2/20	1/10	4	4	2	3
F	2	182	4/30	2/30	2/15	9	4	4	4
F	3	195	6/25	2/25	1/15	12	5	2	4
F	4	198	4/25	2/25	0/15	8	4	0	2
F	5	297	5/25	1/25	0/15	10	2	0	1
F	6	425	11/30	2/30	0/20	21	5	0	3
F	7	703	4/40	8/40	2/30	8	21	4	13
M	1	102	0/20	0/20	0/10	0	0	0	0
M	2	118	2/20	2/20	1/15	4	4	2	3
M	3	121	1/25	0/25	0/15	2	0	0	0

*The experiments at 20°C and less were carried out in an aquarium ($36 \times 18 \times 25$) cm^3 which was placed in the cooling apparatus and illuminated by a 10 W florescent lamp for 12 hours daily. After use in the experiment at 20°C, the same fish were used in the following experiment at 15°C.

Table 1, b Predation of mosquito larvae at 20°C

Guppies			Mosquito larvae (4th instar)						
			Eaten number/Supplied number			Predation amount in mg			
Sex	No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day
F	1	97	13/25	5/20	4/20	27	22	9	16
F	2	173	22/40	6/30	14/30	40	27	29	28
F	3	192	16/40	2/25	8/25	35	9	16	13
F	4	195	13/40	5/25	11/25	61	22	26	24
F	5	290	14/40	5/25	5/25	30	22	5	17
F	6	403	23/40	3/30	8/30	53	14	16	15
F	7	700	30/50	6/40	10/40	63	27	23	25
M	1	97	5/30	2/20	4/20	11	9	8	9
M	2	114	9/30	3/20	3/20	18	14	6	10
M	3	122	5/40	1/25	1/25	11	5	2	4

Table 1, c Predation of mosquito larvae by guppies at 25 °C

Guppies			Mosquito larvae (4th instar)						
			Eaten number/Supplied number			Predation amount in mg			
Sex	No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day
F	1	70	37/50	17/25	21/25	44	41	50	46
F	2	90	34/50	16/40	18/40	61	38	43	41
F	3	91	31/50	18/25	23/35	60	43	55	49
F	4	156	9/50	16/25	28/35	40	38	67	53
F	5	170	32/50	34/40	26/65	64	82	62	72
F	6	175	10/50	13/25	18/25	46	31	43	37
F	7	179	44/50	25/40	22/40	96	60	53	57
F	8	209	11/50	13/25	16/25	51	31	38	35
F	9	219	33/50	19/25	32/35	116	46	77	61
F	10	229	16/50	16/40	49/50	70	38	118	78
F	11	235	18/50	24/40	33/40	83	58	79	68
F	12	374	24/70	22/50	40/50	106	53	96	74
F	13	474	28/70	24/50	28/50	125	58	67	62
F	14*	504	25/70	22/50	28/50	114	53	67	60
F	15	581	18/70	32/50	44/50	87	77	106	91
M	1	69	11/30	8/20	8/20	29	19	18	19
M	2	89	12/50	13/30	12/30	32	30	28	29
M	3	93	14/50	9/30	9/30	38	23	20	22

*This fish was placed in a large vessel.

Table 1, d Predation of mosquito larvae at 30°C

Guppies			Mosquito larvae (4th instar)						
			Eaten number/Supplied number			Predation amount in mg			
Sex	No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day
F	1	83	24/30	25/30	21/30	55	54	48	51
F	2	125	24/30	25/30	24/30	62	63	57	60
F	3	140	24/30	22/30	24/30	66	51	51	51
F	4	208	27/50	30/30	28/30	70	69	65	67
F	5*	223	24/40	21/30	28/30	59	48	61	54
F	6	246	27/40	20/40	15/40	50	44	35	39
F	7	314	29/50	33/40	20/40	72	72	45	59
F	8	509	35/50	38/50	22/50	100	82	59	71
F	9**	542	38/50	21/30	6/30	88	47	13	30
M	1	69	11/30	8/20	8/20	29	19	18	19
M	2	89	12/50	13/30	12/20	32	30	28	29
M	3	93	14/50	9/30	9/30	38	23	20	22

*This fish was placed in a large vessel.
**This fish spawned in the third day.

quito larvae than femals. At 15°C the guppies consumed so few larvae that they scarcely maintained their initial body weight. All dead larvae, whether entire or in pieces, could not be regarded as killed by fish, since some might die from other causes such as higher or lower temperatures, or injury suffered in handling. No significant difference was observed in predation between the fish in large and in small vessels.

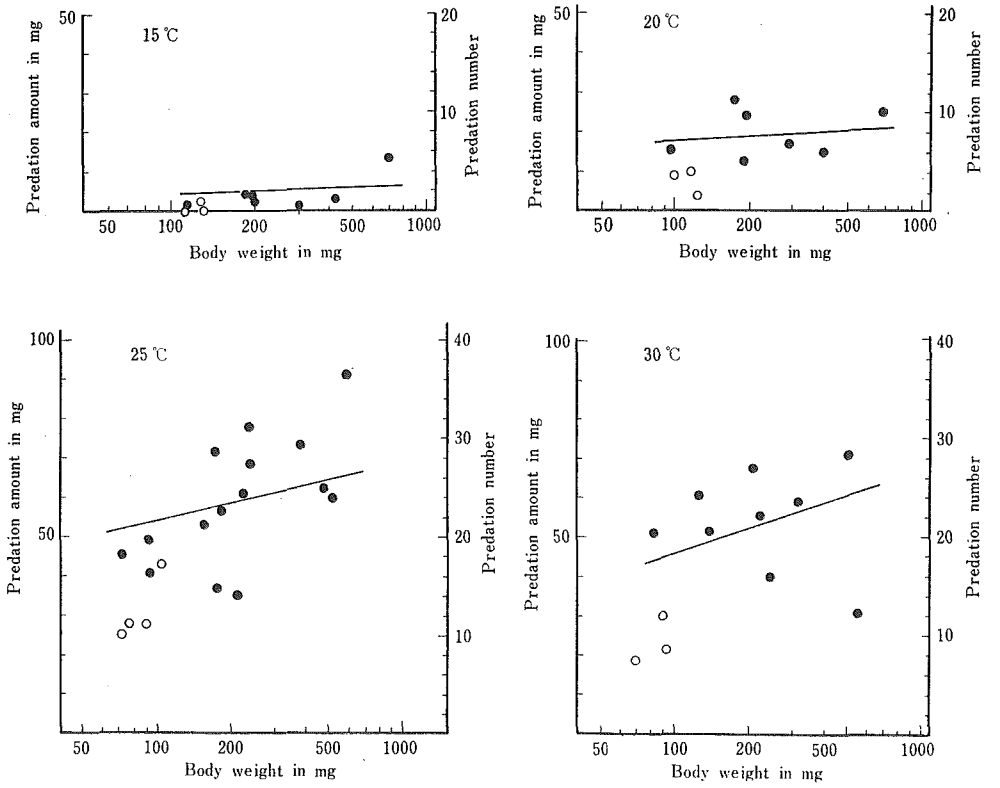
2 *Predation of mosquito larvae by guppies in aggregation*

The above experiments were made in complete isolation. However, in some fish species maintaining more or less contact among individuals during life, amounts of eaten food may be greater when in aggregation than when in isolation (WELTY, 1934). According to the author's observations, guppies live in their hot spring habitats more or less in aggregation, though slightly aggressive behaviour often occurs homosexually

In order to determine whether group effects on predation occurs, three guppies each of the same sex were placed in the vessels used in the former experiments, and kept at 25°C. In comparing predation amounts in aggregation (**Table 2**) with those in isolation at the same temperature (**Table 1, a**) no significant difference was observed in female fish but in males some increase in predation was observed in aggregation.

3 *Predation of mosquito larvae by guppies in darkness*

It is important to know whether guppies can prey upon mosquito larvae in darkness, since in recent years emergence of *Culex pipiens molestus* often occurs



○ male; ● female.

Fig. 1 Relationships between average predation amounts and body weights of guppies at different water temperatures. For females the equations, $Y=-0.257+1.892X$, $Y=10.903+3.656X$, $Y=24.708+14.770X$ and $Y=4.586+20.776X$ are suitable at 15°C, 20°C, 25°C and 30°C respectively. X was calculated as $\log_{10}x$. Predation numbers were shown as 2.5 mg per larva.

in underdrains, basement pools, or water tanks with lids.

The experiment in darkness was carried out in the same apparatus as the former experiments, excepted that light was excluded by covering them with a black vinyl bag. Water temperature was kept at 25°C. Anaesthetized guppies were introduced into the polystyrene vessels, into which weighed mosquito larvae previously were placed. The apparatus was covered before anaesthesia left the fish, and therefore feeding in light was prevented.

As shown in **Table 3** and in **Fig. 2**, predation was observed also in darkness, though the predation amounts decreased in comparison that in light, especially in the males and smaller females. It is possible that the experiment was not adeqeate to investigate actual feeding activity of guppies. The fish might feed not

Table 2 Predation of mosquito larvae by aggregated guppies at 25°C

Guppies			Mosquito larvae (4th instar)						
			Eaten number/Supplied number			Predation amount in mg			
Sex	No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day
F	1	101	98/100	60/120	49/120	130	169	137	153
	2	112							
	3	87							
	Average	100							Average amount per fish 51
F*	4	190	78/150	37/150	59/120	133	111	177	144
	5	176							
	6	180							
	Average	182							Average amount per fish 48
M*	1	78	43/100	34/100	35/100	75	98	92	95
	2	79							
	3	79							
	Average	79							Average amount per fish 32

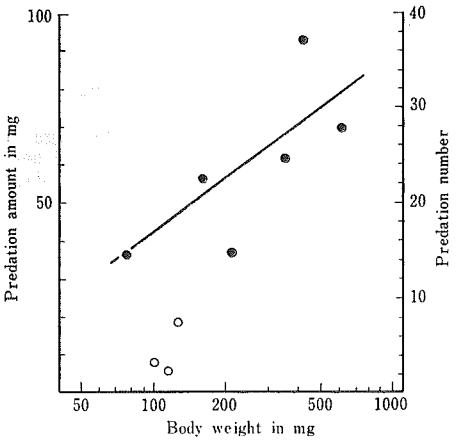
*These fish were placed in a large vessel.

Table 3 Predation of mosquito larvae by guppies in darkness at 25°C

Guppies			Mosquito larvae (4th instar)						
			Eaten number/Supplied number			Predation amount in mg			
Sex	No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day
F	1	77	12/25	13/25	18/20	30	34	38	36
F	2	162	23/40	23/40	24/40	62	52	60	56
F	3	210	13/30	12/30	16/30	37	33	39	36
F	4*	349	30/50	23/50	50/50	70	76	62	62
F	5**	412	42/50	34/50	50/50	113	76	109	93
F	6	605	31/50	29/50	31/50	79	68	69	69
M	1	99	8/40	2/40	4/20	22	5	10	8
M	2	114	8/40	1/40	4/20	18	2	10	6
M	3	125	5/40	13/40	4/20	14	28	9	19

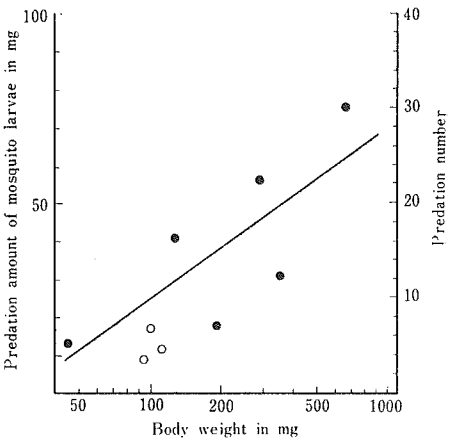
*This fish spawned in the third day.
**This fish was placed in a large vessel.

by active orientation to mosquito larvae, but by spontaneous contacts with larvae which may have been in greatly limited space in the small polystyrene glasses.



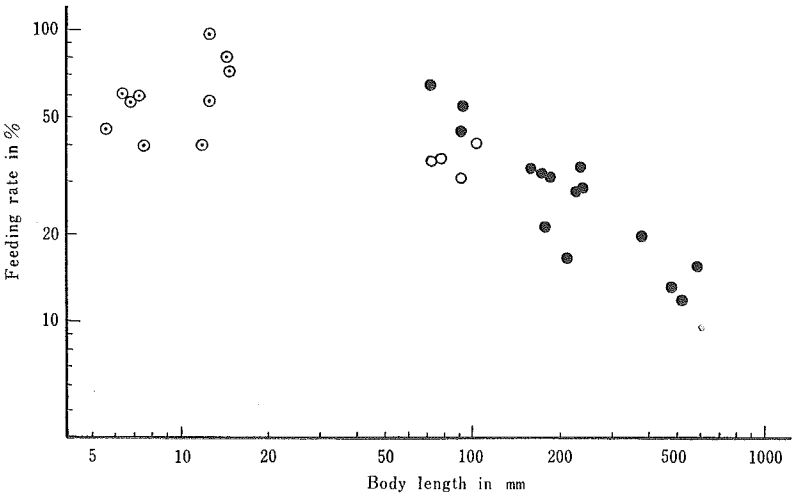
○ male; ● female.

Fig. 2 Relationship between average predation amounts in darkness at 25°C and body weights of guppies. For females the equation, $Y=-55,554+47.691X$ is suitable. X was calculated as $\log_{10}X$. Predation numbers were shown as 2.5 mg per larva.



○ male; ● female.

Fig. 3 Relationship between average predation amounts of mosquito larvae and body weights of guppies when fresh daphnids were given at the same time (at 25°C). For females the equation, $Y=-63.781+44.242X$ is suitable. X was calculated as $\log_{10}X$. Predation numbers were shown as 2.5 mg per larva.



○ male; ● female; ◉ fry.

Fig. 4 Relationship between feeding rates and body weights of guppies at 25°C. Guppy fry were fed with 2nd and 3rd instar larvae and adult fish were fed with 4th instar larvae respectively.

Another experiment was made in much larger space. Twenty mosquito larvae and an anaesthetized female guppy (317 mg in weight) were introduced directly

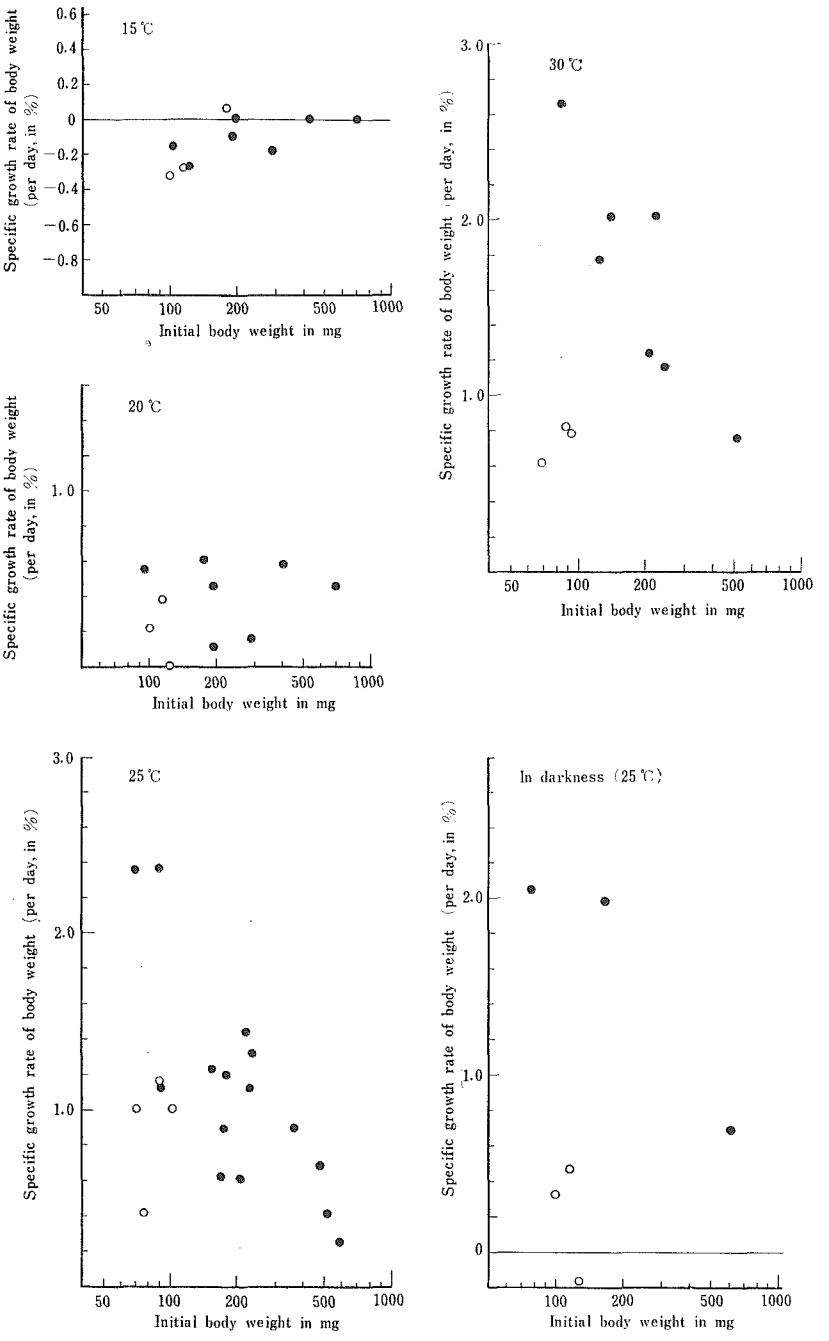
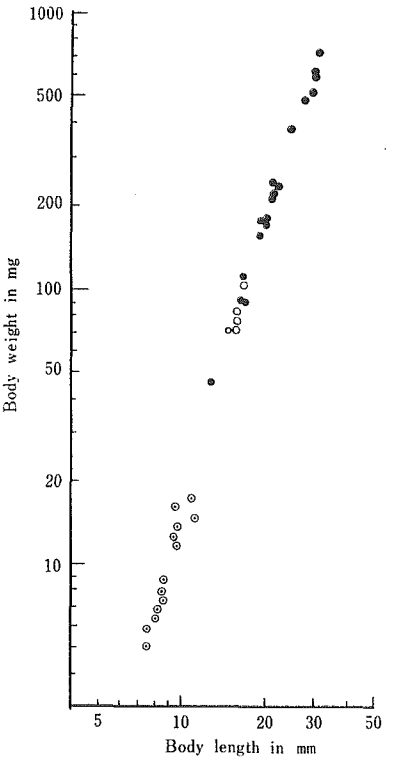


Fig. 5 Relationships between specific growth rates of body weight (per day, in %) and initial body weights of the guppies fed with mosquito larvae at different temperatures and in darkness.

into the water (25°C) in an aquarium (60×30×30) cm^3, and then the aquarinm covered by the vinyl bag. The female guppy ate 10 (32 mg), 13 (41 mg) and 17 (44 mg) larvae on the first, second and third days, respectively. Thus, predation in darkness was demonstrated again.

4 *Selection of mosquito larvae by guppies in preference to other food organisms present*

As mentioned in the author's previous paper (YAMAGISHI ET AL., 1966) the guppy should be regarded as an euryphagous fish. This capability is probably advantageous for feeding under conditions of limited kinds of food. But in mosquito control in bodies of water where mosquito larvae usually emerge along with zooplankton, such as in rice fields, it should be determined whether guppies prefer mosquito larvae or not. Therefore, mosquito predation by guppies was studied in company with a supply of fresh daphnids, *Daphnia carinata* in approximately equal weight as the mosquito larvae. The experiment was done at 25°C. Selectivity was determined by using Ivlev's index, $E=ri-pi/ri+pi$, where E is the index of selectivity; ri is the relative content of given component (i) in whole amount of eaten food; and pi is the relative content of i in the enviromental or supplied food. The value of E varies from -1 to $+1$. If fish ate only i, E would be equal to $+1$. on the contrary, if fish did not eat i at all, E would be equal to -1. As shown in **Table 4**, E values of nine fish during three days varied from -1.00 to $+0.20$, and were concentrated mostly in the range from 0 to -0.1 (average value of E was -0.19). These results show that the guppy slightly prefers daphnids over mosquito larvae, but the possibility of mosquito control with guppies still remains.



○ male; ● female; ◉ fry.

Fig. 6 Weight-length relationship of guppies. For adults the equation, $W=0.017\ L^{3.122}$, where W is body weight and L is body length is suitable.

5 *Predation of immature mosquito larvae by young guppies*

Larvae of viviparous fishes as a rule are born at a more developed stage than larvae of oviparous fishes. The guppy larva is entirely covered with scales, has an already motile mouth even on the day of the birth, and can swim and eat actively. So it may be supposed that the guppy is capable of taking a part in the controlling of immature mosquito larvae from early life.

An experiment was performed by using beakers with 400 cc water placed in

Table 4 Predation of mosquito larvae by guppies with fresh daphnids available

Guppies			Mosquito larvae (4th instar)							Daphnids			E (Index of selectivity)		
			Eaten number/supplied number			Predation amount in mg				Eaten amount/Supplied amount in mg					
Sex	No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day	First day	Second day	Third day	First day	Second day	Third day
F	1	45	6/10	8/10	4/10	12	17	9	13	30/40	20/22	22/30	−0.08	−0.20	−0.03
F	2	128	15/20	18/20	16/30	28	39	43	41	37/60	31/31	31/40	+0.03	−0.07*	−0.02
F	3	192	11/30	6/30	8/30	27	14	21	18	60/100	39/40	40/60	−0.15	−0.18	−0.45
F	4	249	20/30	30/30	18/30	46	14	48	56	65/85	30/30	30/45	−0.04	−0.02*	0
F	5**	353	0/30	21/30	8/20	0	41	20	31	92/100	49/55	55/92	−1.00	−0.23	-0.05
F	6***	682	30/30	30/30	35/40	65	64	85	75	85/85	76/76	76/100	0*	−0.04*	0
M	1	97	4/20	5/20	3/20	9	11	7	9	40/60	19/22	22/40	−0.40	−0.38	−0.38
M	2	110	9/20	7/20	8/20	22	14	20	17	35/60	29/31	31/35	−0.08	+0.20	−0.26
M	3	112	7/20	4/20	5/20	17	10	13	12	20/60	18/21	21/30	−0.01	−0.25	−0.33

*These values of E were excluded from the calculation of average of E because all mosquito larvae or daphnids supplied were consumed.

**This fish was placed in a large vessel.

***This fish spawned in the second day.

the water-bath at 25°C. At first, mosquito larvae in first and second instars were supplied to young guppies on the day of birth, and on the second day after birth. After 13 days, guppies were fed with early third instar mosquito larvae. The results are shown in **Table 5**. It was established that the guppy fry intensively prey upon immature mosquito larvae. But as shown in Fig. 5, the feeding rates of the fry did not attain such a level as to fit the correlation between feeding rates and body weights in adult fish.

Table 5, a Predation of immature mosquito larvae by new-born guppies

Guppies		Mosquito larvae					Body weight of guppies after the experiment in mg**
No.	Weight in mg	Instar	Eaten number/ Supplied number		Predation amount in mg		
			First day	Second day	First day	Second day	
1	5-6*	1	77/80	98/100			8.7
2		1	79/80	85/100			7.3
3		1	79/80	98/100			6.8
4		1	79/80	94/100			7.9
5		1	76/80	100/100			7.3
6		2	43/50	46/50	2.8	2.9	7.3
7		2	38/50	60/60	2.4	3.8	6.7
8		2	42/50	59/60	2.7	3.8	6.3
9		2	35/50	56/60	2.2	3.6	6.1
10***		2	28/50	27/60	1.8	1.7	6.2

*Body weight of the guppies was estimated from weighning of other individuals of the same brood.

**Weighning was done in the fourth day.

***This fish died in the second day. Body weight was measured soon after death.

Table 5, b Predation of immature mosquito larvae by 13 days guppy fry

Guppies		Mosquito larvae (3rd instar)						
		Eaten number/Supplied number			Predation amount in mg			
No.	Weight in mg	First day	Second day	Third day	First day	Second day	Third day	Average of second and third day
1	11.7	8/30	4.5/20	3/20	8.0	5.0	4.4	4.7
2	12.2	6/30	5/20	5.5/20	7.2	6.0	8.0	7.0
3	14.6	10/30	7.5/20	8/20	11.3	10.1	10.8	10.5
4	14.1	16/30	8/20	9/20	20.0	10.8	11.7	11.3
5*	12.3	13/30	11.5/20	7.5/20	13.0	13.8	11.1	11.9

*This fish died from asphyxia caused by eaten mosquito larva in the throat on the third day.

6 *Growth of guppies fed with mosquito larvae at different water temperatures and under different levels of illumination*

With the guppy, a fish maturing in a short time, it is important to know the growth rate of individual as an index showing the possibility of reproduction. Therefore, a comparison of growth of guppies in the above mentioned experiments was made. The specific growth rates were calculated from the usual formula, $100 (\log x_1 - \log x_0) / (t_1 - t_0)$, where x_1 and x_0 are the body weight at times t_1 and t_0 ($t_1 > t_0$). The female fish which spawned during the course of the experiment were excluded from calculations. In **Fig. 6** the relationships between the specific growth rates of individuals and the initial body weights are shown. At 25°C, 30°C and in darkness at 25°C, clear negative correlation was demonstrated in females, that is to say, the fish showed normal growth. However, at 20°C the correlation became indistinct, and at 15°C allmost all the individuals ceased growing. It is necessary to point out that the growth of males was usually less than that of females. This fact is related to the much smaller maximum size attained by the male fish.

In **Fig. 7** the relationship between body weight and body length (from the tip of snout to the terminal scales) in guppies is shown. The equation, $W = 0.017 L^{3.122}$, where W is body weight and L is body length, is suitable for adults but not for fry. This demonstrates the presence of change in body form between fry and adult.

Discussion

As mentioned above, guppies ate mosquito larvae even in darkness or in the presence of other food organism so long as suitable water temperature was maintained. At 25°C guppies showed maximum predation. The average amounts of predation by adult fish at 25°C reached to 22–91mg in 24 hours. This corresponds to 9–36 larvae (4 instar) when the larvae are 2.5 mg in weight per individual. Moreover, as shown in the author's previous paper (Yamagishi et al., 1966), the guppy grows and increases in numbers rapidly in rice fields and other small bodies of water even when they are polluted. These facts indicate that the guppy is one of the effective mosquito-eating fish. But the guppy is not cold-resistant. So in the temparete zone, preservation of guppies in the warm water during winter is necessary. The hot springs, where water quality is not harmful to fishes, will offer suitable propagation places.

If guppies are placed in darkness for long time, inhibition of reproduction may take place. It is known that *Gambusia affinis*, placed in complete darkness, has its reproduction interfered with vitamin D avitaminosis (Samokhvalova, 1939). The guppy is an euryphagous fish. Guppies increase in number even when fed with attached bacteria and deposits in sewage water. Moreover, the guppy preys upon mosquito larvae in the presence of daphnids. This peculiarity would be very useful for mosquito control. Male guppies ate as a rule less mosquito larvae than females of the same size, as is the case in *Gambusia affinis*, as established by Hess and Tarzwell (1942). Consequently many females must be used in mosquito control. Moreover, it is well known that female guppies produce several broods

after a single copulation.

Feeding rates of guppy fry were not much greater than those of adults. According to CHIBA (1961) carp fry consumed fresh daphnids at about 2–3 times their body weight for one day. It seemed that mosquito larvae were too large for guppy fry to eat, since the fry died from asphyxia caused undoubtedly from ingested mosquito larva in the throat.

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