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Impact of the Desert Pupfish, *Cyprinodon macularius*, and *Gambusia affinis affinis* on Fauna in Pond Ecosystems

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Effects of populations of *Cyprinodon macularius* Baird and Girard (desert pupfish) and *Gambusia affinis affinis* (Baird and Girard) on aquatic insects and zooplankton were investigated in shallow ponds clear of emergent vegetation and those planted with rice. Presence of both fish caused equivalently lower densities of beneficial insect predators, mosquitoes, and zooplankton.

Except for some behavioral feeding differences, food consumption appeared to be related to the density of food organisms available. *Cyprinodon macularius* foraged primarily in the benthos, where it consumed chironomid midge larvae, aquatic vegetation, snails, detritus, and some zooplankton and insect predator groups; whereas, *G. affinis,* foraging more at the surface, consumed a diverse selection of terrestrial insects as well as chironomid midge larvae, more planktonic zooplankters, snails, and some insect predators.

Cyprinodon macularius appears to be an acceptable substitute for *G. affinis* in mosquito control, and may be preferred in habitats harboring other native fishes due to minimal piscivorous behavior.

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Impact of the Desert Pupfish, *Cyprinodon macularius*, and *Gambusia affinis affinis* on Fauna in Pond Ecosystems[1,2]

INTRODUCTION

THE LARVIVOROUS MINNOW, *Gambusia affinis* (Baird and Girard), has figured prominently in mosquito abatement worldwide (de Buen, 1940; de Buen and de Buen, 1922; Howard, 1920; Krumholz, 1948; Sella, 1929; Sokolov and Chvaliova, 1936). However, recently the impact of this species on endemic aquatic fauna has caused concern with the discovery that it destroys juvenile native mosquito-eating fishes (Deacon and Bunnell, 1970; Mallars and Fowler, 1970), cyprinid fishes (Miller and Hubbs, 1960) and juvenile black bass in hatcheries (Myers, 1965). Additionally, *Gambusia* spp. may force out native fish species by competition for food and space (Deacon and Bunnell, 1970).

The impact of *G. affinis* on organisms other than mosquitoes in fresh-water ecosystems has been speculative (Hoy, Kauffman, and O'Berg, 1972) or determined from experiments in simulated environments (Hurlbert, Zedler, and Fairbanks, 1972). Such studies have associated *G. affinis* with detrimental selective predation on beneficial insect predators of mosquito larvae such as notonectids, dytiscids, and on zooplankton, causing ecosystem disruption and mosquito resurgence.

Recently, the use of alternative native fishes of the genus *Cyprinodon* has been considered as a biological mosquito control substitute (Legner and Medved, 1974; Legner, Medved, and Hauser, 1975), because ecological upsets have not previously been associated with these fishes. The desert pupfish *Cyprinodon macularius*, is known to cohabit with a great variety of fishes and aquatic organisms in native habitats throughout the Lower Sonoran Desert of California, Arizona and northwestern Mexico, with apparent minimal ecosystem disruption (Legner and Medved, 1974; Walker, Whitney, and Barlow, 1961).

The present study was designed to compare the effects of the introduced *G. affinis* with a native southern California strain of *C. macularius* on principal biotic elements in natural pond and rice ecosystems and to demonstrate the basis of food selection in these habitats.

METHODS AND MATERIALS

Studies were performed in 12 earthen 4 x 7 m experimental ponds provided with well water (Legner and Medved, 1973). Ponds were graded so that water depth was equivalent throughout, excepting a 0.5 m periphery of shallower depths. Three treatments were used, the first treatment containing *C. macularius* only, the second *G. affinis* only, and the third no fish (control). Four replicates of each treatment were assigned at random to ponds in 1974 and reassigned in 1975. Ponds were flooded 3-4 weeks before the study periods June 8 to October 24,

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1974, and June 17 to September 5, 1975, and were drained and refurbished between periods. Pond temperature was continuously recorded at the benthic surface in a centrally located pond, and coverage of predominant aquatic vegetation was noted periodically.

In 1974, two replicates of each treatment group were planted mid-May with two to four Colusa 1600 rice seedlings at each point of a ca. 25 cm grid. In that year, ponds initially received 750 g of chick starter mash as an ovipositional and nutritional stimulus for aquatic insects. This was followed by a lower rate of 250 g per pond biweekly throughout the study period. Water level was maintained at 0.25 m depth in 1974 to favor rice culture. In 1975, no rice was planted, and water depth was maintained at 0.36 m. Ponds were initially fertilized with 1500 g starter mash supplemented at a higher rate of 375 g per pond each week.

Fish were introduced on June 17, 1974, and June 22, 1975, at the rate of 10 mature ♂♂ and 15 mature ♀♀ of each species per replicate. This was equivalent to 3,625 total fish per acre (8,957/hectare). Fish recovered from drainage of ponds in 1974 were overwintered in smaller outdoor ponds.

Densities of aquatic insects were sampled with a water column sampler (Legner, Medved, and Sjogren, 1975) encompassing both vegetated and open water habitats. Due to different pond depths between years, the volume of most samples taken in 1974 was 1.8 liters and in 1975, 2.5 liters. Peripheral areas were incorporated into the total random sample without adjustment. In both years, samples were divided over 2 consecutive days. In 1974, 10 random subsamples per pond were taken at 3- to 4-week intervals. Pretreatment samples were taken on June 8–9. In 1975, 20 subsamples per pond were taken every 23 to 25 days and pretreatment samples on June 17–18.

Immature chironomid densities in 1974 were measured by means of trays placed in the benthos (Bay and Anderson, 1966), three trays per replicate monthly. However, due to the heavy growth of algae and other aquatic plants that precluded random tray placement, use of the water column sampler was implemented in 1975 to improve the random sampling of midge larvae.

Zooplankton were sampled in both years with a specially devised qualitative zooplankton sampler (Walters, 1976). Ten dipper samples were randomly collected from each pond to make 8 liters and concentrated by pouring through a 1 m (64μ aperture mesh) Kahlsico® Standard Plankton Net with plankton bucket attachment. The 236 ml plankton concentrate was preserved as a 10 percent formalin solution.

In 1974, all zooplankters in the concentrate were counted under a dissecting microscope using a 10 × 5 × 2 cm plexiglass counting chamber. Due to increased numbers of zooplankters in ponds in 1975, the concentrate was reduced to 100 ml and 10 1-ml aliquots were transferred into a Sedgwick-Rafter® counting chamber and examined under a compound microscope.

Zooplankters were sampled during the crepuscular period on two consecutive evenings for each sample period, every 14 days in 1974 and every 23 to 25 days in 1975. During 1975, zooplankton were sampled on the 2 days following insect sampling periods.

Fish were trapped from ponds with Standard Gees® minnow traps during pond fauna sampling periods (4 consecutive days) in 1975 to determine the number and kind of pond organisms in their stomachs. The densities of organisms in stomachs and in ponds were compared for the same interval.

A preliminary study established diel feeding patterns in each fish species. Stomach analyses of *C. macularius* trapped every 3 hours showed that stomachs were most apt to be full during a 3 hour period beginning 2 hours before sunset. A secondary peak appeared in the early morning, 1 to 2 hours after sunrise. Examination of *G. affinis* stomachs revealed a peak feeding activity from 1 to 5 hours after sunrise, with a secondary peak around sunset. Therefore, on the first and second days of each sampling interval, *G. affinis* were trapped from experimental ponds 2 to 4 hours after sunrise, before insects were sampled, while *C. macularius* were trapped just before sunset (after insect sampling). On days 3 and 4, trapping of both fish was done close to the time of zooplankton sampling, 1 hour before sunset. Fish taken from ponds varied with the availability of mature replacements and success in trapping; a minimum of two ♀♀ and one ♂ from each pond per day of sampling was attempted.

Trapped fish were anesthesized in Tricaine Methanesulfonate® (0.375 g/liter) to prevent food regurgitation. Fish were then injected near the liver with 10 percent formalin until the body cavity was visibly bloated and stored in 10 percent formalin for later stomach analysis.

Fish were examined for stage of maturity and measured for total length. The nearly undifferentiated "stomach" of *C. macularius* was considered to be the symetrical U-shaped area around the liver, beginning at the termination of the esophagus. In *G. affinis*, the stomach is distinct and approximates only one side of the U-shaped intestine around the liver. The respective stomach sections were dissected from the rest of the intestinal tract, removed to a Sedgwick-Rafter® counting chamber, cut lengthwise, and the contents flushed into the chamber with 10% formalin. Macroscopic portions were identified and counted under a dissecting microscope. Zooplankters were more easily identified and enumerated under a compound microscope at higher magnifications.

During 1975, 105 *C. macularius* (47♀♀, 28♂♂, 30 juveniles) and 108 *G. afffinis* (42♀♀, 38♂♂, 28 juveniles) were taken from ponds for stomach analyses. Although sexes were examined separately (Walters, 1976), for this discussion mature ♀♀ and ♂♂ were grouped.

Analyses of insect and zooplankton densities were performed on the basis of a completely random design. Sample mean differences were tested with Duncan's New Multiple Range Test, after data were transformed to $\sqrt{X + 0.5}$. Comparisons shown to be different at the 10 percent level of confidence (in terms of probability) were considered significant in this study; comparisons significant at higher levels ($P = .05$ or $.01$) are so notated in the text and tables. Fish stomach contents were compared with pond fauna densities using bivariate correlation analyses tested at the 5 percent level (t statistic) (Steel and Torrie, 1960); only significant correlations are mentioned in the text.

RESULTS AND DISCUSSION

Water temperatures in ponds ranged from 18 to 36 °C in 1974 and 20 to 33 °C in 1975. Macroscopic Chlorophyta (predominantly *Chara* sp., *Cladophora* sp., and *Spirogyra* sp.) proliferated into the summer and by mid-July of each year the bottom of ponds were 60 to 90 percent infested, with some *Cladophora* floating in mats at the surface. The horned pondweed, *Zannichellia palustris* L., invaded most ponds by mid-season during both years. Chlorophyta and *Z. palustris* covered greater than 80 percent of all pond bottoms by September, the weed mass filling 30 to 90 percent of the water volume. In 1974, the rice stand became dense and headed out at 1 m by early August, followed by a decline in vigor and lodging in October.

Reproduction of both fish species occurred within 20 days in 1974. More specific observations in 1975 revealed the appearance of *G. affinis* fry 10 days after fish introduction; *C. macularius* fry appeared 10 days later. Territorial and spawning behavior of oviparous *C. macularius* was noted several weeks after introduction of mature fish and continued through September. Young-of-the-year females (2.5 cm total length)

TABLE 1
STOMACH ANALYSIS OF 75 MATURE *C. MACULARIUS* AND 80 MATURE *G. AFFINIS* FROM EXPERIMENTAL PONDS DURING 1975, RIVERSIDE, CALIFORNIA

	C. macularius[1]						***G. affinis***[2]					
	Mean no./ stomach			**Freq. of occurrence**[4]			**Mean no./ stomach**			**Freq. of occurrence**		
Food organisms	**Jul. (24)**[3]	**Aug. (24)**	**Sept. (27)**	**Jul.**	**Aug.**	**Sept.**	**Jul. (19)**	**Aug. (19)**	**Sept. (42)**	**Jul.**	**Aug.**	**Sept.**
Total zooplankton	21.3	2.3	13.2	63	25	52	12.4	1.3	2.9	37	32	33
Total Cladocera	21.2	0.6	10.0	63	13	41	11.1	0.6	1.7	37	11	43
Ceriodaphnia pulchella	20.2	0.0	0.2	58	0	7	8.8	0.3	0.0	21	5	0
Alona sp.	0.7	0.6	9.8	25	13	41	0.5	0.3	1.7	16	11	33
Other[5]	0.3	<0.1	<0.1	17	4	4	1.8	0.0	0.0	16	0	0
Copepoda												
Eucyclops agilis	0.1	0.3	1.6	8	13	37	0.0	0.0	0.8	0	0	17
Diaptomus pallidus	0.0	0.0	0.0	0	0	0	1.3	<0.1	0.0	16	5	0
Ostracoda	<0.1	1.2	1.6	4	25	44	<0.1	0.6	0.4	5	21	12
Total insect predators	1.0	0.1	0.0	29	8	0	0.4	<0.1	0.0	21	5	0
Coleoptera (larvae)												
Laccophilus terminalis	0.9	0.0	0.0	29	0	0	<0.1	0.0	0.0	5	0	0
Tropisternus lateralis	0.0	0.0	0.0	0	0	0	0.3	0.0	0.0	21	0	0
Berosus punctatissimus	0.0	<0.1	0.0	0	4	0	0.0	0.0	0.0	0	0	0
Odonata (naiads)												
Libellula pulchella	0.0	<0.1	0.0	0	4	0	0.0	0.0	0.0	0	0	0
Anomalagrion hastatum	0.0	0.0	0.0	0	0	0	0.0	0.1	0.0	0	5	0
Corixidae	0.1	0.0	0.0	4	0	0	0.1	0.0	0.0	11	0	0
Ephemeroptera (nymphs)												
Callibaetis sp.	0.0	0.0	0.0	0	0	0	0.1	0.0	<0.1	5	0	2
Chironomidae (larvae)	2.7	3.0	0.6	63	58	33	4.3	0.2	0.1	84	11	10
Culex tarsalis (larvae)	0.3	0.0	0.0	13	0	0	0.1	0.0	0.0	5	0	0
Mollusca												
Physa virgata	0.0	1.8	0.1	0	33	7	0.1	2.8	0.6	5	58	21
Chlorophyta/ *Z. palustris*				4	65	85				0	16	26

[1] 47 ♀♀, 28 ♂♂ (3.5–5.5 cm total length).

[2] 42 ♀♀ (4.0–6.0 cm total length), 38 ♂♂ (2.0–2.9 cm total length).

[3] No. of stomachs dissected per month.

[4] Percent of stomachs containing organism or group, to nearest whole number.

[5] *Simocephalus vetulus* and *Macrothrix hirsuticornis*.

developed during August. Although mating behavior of ovoviviparous *G. affinis* was not specifically noted in ponds, fry were observed throughout both sampling seasons with young-of-the-year females (2.5 cm total length) developing about one month earlier than *C. macularius*. Some originally stocked *G. affinis* ♀♀ were undoubtably pregnant as evidenced by fry appearance in ponds earlier than normal gestation periods (Hubbs, 1971).

Predominant pond organisms and population trends.— The Dytiscidae predominated among the predatory Coleoptera. In 1975, the Dytiscid, *Laccophilus terminalis* Sharp, and the hydrophilid, *Berosus punctatissimus* LeConte, dominated with larval and adult populations peaking in mid-July and in mid-September, respectively. Among the Odonata, Anisoptera represented the greatest proportion of individuals each year with *Libellula pulchella* Drury dominant in 1975, along with the zygopteran, *Anomalagrion hastatum* Selys. Hemipterans were few and poorly distributed with only the notonectid, *Buenoa scimitra* Bare, representing the group in 1974 and in 1975, only the corixids, *Corisella* sp. and *Hesperocorixa laevigata* (Uhler).

Benthic chironomid larvae, predominately *Chironomus* spp., showed a steady decline in population densities from June to September in both years, with peak densities in control ponds of ca. 270 to 360 larvae per square meter. Larval *Culex tarsalis* Coquillett and Ephemeroptera (*Callibaetis* sp.) populations occurred at relative low densities each year.

Zooplankton populations were four to eight times more abundant in 1975 than in 1974, possibly a result of higher nutritional supplementation of ponds in 1975. The same zooplankters predominated in both years, with Cladocera more numerous than either Copepoda or Ostracoda. Within the Cladocera, *Ceriodaphnia pulchella* Sars and *Alona* sp. were present in greatest abundance. *Diaptomus pallidus* Herrick (Calanoida) of the Copepoda was dominant early in the season, but *Eucyclops agilis* Koch (Cyclopoida) dominated after July. Most zooplankters exhibited increasing population densities as the season progressed; however, some, such as *D. pallidus*, generally exhibited peak population densities in July, declining in numbers by mid-August in both years.

Fish impact on aquatic insects.-Ponds stocked with fish contained fewer total insect predators in both years ($P \leq 10$) especially later in the season (August to October), with some reductions observed in early July (Fig. 1). The reduction of insect predators in the presence of both fish was due to predation, particularly early in the season. Detailed fish stomach analysis in 1975 substantiated predation on these insect groups in July, primarily fish foraging on coleopterous larvae (Table 1). Adults of *C. macularius* consumed substantial numbers of larval dytiscids, with *Laccophilus terminalis* averaging ca. one per stomach examined. *Gambusia affinis* exhibited slightly more diverse feeding on insect predators, consuming larvae of the hydrophilid, *Tropisternus lateralis* (Fab.) as well as *L. terminalis*. Both fish foraged to a minor extent on low densities of corixids present during July. Foraging for insect predators, as indicated by stomach analyses, was much reduced during August and September. However, some feeding on Odonata by both fish was apparent in August and *C. macularius* fed on the hydrophilid, *Berosus punctatissimus*, at that time also.

In both years, *G. affinis* appeared to have more effect on populations of aquatic Coleoptera than did *C. macularius*, although significant evidence was found only in 1974 (Fig. 2). Major feeding by fish on coleopterous larvae, in both years appeared to be density-dependent. Stomach analyses in 1975 revealed primary feeding in July at the time of peak abundance of the predominant dytiscid, *Laccophilus terminalis*, and hydrophilid, *Tropisternus lateralis* (Table 1). As the density of these food sources was reduced by fish and by adult

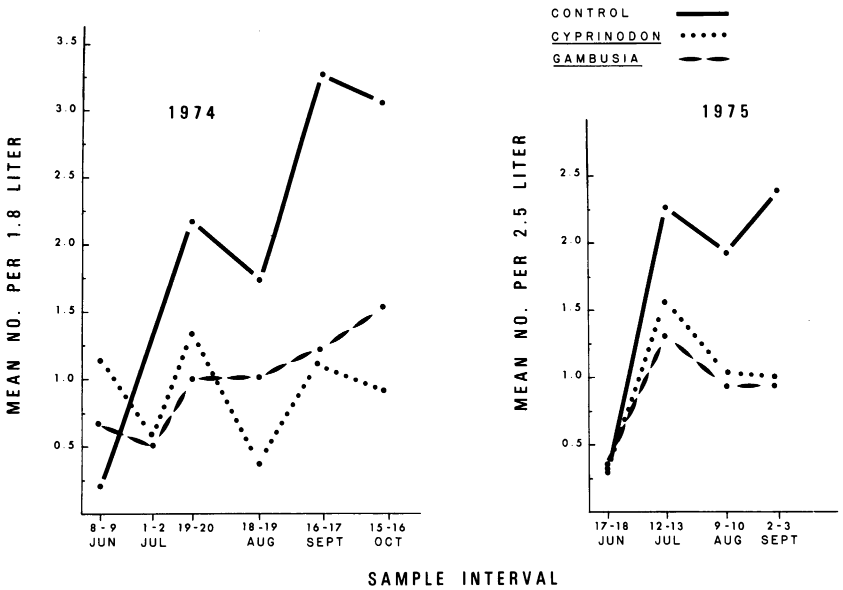

Fig. 1. Density of total insect predators sampled from experimental ponds in the presence of *C. macularius* and *G. affinis*, compared to a control. (A) = Adults, (L) = Larvae. Insect predators included the Coleoptera—*Laccophilus terminalis* (A and L), *Rhantus gutticollis* (Say) (A), *Berosus punctatissimus* (A and L), *Tropisternus lateralis* (A and L), *Laccobius californicus* d'Orchymont (A); Odonata—*Libellula pulchella*, *Pantala hymenaea* (Say), *Tramea lacerata* Hagen, *Anax junius* (Drury), *Anomalagrion hastatum*, *Ischnura perparva* Selys; Hemiptera—*Buenoa scimitra*, *Corisella* sp., *Hesperocorixa laevigata*.

emergence, foraging apparently shifted to more abundant prey. Foraging has been directly correlated with food availability for *G. affinis* previously (Bay and Anderson, 1966; Hess and Tarzwell, 1942; Washino, 1968; Washino and Hokama, 1967). This behavior was further substantiated in the present study by a significant level of correlation between stomach contents and pond coleopterous larval density ($r = 0.975$). It was also shown that the same food selection behavior might be true for *C. macularius* as indicated by Cox (1972). Adult Coleoptera appeared not to have been utilized as food by either fish under the conditions of the experiment.

No intensive foraging on benthic Odonata naiads by either fish was shown by stomach analysis (Table 1) although it has been observed previously with *G. affinis* (Beesley, 1972; Hess and Tarswell, 1942). Relatively low population densities of Odonata in ponds may have precluded adequate representation of these insects in the gut, although significant evidence ($P = .05$) of naiad reduction in ponds compared to controls was found late in the season both years (Fig. 2). Fish feeding appeared coincident with the appearance of second generation young instars in ponds during August and the relative non-availability of other predator groups during this month.

Although no significant evidence for a reduction of the notonectid, *Berosus scimitra*, was found, some predation on this small species by both fish probably occurred. In control ponds in 1974, *B. scimitra* developed throughout the season, whereas in fish ponds its population disappeared about one month after first occurrence. Hoy, Kauffman, and O'Berg (1972) suspected *G. affinis* to forage early in the season on Notonectidae in rice fields, resulting in mosquito upsets.

Corixid populations in 1975 were little

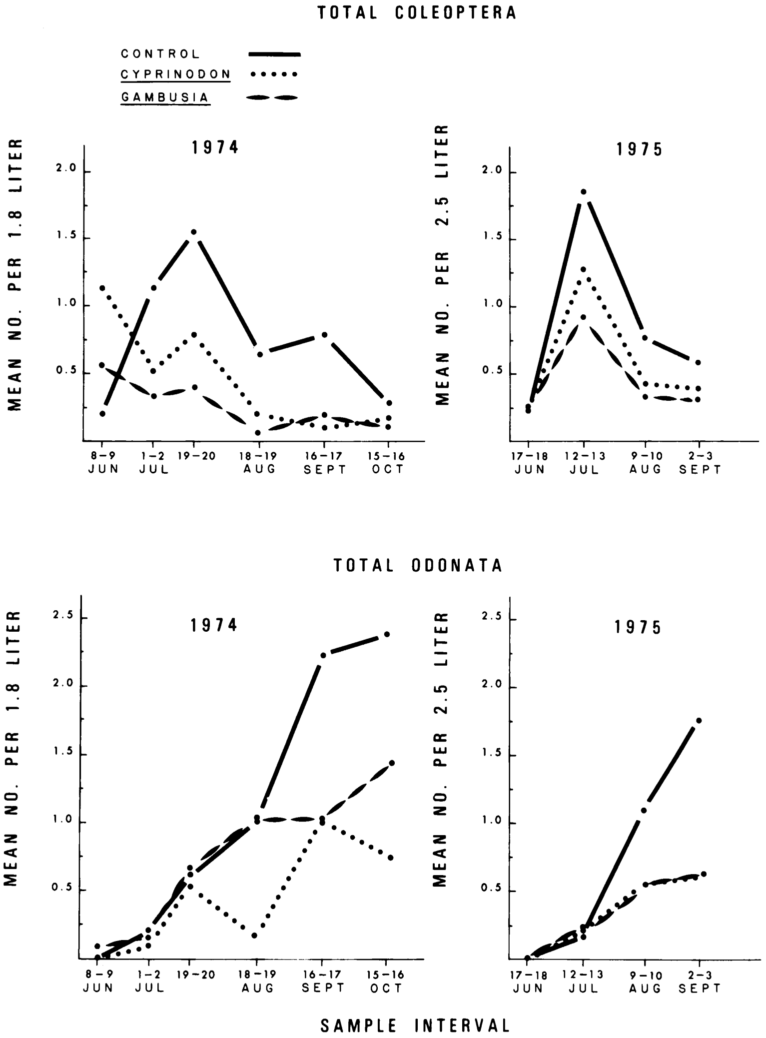

Fig. 2. Density of total Odonata and Coleoptera sampled from experimental ponds in the presence of *C. macularius* and *G. affinis*, compared to a control.

affected by fish introductions, although both fish used them for food as determined from stomach analyses (Table 1). Feeding on corixids by *C. macularius* has not been reported previously, although *G. affinis* has been associated with feeding on several species in different habitats (Hess and Tarzwell, 1942; Washino and Hokama, 1967).

Among prey organisms, populations of chironomid midge larvae were not notably reduced by fish either year (Table 2). Stomach analyses in 1975 demonstrated that chironomid larvae were the primary insect food present in the gut of *C. macularius* at all sample intervals—but in *G. affinis* only during July (Table 1). Chironomid larvae were the most abundant insect group in ponds during July, and in August, they were as abundant as mosquito larvae. In September, both *G. affinis* and *C. macularius* fed less upon chironomids, as midge density declined (Tables 1, 2). Washino and Hokama (1967) also found maximum predation by *G. affinis* on

TABLE 2
EFFECTS OF *C. MACULARIUS* AND *G. AFFINIS* ON THE DENSITY OF LARVAL CHIRONOMIDAE IN EXPERIMENTAL PONDS DURING 1974 AND 1975, RIVERSIDE, CALIFORNIA

Fish species	Sample month[1]	Mean no. larvae/sample[2] 1974	Mean no. larvae/sample[2] 1975
Control (no fish)	Jun.	33.9	14.0
	Jul.	20.7	6.8
	Aug.	6.8	2.7
	Sept.	2.7	1.1
	Oct.	2.8	
C. macularius	Jun.	28.2	12.6
	Jul.	5.8	16.0
	Aug.	5.3	2.6
	Sept.	4.6	1.2
	Oct.	3.5	
G. affinis	Jun.	44.8	11.9
	Jul.	10.3	16.1
	Aug.	11.2	4.1
	Sept.	7.8	2.4
	Oct.	3.3	

[1] Sample periods Jun. 17, Jul. 22, Aug. 20, Sept. 18, Oct. 24, 1974 and Jun. 17–18, Jul. 12–13, Aug. 9–10, Sept. 2–3, 1975.

[2] 1974 tray sample = 231 $(cm)^2$; 1975 column sample = 71 $(cm)^2$.

chironomid larvae following peaks of abundance in rice. Minimal feeding by *G. affinis* upon chironomids at low densities may have been associated with the primarily surface-foraging behavior of this species and the benthic existence of *Chironomus* spp. predominating in ponds. Behaviorally, *G. affinis* would be expected to consume benthic midges when larvae temporarily and periodically become nektonic in ponds or are free-swimming as first instars.

Bay and Anderson (1966) considered *G. affinis* of no practical value in the reduction of chironomids; in fact, larvae averaged 21 percent higher in ponds with *Gambusia* present. A similar trend was observed during some months in the present study with both fishes (Table 2). Some minor field reductions of chironomid midges by *C. macularius* have been reported (Legner, Medved, and Hauser, 1975), the impact varying seasonally with different species.

Both fish appeared to have the same effect on mosquito populations in both years. In 1974, reduction of larvae ($P \leq .05$) occurred in all fish-treated ponds throughout the season (Fig. 3). In 1975, the very low mosquito densities made it difficult to assess impact; however, some decrease was observed during August and September.

With rice present in 1974, *G. affinis* was apparently able to control low densities of mosquitoes as effectively as did *C. macularius*, although this species has not been noted for good foraging ability in emergent weed habitats (Danielson, 1968). Stomach analyses also established foraging on mosquito larvae by both species, but only in July (Table 1). This, however, did not correlate with the peak availability of larvae in August (Fig. 3).

The density of Ephemeroptera (*Callibaetis* sp.) nymphs in fish ponds was significantly reduced ($P = .05$) compared to controls in August of each year and some reduction was observed in *G. affinis*-treated ponds again in October,

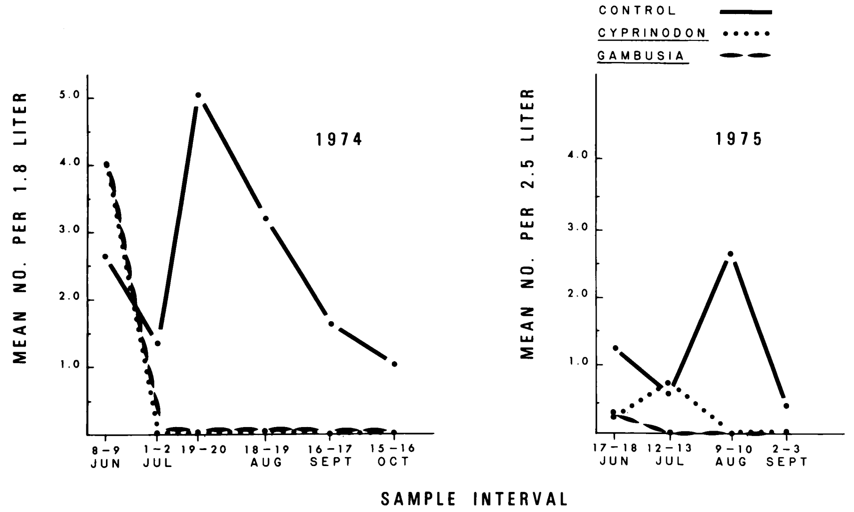

Fig. 3. Density of *Culex tarsalis* larvae sampled from experimental ponds in the presence of *C. macularius* and *G. affinis*, compared to a control.

1974, when a secondary peak in *Callibaetis* was apparent. Stomach analyses showed that mature *G. affinis* foraged on nymphs in July and September (Table 1); juvenile *C. macularius* fed on nymphs during September (Walters, 1976).

Fish impact on zooplankton.—Reductions of zooplankton as a group were observed in all fish ponds during both years (Fig. 4, Table 3). In 1974, reductions below controls were observed by both species only in late July, whereas in 1975 reductions were apparent during August and September. Only *G. affinis*

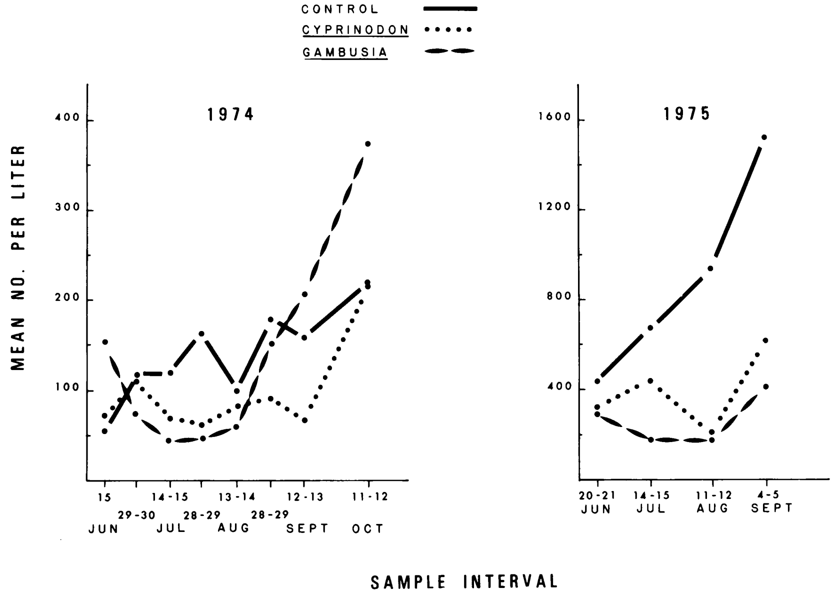

Fig. 4. Density of zooplankton sampled from experimental ponds in the presence of *C. macularius* and *G. affinis*, compared to a control.

TABLE 3
EFFECTS OF *C. MACULARIUS* AND *G. AFFINIS* ON THE DENSITY OF ZOOPLANKTON IN EXPERIMENTAL PONDS DURING 1974 AND 1975, RIVERSIDE, CALIFORNIA

		Mean no. / liter[1]							
		Total zooplankton		**Cladocera**		**Copepoda**		**Ostracoda**	
Fish species	**Sample month**[2]	**1974**	**1975**	**1974**	**1975**	**1974**	**1975**	**1974**	**1975**
Control (no fish)	Jun.	55	441	45	333	9	78	2	30
		118		78		40		1	
	Jul.	119	673	86	481	30	171	3	21
		163		99		55		10	
	Aug.	99	943	43	730	38	154	19	58
		179		119		50		11	
	Sept.	157	1523	94	1153	54	273	10	97
	Oct.	219		130		70		19	
C. macularius	Jun.	71	319	54	223	13	75	4	21
		109		61		47		1	
	Jul.	70	439	17^{x}	82^{**}	51	349	2	8
		62^{**}[3]		16^{**}		41		5	
	Aug.	83	203^{*}	21	25^{**}	55	149	6	29
		91		26^{x}		62		3	
	Sept.	67	624^{x}	15^{x}	244^{*}	48	293	5	88
	Oct.	216		58^{x}		140		18	
G. affinis	Jun.	153	291	137	199	14	55	2	37
		73		39		34		1	
	Jul.	44^{x}	179^{*}	29	32^{**}	13^{*a}[4]	128	2	19
		46^{**}		21^{**}		19^{*}		6	
	Aug.	60	176^{*}	17	33^{**}	32	101	11	41
		149		65		65		20	
	Sept.	205	410^{*}	56	118^{**}	83	223	66	68
	Oct.	375		134		168		73	

[1] To nearest whole number.

[2] Sample periods Jun. 15, 29–30, Jul. 14–15, 28–29, Aug. 13–14, 28–29, Sept. 12–13, Oct. 11–12, 1974, and Jun. 20–21, Jul. 14–15, Aug. 11–12, Sept. 4–5, 1975.

[3] Significantly lower than control: ^{x} at Duncan's .10; * at .05; ** at .01.

[4] a = significantly lower than *C. macularius* at Duncan's .05.

reduced populations of zooplankton earlier in the season (mid-July) of each year. Additionally, some significant zooplankton increases occurred in ponds with *G. affinis*.

The different effects on zooplankton populations that were observed in 1974 and 1975 are probably related to the presence of emergent rice in half of the 1974 ponds, which resulted in behavioral adaptations and orientations by some species of zooplankters. This behavior

TABLE 4
EFFECTS OF *C. MACULARIUS* AND *G. AFFINIS* ON THE DENSITY OF CLADOCERA SPECIES IN EXPERIMENTAL PONDS DURING 1974 AND 1975, RIVERSIDE, CALIFORNIA

Fish species	Sample month[2]	Mean no./liter[1]								
		Ceriodaphnia pulchella		***Alona* sp.**		***Simocephalus vetulus***		***Macrothrix hirsuticornis***	**Other spp.**[3]	
		1974	**1975**	**1974**	**1975**	**1974**	**1975**	**1975**	**1974**	**1975**
Control (no fish)	Jun.	27	113	<1	86	0	0	4	18	130
		20		6		1			51	
	Jul.	33	280	7	190	1	8	<1	45	2
		54		26		3			17	
	Aug.	19	475	19	253	5	3	0	0	0
		79		30		10			0	
	Sept.	64	589	19	542	11	23	0	0	0
	Oct.	62		63^{a} [5]		5			0	
C macularius	Jun.	48	86	<1	86	1	1	3	5	48
		8		30		0			23	
	Jul.	5	34**	10	31*	<1	1	0	2	16
		6		6^{x}		<1			3	
	Aug.	6	5**	14	19**	1^{x}	0	0	0	1
		1**[4]		24		1**			0	
	Sept.	5*	13**	10	230	<1^{x}	1*	0	0	0
	Oct.	1**		57^{a}		1*			0	
G. affinis	Jun.	114	65	0	65	0	<1	16	24	52
		11		4		<1			24	
	Jul.	5	13**	21	15*	1	2	0	2	2
		5		13		<1			3	
	Aug.	1	14**	14	19**	1^{x}	0	<1	0	<1
		2**		62		2**			0	
	Sept.	4*	31**	49	85*	2	3*	<1	0	0
	Oct.	3**		130		1^{x}			0	

[1] To nearest whole number.

[2] Sample periods Jun. 15, 29–30; Jul. 14–15, 28–29; Aug. 13–14, 28–29; Sept. 12–13; Oct. 11–12, 1974 and Jun. 20–21, Jul. 14–15, Aug. 11–12, Sept. 4–5, 1975.

[3] *Moina micrura* Kurz, *Daphnia pulex* Leydig (1974); *M. micrura, D. pulex, Bosmina longirostris* (Muller) (1975).

[4] Significantly lower than control: ^{x} at Duncan's .10 ; * at .05; ** at .01.

[5] a = significantly lower than *G. affinis* at Duncan's .05.

may account for the different impact each fish had in the two habitats. Major differences in Cladocera density were observed between rice and non-rice habitats. In 1974, rice culture may have effectively sheltered at least one littoral cladoceran, *Alona* sp. (Brooks, 1959), while apparently not affecting predation on another, *Simocephalus vetulus* Schødler (Fig. 5, Table 4). The large relative size of *S. vetulus* perhaps favored its being taken as food, especially by *C. macularius* in rice. Algae and *Zannichellia palustris* apparently did not interfere with effective predation on *Alona* sp. in 1975 where reductions below the controls were observed during most of the season (Fig. 5, Table 4).

Although significant reduction of *Alona* sp. was not demonstrated by *C. macularius* in September, 1975, as it was with *G. affinis*, subsequent stomach analyses revealed major feeding on this group with 41 percent frequency in stomachs (Table 1). Feeding on *Alona* sp. and *Simocephalus vetulus* during September by both fish appeared related to peak pond densities of these species.

The presence of rice in 1974 appeared to afford some shelter to *Ceriodaphnia pulchella*, which is both littoral and limnetic in pond habitats (Brooks, 1959)

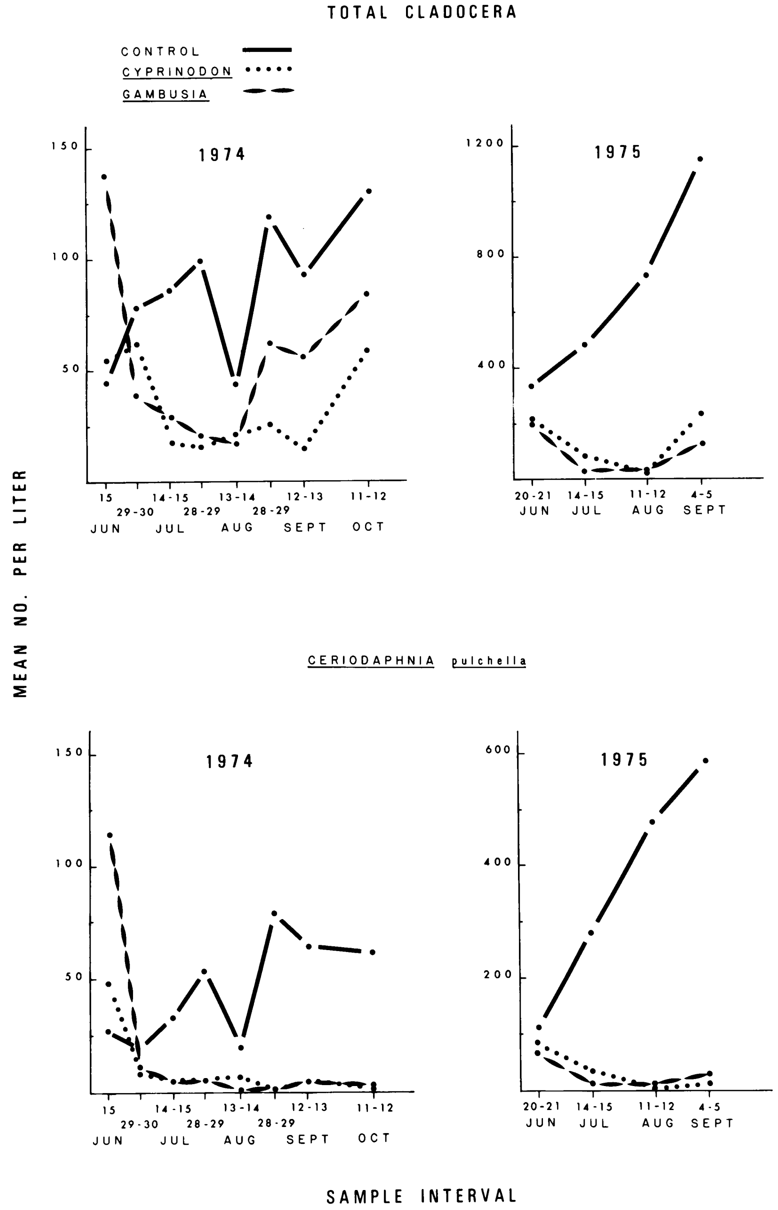

Fig. 5. Density of Cladocera species sampled from experimental ponds in the presence of *C. macularius* and *G. affinis*, compared to a control.

(Fig. 5, Table 4). *Ceriodaphnia pulchella* populations were reduced below controls during each month following fish introduction in 1975, whereas reductions in 1974 began to occur only at peak densities of this species. Regardless of the habitat present, *C. pulchella* appeared to be the major cladoceran taken as food each year, which was substantiated by stomach analysis in 1975 (Table 1). Feeding on this species by both fish occurred primarily in July, when it was the most abundant zooplankter in ponds. Populations may have been reduced to non-recovery levels as foraging in subsequent months was minimal.

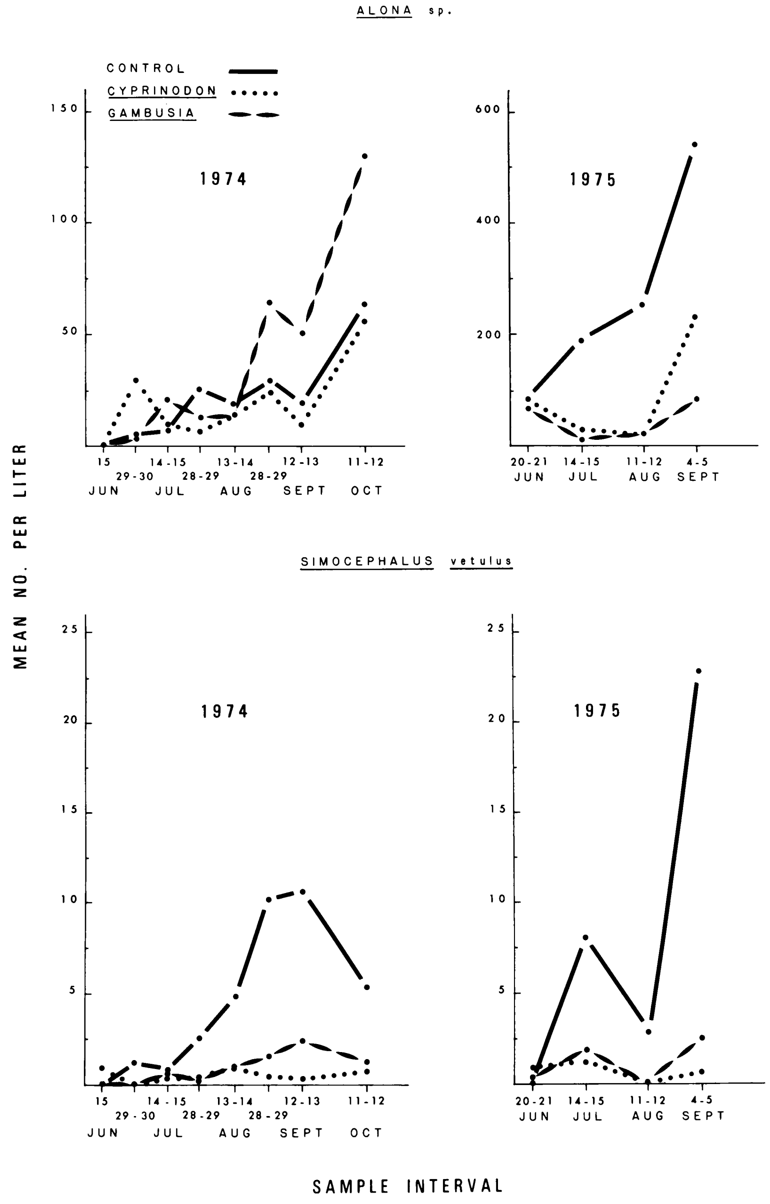

Fig. 5. *(cont.)* Density of Cladocera species sampled from experimental ponds in the presence of *C. macularis* and *G. affinis*, compared to a control.

Copepods were reduced below control densities only in *G. affinis* ponds in July, 1974 (Table 3). In addition, copepod densities in *G. affinis* ponds were significantly reduced below those in *C. macularius* ponds (Table 5). Reduction below controls was due to foraging on both copepod species; however, differences between fish were related to foraging on *Diaptomus pallidus*. Greatest reductions of this species throughout the season were observed in *G. affinis* ponds, while maximum densities were noted in *C. macularius* ponds. These same trends were also observed in 1975 and probably were correlated with normal differences

TABLE 5
EFFECTS OF *C. MACULARIUS* AND *G. AFFINIS* ON THE DENSITY OF COPEPODA IN EXPERIMENTAL PONDS DURING 1974 AND 1975, RIVERSIDE, CALIFORNIA

Fish species	Sample month[2]	Mean no./liter[1]			
		Diaptomus pallidus		*Eucyclops agilis*	
		1974	1975	1974	1975
Control (no fish)	Jun.	5	45	4	33
		27		12	
	Jul.	12	64	18	107
		17		37	
	Aug.	1	59	37	95
		1		49	
	Sept.	1	13	53	259
	Oct.	<1		70	
C. macularius	Jun.	7	44	7	31
		25		22	
	Jul.	34	231	17	118
		15		27	
	Aug.	2	38	53	111
		2		61	
	Sept.	2	21	47	272
	Oct.	<1		140	
G. affinis	Jun.	8	48	6	6
		16		18	
	Jul.	4[a] [3]	104	10	44
		2		17	
	Aug.	0	12	32	89
		<1		65	
	Sept.	<1	3	83	220
	Oct.	0		168	

[1] To the nearest whole number.

[2] Sample periods Jun. 15, 29–30; Jul. 14–15, 28–29; Aug. 13–14, 28–29; Sept. 12–13, Oct. 11–12, 1974, and Jun. 20–21, Jul. 14–15, Aug. 11–12, Sept. 4–5, 1975.

[3] a = significantly lower than *C. macularius* at Duncan's .05.

in feeding behavior. *Gambusia affinis* perhaps grazed on more accessible planktonic forms such as *D. pallidus*, whereas *C. macularius* apparently foraged on more littoral and benthic zooplankters, allowing relative increases of *D. pallidus*. Stomach analyses in 1975 substantiated these differences (Table 1). No evidence of *C. macularius* feeding on *D. pallidus* was demonstrated by any age group, whereas *G. affinis* foraged on this species during several months, especially juveniles (Walters, 1976).

Populations of the ostracods, *Cypridopsis vidua* (Muller) and *Physocypria pustulosa* Sharpe, were not significantly reduced by either fish (Table 3). However, 1975 stomach analyses (Table 1) revealed apparent selective feeding on these species during August and September by *C. macularius* and to a lesser extent by *G. affinis*. Although ostracods were the least abundant zooplankton group present throughout the sample period in 1975, they appeared to be an important food item for mature fish and additionally they appeared in 90 percent of juvenile *C. macularius* stomachs in August at a rate of 6.4 per stomach. As most Ostracoda live a benthic existence (Tressler, 1959), it would appear that *C. macularius* might be most capable of consuming this group.

Both *C. macularius* and *G. affinis* exerted greater environmental impact on total zooplankton biomass and community diversity in ponds compared to the reported impact of bluegill sunfish, *Lepomis macrochirus* Rafinesque on zooplankton in these habitats (Hall, Cooper, and Warner, 1970). Bluegill, a common omnivorous cohabitant with *G. affinis* in California, exhibited no predatory effect on total zooplankton density; whereas, selective feeding on *Ceriodaphnia* resulted in increased diversity of both cladoceran and copepod communities.

Additional food sources. —Aquatic vegetation and the snail *Physa virgata* Gould, were important additional food sources for both fish (Table 1), with the snail being of primary importance compared to insect or zooplankton food due to its relatively large size. This species occurred in 58 percent of *G. affinis* and 33 percent of *C. macularius* stomachs during August. Macroscopic vegetation constituted much of the bulk of *C. macularius* stomachs during August and September (65 to 85 percent frequency), while *G. affinis* fed on vegetation to a much lesser extent (Table 1). Utilization of vegetation by both species appeared to be density-related with greater consumption occurring as algae accumulated in ponds.

Miscellaneous food items such as seed capsules and nematodes were more prevalent in the stomachs of *G. affinis*; however, detritis was more commonly found in *C. macularius* stomachs. *Gambusia affinis* also consumed large numbers of terrestrial insects on the pond surface such as aphid nymphs, thrips, springtails, ants, adult chironomids and other Diptera. Significant feeding on terrestrial insects by *G. affinis* has been reported previously (Harrington and Harrington, 1961; Washino and Hokama, 1967).

Gambusia affinis were piscivorous in ponds with 6 percent of stomachs containing *G. affinis* fry; fry were not found in *C. macularius* stomachs.

SUMMARY

Limited studies in shallow, natural ponds indicated a density-dependent trend of food consumption by both *C. macularius* and *G. affinis*. In these ecosystems, *C. macularius* foraged mostly in the benthos, consuming large numbers of benthic chironomid midge larvae, much detritus, aquatic vegetation, and snails. *Gambusia affinis*, foraging mostly at the surface, consumed large numbers of floating terrestrial insects, immature chironomids and snails which were found on vegetation throughout the ponds.

Consumption of zooplankters by *C. macularius* was common in weedy or benthic habitats, whereas minimal consumption of planktonic forms was demonstrated. Foraging on zooplankton by *G. affinis* in the benthos or in emergent vegetation was restricted compared to *C. macularius*, but moderate foraging on planktonic forms occurred. These foraging differences would explain the greater impact of *C. macularius* on some littoral Cladocera in dense rice culture during 1974. Greater reductions of zooplankton were produced by both fish in 1975 nonrice habitats, even where heavy algal densities were present; *G. affinis* foraged slightly more effectively in some cases.

Generally, both fish exhibited comparable consumption rates of beneficial insect predators, foraging primarily on coleopterous larvae. Some measure of direct mosquito predation occurred each year. Foraging on mosquito predators and zooplankton did not cause mosquito upsets or phytoplanktonic blooms at the stocking rate used.

Although *G. affinis* reproduced more rapidly in ponds, and a greater environmental impact might have been expected, the effect on organisms in the pond ecosystem appeared to have been similar to that with comparatively lower numbers of *C. macularius*.

We conclude that the native *C. macularius* would be an acceptable substitute to *G. affinis* for use in mosquito control in southwestern states. The use of *C. macularius* may be preferred in habitats containing other native fishes, as this species exhibits little piscivorous behavior compared to *G. affinis*, a known piscivore. Although both fishes are eurythermal, *C. macularius* is considerably more euryhaline in its natural habitat, easily adaptable to both fresh and saline (4.6 to 6.8 percent) conditions (Barlow, 1958*a*,*b*). *Gambusia affinis* exhibits high mortality at these salinities in the laboratory (Ahuja, 1964) and is normally found in field salinities below 2.5 percent. *Cyprinodon macularius*, therefore, may be a more practical candidate for use in diverse saline environments.

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