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Food and Feeding Habits of an Introduced Mosquitofish, *Gambusia holbrooki* (Girard) (Poeciliidae) in a Subtropical Lake, Lake Nainital, India

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Abstract

The feeding ecology and diet composition of an introduced mosquito fish, *Gambusia holbrooki* was studied in Lake Nainital for a period of one year from February, 2005 to January, 2006. The study confirmed the zooplanktivorous nature of the fish. The fish showed preference towards cladocerans, though copepods and insects also formed the significant proportion of its diet. Mosquito larvae constituted a negligible proportion of its diet. There was no significant variation in diet composition of males and females; however, diet of juvenile fish differed significantly from adults. Significant temporal variations also occurred in the diet composition of adults and juvenile fish. The study also revealed the positive relation between fish size and prey size selection. Higher values of fullness of gut were found for juvenile fish than that of adult *Gambusia*.

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Introduction

Gambusia holbrooki (Girard, 1859), Eastern Gambusia (previously called mosquito fish), is a small, viviparous fish. The fish is native to the eastern U.S.A. and has been introduced to various water-bodies worldwide as a mosquito control agent (Krumholz, 1948). In India, the fish was brought from Italy in 1928 (Sharma, 1994). In Lake Nainital, it was probably introduced by the Malaria Control Department in the 1990s s (Nagdali and Gupta, 2002). Although the fish was supposed to be a useful biological agent to control mosquitoes in the past (Chandra et al. 2008), recent studies have indicated its negative impacts on aquatic biodiversity (Ling and Wills, 2005; Weihrauch, 2006). In some studies it has also been reported that the fish may indirectly encourage the growth of mosquito larvae by feeding on cladocerans, which are strong competitors of mosquito larvae.

Although, a considerable amount of literature has accumulated on the feeding ecology of *Gambusia* in several countries (e.g. Mansfield and McArdle 1998; Blanco et al. 2004) such studies are scanty in India. The National Institute of Malaria Research, New Delhi (India) has informed the authors that *Gambusia* is distributed in many parts of India and is now being successfully used as ‘biological’ malaria control agent (Personal Communication). However, there is surprisingly lack of data on the dietary ecology of this fish in areas of introduction in India. This prompted us to carry out an extensive study on gut content analysis of *Gambusia* so that its potential for mosquito control could be evaluated. Moreover, the aims of the present investigation also included: (I) the identification of the main prey items of the fish (II) seasonal variation in diet composition of female, male and juvenile fish and (III) relationship between diet composition and fish length.

Materials and Methods

Study area

The study was conducted in Lake Nainital which is one of the national lakes of India. The lake is situated at 1937 m above sea level ($29^{\circ}24'$ N latitude and $79^{\circ}28'$ E longitude) and is a subtropical eutrophic water body. Based on thermal criteria, it is a warm monomictic lake and undergoes circulation during the winter months. The surface water temperature ranges from 9 ^{o}C in winter to above 24 ^{o}C in summer. The surface area of the lake is 48 h, mean depth 16.2 m and shoreline development is 1.20. The catchment area is 3.96 km^2, and *G. holbrooki* numerically dominates the fish community in the lake showing monospecific population in the shore area.

Sampling method

Sampling was done at monthly intervals using hand net between 10 a.m. and 1 p.m., peak feeding hours (Crivelli and Boy, 1987) of the fish, from February 2005 to January 2006. Fish were collected from the littoral zone of the lake. All mosquito fish caught were immediately preserved in 4% formaldehyde and brought to the laboratory within an hour.

In the laboratory, fish were washed and separated as males and females on the basis of sexual dimorphism. Fish smaller than 20 mm could not be distinguished as males or females, therefore, they were regarded as juveniles. Ten specimens of each category, i.e. female, male and juvenile were arbitrarily selected for dietary analysis. Length and weight measurements of each fish were taken. The entire gut was then removed and the gut contents were examined under a high magnification. Prey items were identified usually to genus or species level. Dietary importance of each food category was determined by percent number and percent frequency of occurrence. Percent number was the number of individuals expressed as a percentage, after pooling the gut contents of all fish. Frequency of occurrence was the percentage of guts where a food category was present. Time constraints prevented the use of volumetric methods. To see the seasonal variations months were categorized into three seasons: summer (March to June), monsoon (July to October) and winter (November to February). To determine the variations in diet composition caused by fish size, females were categorized into 4 size groups, viz. 20-30 mm, 31-40 mm, 41-50 mm and 51-60 mm, while males were divided into two size groups, viz. 20-30 mm and 31-40 mm. Gut fullness index was assessed after Robotham (1977): gut was divided into ten equal regions and expressed as percentage of the number of full sections.

Results

The diet of the fish composition belonged to 7 major groups namely, Insecta, Copepoda, Cladocera, Rotifera, Algae, Zoobenthos and Protozoa (Table 1). Apart from these, miscellaneous food items such as unidentified eggs, spore/cyst, etc. also contributed to the food items of the fish. Out of the various groups, Cladocera contributed 29.7 % to the total food by number. Among Cladocera, *Daphnia longispina* dominated over others. Copepoda occupied second position and contributed about 24.1 % to the total number of prey. Within copepods, *Cyclops* sp. was dominant. The third important group was Insecta (18.3 %) in which insects other than mosquitoes were significant (Table 1). Miscellaneous group (14.8 %) and Rotifera (8.2 %) were the other important sources of food for *G. holbrooki*. Algae, zoobenthos and protozoa contributed a negligible proportion to its diet. A similar trend was found in percent frequency of occurrence: the group Cladocera dominated over others and was followed by Copepoda, Insecta, Miscellaneous, Rotifera, Algae, Zoobenthos and Protozoa. Individually, *Cyclops* sp. dominated the prey items in terms of both percent number and percent frequency of occurrence, whereas *Astrameoba* sp. constituted the least proportion (Table 1). Larvae, pupae or adults of mosquitoes constituted less than 1% of the diet of *Gambusia* in Lake Nainital.

Table 1. Diet composition of *Gambusia holbrooki* in terms of percent number and percent frequency of occurrence in Lake Nainital, India.

S. No.	Prey items	% number	Frequency of occurrence
	Insecta		
1	Aerial insect	6.5	24.6
2	Insect larvae (aquatic)	12.2	35.2
3	Aerial mosquito	0.1	0.8
4	Mosquito larvae	0.33	1.6
5	Mosquito pupae	0.1	0.8
	Copepoda		
6	*Cyclops* sp.	21.8	65.4
7	Nauplius of *Cyclops*	4.2	8.1
	Cladocera		
8	*Chydorus* sp.	9.5	36
9	*Daphnia longispina*	15.2	40
10	*Ceriodaphnia* sp.	2.7	10
	Rotifera		
11	*Brachionus* sp.	2.7	11.3
12	*Keratella* sp.	4.1	16.6
13	*Filinia* sp.	0.59	4.5
	Algae		
14	Filamentous algae	1.3	15.2
15	Colonial algae	0.85	7.2
16	Diatoms	2.1	3.6
	Zoobenthos		
17	*Chironomus* sp.	0.96	9.4
	Protozoa		
18	Astrameoba	0.13	2.5
	Miscellaneous		
19	Unidentified protozoans	4.6	12
20	Eggs	6.4	18.6
21	Spores/Cyst	3.7	14.4

Number of gut analyzed= 330, number of prey in the gut contents= 4014; 8 guts were found empty.

Table 2. Diet composition of female, male and juvenile *Gambusia holbrooki* in terms of percent number and percent frequency of occurrence in Lake Nainital, India.

S. No.	Prey items	% number			% frequency of occurrence		
		Female	**Male**	**Juvenile**	**Female**	**Male**	**Juvenile**
	Insecta						
1	Aerial insect	10	4.4	0	21.7	13.6	0
2	Insect larvae	14.7	13.7	0.84	26.9	31.6	6.2
3	Aerial mosquito	0.05	0.21	0	1.7	1.7	0
4	Mosquito larvae	0.48	0.28	0	4.3	2.5	0
5	Mosquito pupae	0.16	0.07	0	1.7	0.85	0
	Copepoda						
6	*Cyclops sp.*	25.6	24.1	4.5	56.5	58.9	20
7	Nauplius of *Cyclops*	0.32	0.14	24.5	1.7	1.7	53.7
	Cladocera						
8	*Chydorus* sp.	7.7	13.4	6	33.9	29	30
9	*Daphnia longispina*	18.5	12.1	8.7	38.2	27.3	30
10	*Ceriodaphnia* sp.	2.7	3.7	0	17.3	10.2	0
	Rotifera						
11	*Brachionus* sp.	1.3	1.22	10.4	6.9	5.1	33.7
12	*Keratella* sp.	0.53	4.1	15.1	2.6	8.5	41.2
13	*Filinia* sp.	1.12	0.14	0	5.2	1.7	0
	Algae						
14	Filamentous algae	1.8	1.15	1.6	20	10.2	8
15	Colonial algae	1	0.93	0	11.3	6.8	0
16	Diatoms	0	5.9	0	0	5.9	0
	Zoobenthos						
17	*Chironomus* sp.	1	0.28	2.3	12.1	2.5	12.5
	Protozoa						
18	Astrameoba	0.16	0.14	0	0.86	1.7	0
	Miscellaneous						
19	Unidentified	1.9	4.6	12.6	5.2	11.9	45
20	Eggs	6.1	5.4	9.9	18.2	14.5	38.7
21	Spores/Cyst	4.1	3.5	3.1	12.1	8.5	8.7

Female: Number of gut analyzed= 120, number of prey in the gut contents= 2037; 5 guts were found empty.
Male: Number of gut analyzed= 120, number of prey in the gut contents= 1346; 3 guts were found empty.
Juvenile: Number of gut analyzed= 90, number of prey in the gut contents= 631

Food in relation to sex

There was no qualitative difference in the overall diet composition of female and male G. *holbrooki* but quantitatively females ate approximately 1.3 times more than males (Table 2). Cladocerans dominated the diet composition of both sexes in terms of percent number as well as in percent frequency of occurrence. Within cladocerans, females preferred *Daphnia longispina* (18.5 %), while males preferred *Chydorus* sp. (13.4 %). After cladocerans, copepods formed the significant proportion of diet of both sexes. Rotifers, algae, zoobenthos and protozoa constituted a negligible proportion of the diet. Species-wise, *Cyclops* sp. was the dominant prey item found in the guts of both sexes by number as well as by percent frequency of occurrence (Table 2).

Food of juvenile fish

Diet of juvenile fish differed significantly from that of adults. Copepoda (29 %) and Rotifera (27.1 %) dominated the diet of juveniles in terms of percent number (Table 2). Miscellaneous group and cladocerans were the other important components of the diet. The food items, frequently found in the guts of juveniles were: miscellaneous group, rotifers, copepods and cladocerans. Their frequencies of occurrence were: 92.4 %, 74.9 %, 73.7 % and 60 %, respectively. Species-wise, nauplius of *Cyclops* sp. was the abundant prey item in terms of both number and occurrence (Table 2).

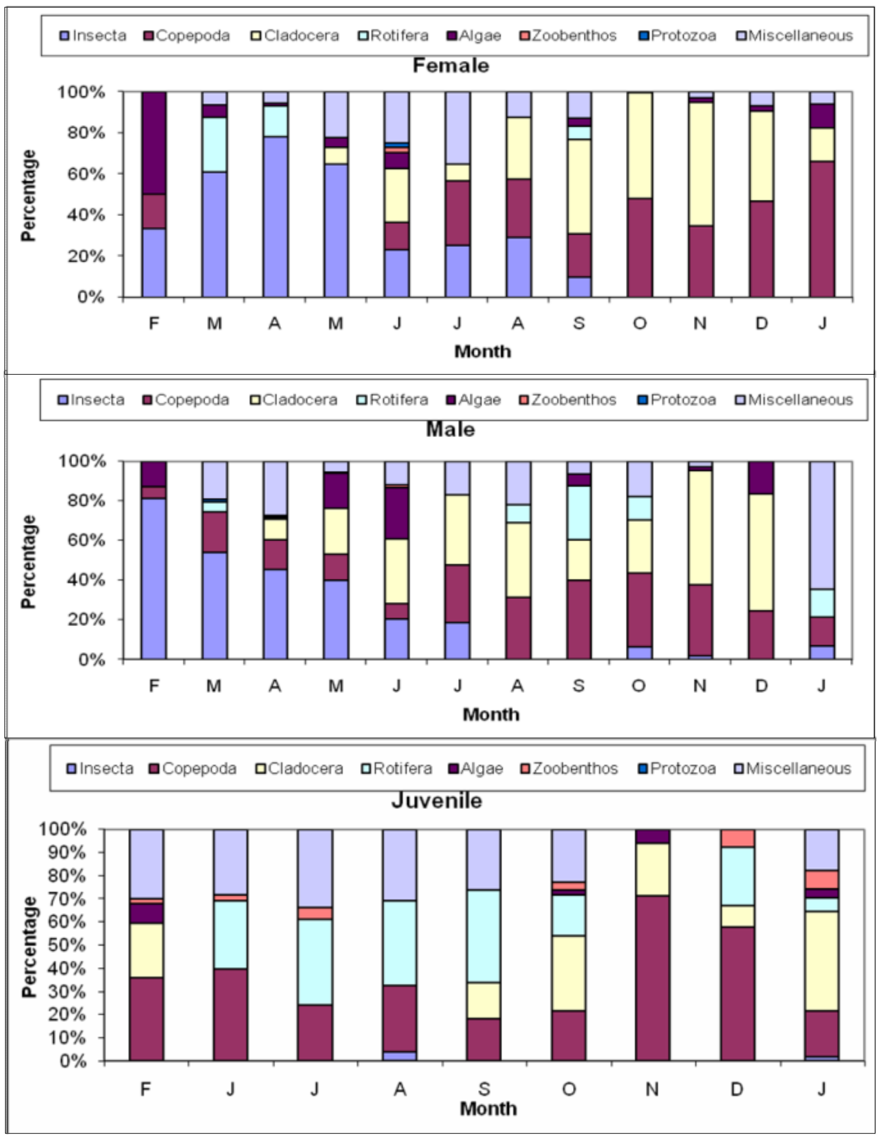

Fig. 1. Seasonal variation in diet composition of female, male and juvenile G. holbrooki in Lake Nainital, India.

Table 3. Percent number and percent frequency of occurrence in different size groups of female and male *Gambusia* in Lake Nainital, India.

S. No.	Prey Items	Size groups 20-30 % number Female	20-30 % number Male	20-30 Frequency of Female	20-30 Frequency of Male	31-40 % number Female	31-40 % number Male	31-40 Frequency of Female	31-40 Frequency of Male	41-50 % Female	41-50 Frequency Female	51-60 % Female	51-60 Frequency Female
	Insecta												
1	Aerial insect	0.53	1.2	3	3.2	4.4	3.3	21	10.4	14.4	37	10.5	20
2	Insect larvae (aquatic)	0.53	6	7	12	11.1	17	35	34.4	16.1	50	12	35
3	Aerial mosquito	0	0	0	0	0	2	0	3.2	0.51	1.6	0.7	1.2
4	Mosquito larvae	0	0.4	0	1.4	0	2.7	0	4	1.4	2.3	1.8	3.8
5	Mosquito pupae	0	0	0	0	0	0.67	0	1.4	0.51	1	1.2	2.3
	Copepoda												
6	*Cyclops* sp.	20	19.7	70	37.5	8.2	12.8	16	35	18.1	36.6	8.8	23
7	Nauplius of *Cyclops*	2.2	2	8	4.3	0	0	0	0	0	0	0	0
	Cladocera												
8	*Chydorus* sp.	28.2	14.6	50	42.3	11.1	10	50	26.3	9.1	45.7	6.1	18
9	*Daphnia longispina*	6.3	5.2	25	14.5	13.6	16.2	20	40	11.1	40	17.6	56
10	*Ceriodaphnia* sp.	0	2.5	0	5.4	3.2	0	8	0	0	0	0	0
	Rotifera												
11	*Brachionus* sp.	2	3.2	11	8.2	0	0	0	0	3	12	9.3	11
12	*Keratella* sp.	2.6	6.6	24	22	3.6	6	22	17.3	0	0	0	0
13	*Filinia* sp.	6.6	1.3	16	4	7.8	0	14	0	0	0	0	0
	Algae												
14	Filamentous algae	0.26	1	15	2.4	5.1	1.3	14	4.5	2.1	8	7.2	24
15	Colonial algae	4.2	1.6	10	5	1.4	2.7	19	8	1	11	0	0
16	Diatoms	0	5	0	6.2	0	0	0	0	0	0	0	0
	Zoobenthos												
17	Chironomus sp.	2.5	2.5	7	8.6	5.9	4	20	15	1	8	1.4	17.4
	Protozoa												
18	Astraameoba	0	0.4	0	1.2	0	0	0	0	0	0	1	2.8
	Miscellaneous												
19	Unidentified	5	8.7	18	10	9.6	8	17	16	4.6	33	1.7	4.5
20	Eggs	16.2	13.1	30	18	12.1	6	40	12	7.8	14	5.3	8.7
21	Spores/Cyst	2.9	5	21	8	2.9	7.4	15	18.4	9.3	27	15.4	34.6

Seasonal variations in the diet of the fish

In general, Cladocera dominated the diet composition of both sexes for the most part of the year. Both sexes showed significant seasonal variations in their diet ($P<0.01$) (Fig. 1). In the case of females, Insecta was the dominant group from February to April, Cladocera dominated from May to August, Copepoda dominated in September and October while Cladocera again showed its preponderance during November and December. Miscellaneous food items dominated the gut contents in the month of January by contributing 75 % of the total diet. The maximum diet diversity was found in June, while the minimum was noticed in December (Fig. 1).

Seasonal variations in diet composition of males showed the dominance of algae in February, insects from March to May, cladocerans from June to November (except July) and Copepods during December-January. The maximum diet diversity was found in June while the minimum was observed in October (Fig. 1).

Qualitatively as well as quantitatively, the food contents of juveniles differed markedly from those of the adults. In general, the juveniles fed mostly on copepods, which dominated the diet in February, June, November and December. During March, April and May, juveniles were not found in the collection. Rotifers were the dominant prey items during July, August and September while cladocerans were dominant during January. Food diversity was maximum during October and minimum during November (Fig. 1).

A perusal of Table 3 indicated that small sized individuals of both sexes ate smaller prey items while the large sized took larger prey items. Thus, both sexes showed size specific predation.

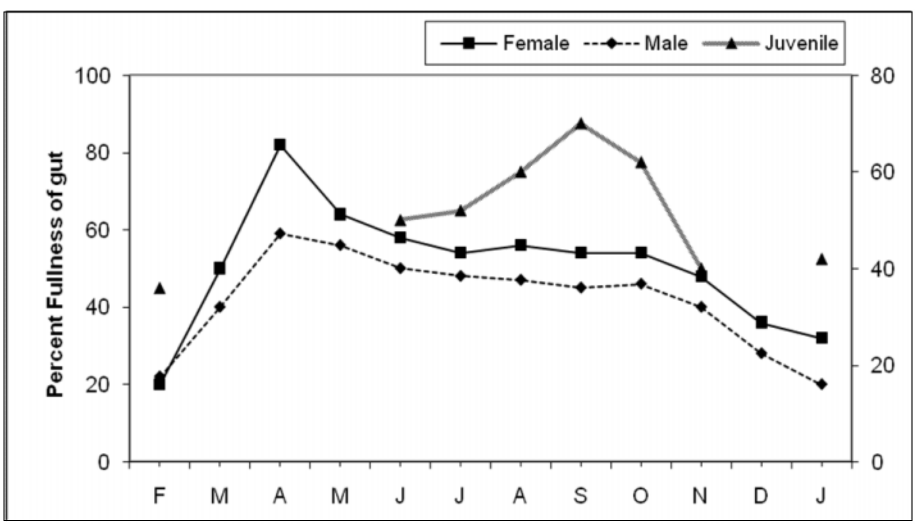

Fig. 2. Seasonal variation in percent fullness of gut of female, male and juvenile G. holbrooki during 2005-06 in Lake Nainital, India.

Fullness of gut

All the three categories of fish, i.e. female, male and juvenile showed almost a similar trend in percent fullness of gut. Higher values of fullness of gut for all the three categories of fish were noted during summer and monsoon seasons while lower values were recorded during winter. Maximum values of gut-fullness for females (70 %) and males (65 %) were recorded in June and May. Juveniles showed maximum value (74 %) of gut-fullness in August (Fig. 2).

Discussion and Conclusion

The striking feature of the diet of *G. holbrooki* is the diversity of prey consumed and the variability of the diet under different circumstances. In one study, the fish fed on zooplankton (Cladocera, Copepoda and Rotifera etc.), snails, larval chironomids, floating terrestrial insects and certain benthic insects and a variety of zoobenthos in pond ecosystems (Hurlbert et al. 1972; Hurlbert and Mulla 1981). In rice field ecosystems, it fed on rotifers, molluscs, crusustaceans, insects, and algae (Chlorophyceae and Desmidaceae) (Sokolav and Chavaliova 1936). Some studies have also reported the high proportions of plant materials in the diet of the fish (Speczier 2004). In the present investigation, *G. holbrooki* fed mostly on zooplankton, aerial insects and zoobenthos. These differences in diet composition could be due to prey availability in different habitats.

Similar to our results, several studies have reported the preference of *Gambusia* for cladocerans (e.g. Crivelli and Boy 1987) (Table 1). However, Cabral et al. (1998) has reported the dominance of copepods in the diet. In this study, copepods and insects were the other important additional sources of energy for the fish. Farley (1980) found that *Gambusia* was a generalist predator with no preference for mosquitoes. In the present study, it was also observed that, aerial mosquitoes, mosquito larvae and pupae formed the negligible proportion of its diet (Table 1). This observation supported the idea that this fish is not suitable for mosquito control. Similar to other studies (Cabral et al. 1998; Stober et al. 1998), benthic animals also formed a part of its diet. Although, *G. holbrooki* is well adapted morphologically to feeding at the water's surface, having a flattened head and terminal, upwardly directed mouth (Scott et al. 1974); the mouth morphology of Poeciliidae in general does not preclude feeding on benthic invertebrates and grazing on algae and detritus (Dussault and Kramer 1981). In *Gambusia* sp., cannibalism had been detected in dense laboratory stocks (Meffe and Snelson 1989), but no cannibalism was found in the present study.

Cabral et al. (1998) had reported differences in diet composition of male and female *Gambusia*. However, in the present investigation there was no qualitative difference in diet composition though, quantitatively females fed more intensively than males (Table 2). Juveniles differed in diet quality composition from males and females and preferred nauplius of *Cyclops* (Table 2). It appeared that in the Nainital Lake, differences in diet quality were due to differences in habitat of adults and juvenile fish.

Seasonal differences in diet reflect changes in prey abundance. Crivelli and Boy (1987) had reported the dominance of insects in the diet of *Gambusia* during summer. It was also observed that both the sexes preferred insects in early summer because insects were abundant during that time (personal observation). Later, adults fed on zooplankton extensively due to easy availability of these prey items. Juveniles preferred smaller food items in all the seasons and showed seasonal variations according to prey availability.

In the present investigation, various size classes of *G. holbrooki* differed in food composition, e.g. smaller females and males preferred smaller prey items (Table 3).This result was similar to other studies (Garcia-Berthou 1999) which had reported size specific predation. Size specific predation could be due to anatomical attributes, such as gape size and visual acuity related to fish size (Lazzaro 1987; Pankhurst 1990) or ecological factors, such as differential habitats used by large and small size fish (Crivelli and Boy 1987; Mansfield and McArdle (1998). In Lake Nainital, smaller fish preferred shallow water towards the margin of the lake, while larger fish preferred the open water away from the shore (personal observation).

The higher values of gut fullness of both the sexes during summer and monsoon could be due to higher requirement of energy for reproduction during that period. It was observed that summer and monsoon seasons were the breeding seasons of the fish. Various factors like high water temperature, abundance of food resources and high metabolic activities of the fish could also be responsible for higher values of fullness of gut during that time. The lower values of gut fullness during winter might be due to depleted food resources, low winter temperature and reduced metabolic rates to a point that digestion was inefficient or impeded (Wurtsbaugh and Ceck 1983). The higher values of gut fullness of juvenile fish than that of adult could be due to their higher metabolic activities.

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