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In Vivo Studies on the Effect of *Gambusia Holbrooki* on Planktonic Community

Nirmal Singh

Department of Zoology, D.S.B. Campus, Kumaun University,
Nainital-263002 Uttarakhand, India.
[*]Present address: Department of Zoology, University of Jammu-180003 (J&K) India.
E-mail: nirmalsingh1981@yahoo.in

Abstract

Experiments were performed in pond ecosystem with different sex of *Gambusia holbrooki* i.e. female, male and juvenile to assess the impact of different categories of fish on planktonic community. Study showed that in all the ponds with fish zooplankton number declined drastically while phytoplankton community showed excessive growth in comparison to control pond. Significant negative correlation was observed between phytoplankton and zooplankton community in ponds with female and male and juvenile fish. Different sex shows size specific predation pressure on zooplankton community. Higher values of pH, DO and low values of secchi disc transparency were noticed in all the ponds with fish in comparison to control pond. Study concludes that the fish showed the 'top-down' control on zooplanktonic community structure and abundance and thus on phytoplankton. It is suggested that the removal of this fish from Lake Nainital, could be one of the key factor in controlling the productivity of lake.
Keywords: *Gambusia holbrooki*, Zooplanktivorus fish, Top-down control, Pond Ecosystem.

Introduction

Gambusia holbrooki (Girard 1859), commonly called as Eastern mosquitofish, is a small viviparous fish. This is native to the Eastern U.S.A. and has been introduced to various water-bodies worldwide as a mosquito control agent (Krumholz 1948; Courtenay and Meffe 1989). In India the fish was brought from Italy by Dr. B. A. Rao in 1928 (Sharma 1994). In Lake Nainital, it was introduced by Malaria Control

Department in nineteen nineties (Nagdali and Gupta 2002). Although the fish was supposed to be useful biological control agent in the past (Wilson 1960), recent studies have indicated its negative impacts on aquatic bio-diversity (Komak and Crossland 2000; Pyke 2005; Rowe *et al.* 2008; Reynolds 2009).

Gambusia is highly carnivorous fish which mainly feeds on zooplankton (Singh and Gupta 2010). Due to selective predation pressure on zooplanktonic community, it results in increased phytoplankton density (Hurlbert *et al.* 1972; Hurlbert and Mulla 1981; Crivelli and Boy 1987). Nagdali and Gupta in 2002 while studying the impact of mass mortality of *Gambusia* on lake ecology has reported that *Gambusia* shows the ‘top-down’ control on zooplankton abundance and thus on phytoplankton community structure. Lake Nainital is one of the national lakes of India. It has undergone eutrophication due to high anthropogenic pressure in the catchment area. Restoration and management programmes including the use of ecotechnologies and biomanipulation are in progress.

Present investigation was designed to strengthen the hypothesis that ‘top-down control’ is equally effective in controlling the productivity of lakes as evidenced in field studies by Lampert *et al.* 1986; Griffin and Rippinle 2001; Nagdali and Gupta 2002; Sommer and Sommer 2005; Low *et al.* 2010. Selective predation of zooplankton community by different size class of *Gambusia* has been reported in literature (Hurlbert and Mulla 1981, Hurlbert *et al.* 1972) but to best of my knowledge, no study related to impact of different sex of *Gambusia* (Female, Male and Juvenile) on aquatic biodiversity has been reported This prompted the author to carry out the present study.

Material and Methods

The experiment was designed to assess the impact of *Gambusia holbrooki* on Zooplankton community and its indirect impact on phytoplankton community structure. Experiment was performed in 4 small ponds of 6× 4 × 6 feet dimension present in vicinity of Zoology Departmental, Kumaun University, Nainital (Uttarakhand) India. Out of four ponds, one pond was kept as control and 3 as experimental. The present study was carried out for a period of 12 months i.e. 1st January, 2007 to 31st December, 2007. First six months were devoted for the establishment and growth of planktonic community in all the ponds and next six months to notice the change in planktonic community and some related physico-chemical parameters (like pH, Dissolved oxygen, Water transparency) after the introduction of the fish. On 1st January, 2007 all the ponds were filled tap water and left for 6 months for proper growth of plankton. During the second month (February) all the ponds where fertilized with cow dung (5 kg in each pond) and even the inocula of plankton from the Lake Nainital collected by horizontal hauling were introduced every month till the sixth month in each pond, so that there should be sufficient food (plankton) at the time of fish introduction. On 1st July, 2007, fish were collected from the Lake Nainital with the help of long handle hand net by horizontal hauling about 3 m away from the shore and kept without feeding for 24 hours, prior to the start of the experiment so as to increase their feeding rate. Next day on 2nd July, 2007, 50 specimens of each category of fish i.e. female (size range 45-60 mm), male (size range 30-40 mm) and juvenile (size range 10-20 mm) of *G. holbrooki* were introduced in experimental ponds. No fish was introduced in control pond.

Regular monitoring of planktonic community was done fortnightly, before and after the introduction of the fish. Plankton were collected with planktonic net of 50 µm pore size fitted with plastic tube of 25 ml capacity at its base. Planktons were collected by filtering 10 liter of water from each pond. Counting of phytoplankton and zooplankton was done in bright line haemocytometer (Stephens and Gillespie 1976) and Sedgwick rafter counting cell (Welch 1948), respectively, before and after the start of experiment. Planktons were identified by following Edmondson 1959; Pennak 1958; Fitter and Manual 1986, etc. After the introduction of fishes, frequency of counting of plankton was increased to 10 days but data presented here on monthly basis for convenience. Triplicate slides were counted for each sample of zooplankton and phytoplankton. Abiotic factors like dissolved oxygen (DO), water temperature and hydrogen ion concentration (pH), were recorded by the YSI water quality monitoring system (600-XL YSI USA) and water transparency was measured by black and white secchi disc, to see is there any change in these parameters before and after the fish introduction. Statistical analysis includes t-test which was performed for comparison of data before and after the introduction of fish. To see the overall variation in population of zooplankton and phytoplankton during the 12 month study analysis of variance (F-test) was performed. The correlation coefficient between Zooplankton and phytoplankton was calculated by the data of last six months (after fish introduction).

Results

The list of zooplankton and phytoplankton identified during experiment was shown in the Table 1. During the 12 month study period a total of 27 species of phytoplankton belonging to 4 taxonomic groups were collected from all the ponds (Table 1). The group Chlorophyceae had maximum number of species (52% of the total). This was followed by Bacillariophyceae (26%), Cyanophyceae (15%) and Dinophyceae (7 %). Seventeen species of zooplankton were recorded from all the ponds belonging to three taxonomic groups namely Rotifera, Cladocera and Copepoda (Table 1). Among these, the group Rotifera had maximum number of species (10) while Copepoda had the least species number (3) (Table 1).

Table 1: List of Phytoplankton species and Zooplankton species recorded from all the experimental (as well as control) ponds during the during the 12 months study period.

S. No.	Phytoplankton Species	S. No.	Zooplankton Species
	Chlorophyceae Species		Rotifera
1	**Chlorella vulgaris**	1	Cephalodella sp.
2	Chlamydomonas sp.	2	Lecane sp.
3	Eudorina elegance	3	Rotaria sp.
4	Clostridium sp.	4	Asplanchna sp.
5	Closterium sp	5	Colurella obtuse
6	Chlorococcum sp.	6	Philodinavus paradoxus
7	Ankistrodesmus falcatus	7	Philodina roseola
8	Scenedesmus sp.	8	Keratella sp.
9	**Pediastrum duplex**	9	Mytilina sp.
10	P. simplex	10	Brachionus nilsoni
11	Actinastrum sp.		Cladocera
12	Desmidium sp.	11	Chydorus sp.
13	Pandorina sp.	12	Daphnia longispina
14	Oocystis sp.	13	Diaphanosoma exisum
	Bacillariophyceae	14	Ceriodaphnia sp.
15	**Navicula sp.**		Copepoda
16	Fragilaria sp.	15	Tropocyclops pracinus
17	Cymbella sp.	16	Cyclops vicinus
18	Tabellaria sp.	17	Eucyclops serrulatus
19	Diatoma sp.		
20	Gomphonema sp.		
21	Synedra sp.		
	Cyanophyceae		
22	**Merismopoedia sp.**		
23	Anabaena spiroids		
24	Chroococcus sp.		
25	Microcystis aeruginosa		
	Dinophyceae		
26	Peridinium sp.		
27	Ceratium sp.		

During the first sixth months, there is an increase in total phytoplankton and total zooplankton number in all the ponds (Figure 1). But after the release of female, male and juvenile *G. holbrooki* in there respective experimental ponds, there is a significant decrease in total zooplankton number at the end of the experiment (Figure 1). The total zooplankton number recorded at the initial stage of experiment with female was 1120 ind./l, with male 900 ind/l and with juvenile 800 ind/l which subsequently reduced to 72 ind./l, 190 ind/l and 240 ind/l respectively, after six months of the experimentation

(Table 2). In contrary to experimental ponds, in control pond significant (p<0.001) increase (60 %) in zooplankton number was observed (Table 2).

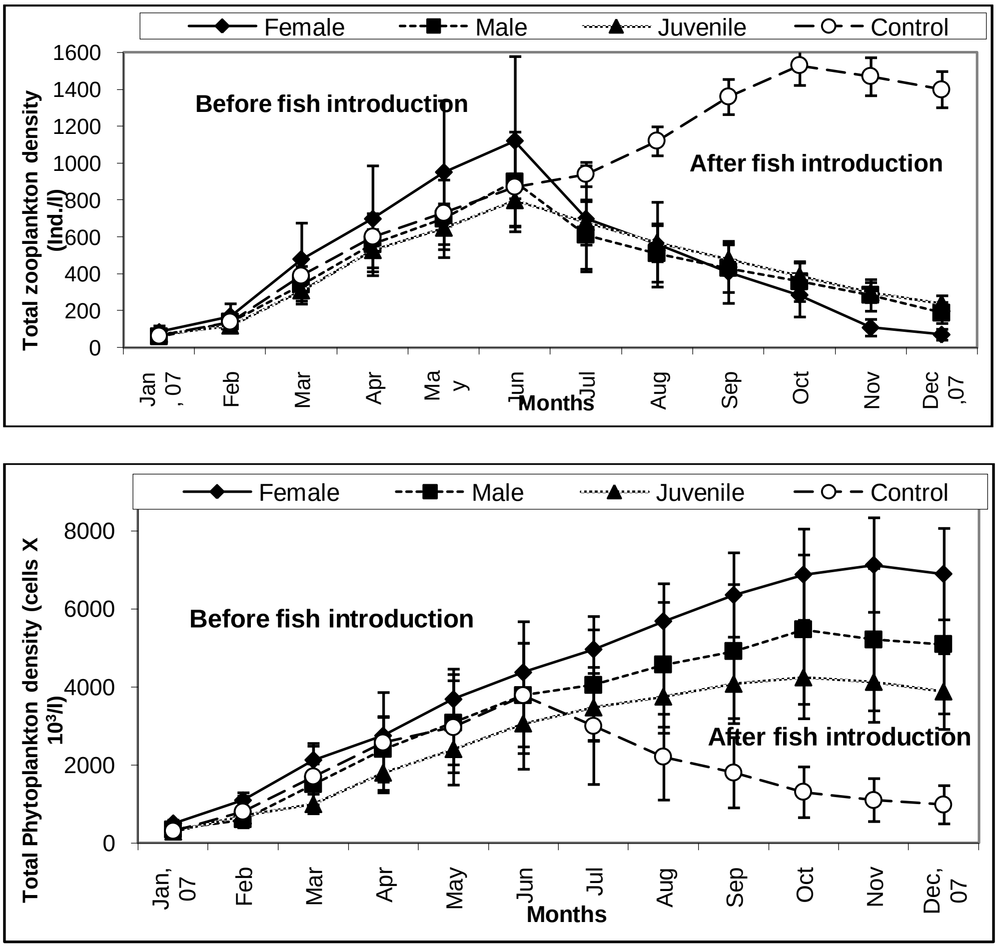


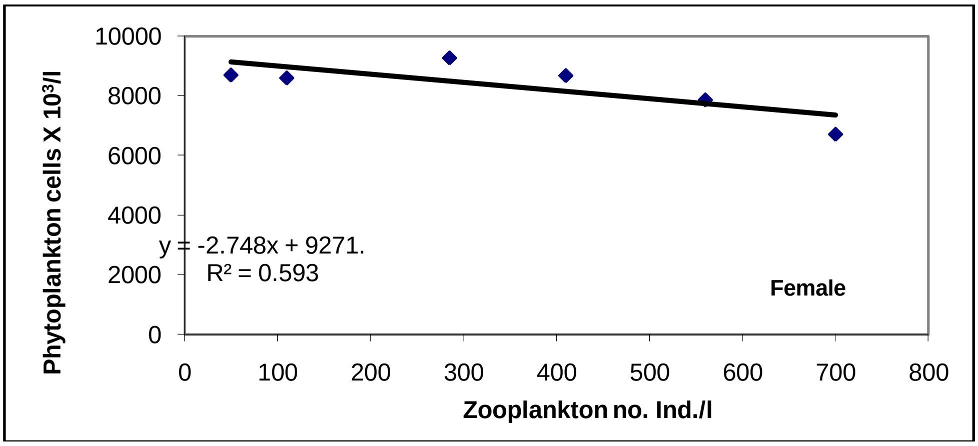
Figure 1: Comparison of total zooplankton and total phytoplankton number before and after the introduction of the fish.

Phytoplankton community abundance was reduced significantly (p<0.001) from 3780 × 10^3 cells per liter to 980 × 10^3 cells per liter after 6 months of experiment in control pond (Table. 2, Figure 1). Contrary to control, in experimental ponds with fish viz. female, male and juvenile the phytoplankton abundance increased from that of initial. In experiment with female *G. holbrooki*, phytoplankton number increased by 58 %, the increase was 35 % in pond with male while with juvenile fish the increase was 27 % after 6 months of experimentation (Table. 2).

A significant (p<0.001) negative co-relation was observed between phyto- and zooplankton density in all the experimental ponds with female and male and juvenile fish (Figure 2). However, all the three categories of the fish (female, male and juvenile) have cascading effect on zooplankton community abundance, even though the amplitude is different and selective (Figure 3). The effect of different categories of the fish on zooplankton community structure was selective in the sense that female *G. holbrooki* rapidly depleted cladocerans and unidentified insect population, male preferred copepods and cladocerans while juveniles had a cascading effect on rotifers (Figure 3). A comparison between the three categories of the fish revealed that females had more cascading effect over zooplankton, thus supported massive growth of phytoplankton in experiment with female fish (Table 2). On the other hand juveniles fed mainly on rotifers, and let the large sized zooplankton to graze upon phytoplankton. Hence phytoplankton population did not show excessive growth in experimental pond with juvenile. Males have a moderate impact on zooplankton density. In general, female, male and juveniles of *G. holbrooki* have reduced the zooplankton population and thus enhanced the phytoplankton growth (Table 2).

Table 2: Showing the details of change in total phytoplankton and zooplankton number before and after the fish introduction in experimental ponds (viz. female, male and juvenile) in comparison to control pond (without fish).

Fish Category	Total number of phytoplankton (Cell × 103 per litre) in control & experimental ponds				Total number of zooplankton (Ind. per litre) in control & experimental ponds			
	Initial reading before fish introduction (June)	Final reading after 6 months (December)	% of Increase or Decrease	Significant level	Initial reading before fish introduction (June)	Final reading after 6 months (December)	% of Increase or Decrease	Significant level
	Mean values ± S.D				Mean values ± S.D			
Control	3780 ± 190	980 ± 57	74 % Decrease	p<0.001	870 ± 33	1400 ± 54	60% Increase	p<0.001
Female	4375 ± 243	6890 ± 290	58 % Increase	p<0.001	1120 ± 70	72 ± 9	94% Decrease	p<0.001
Male	3789 ± 165	5090 ± 186	35 % Increase	p<0.001	900 ± 33	190 ± 14	79% Decrease	p<0.001
Juvenile	3056 ± 142	3880 ± 210	27 % Increase	p<0.01	800 ± 41	240 ± 11	70% Decrease	p<0.001



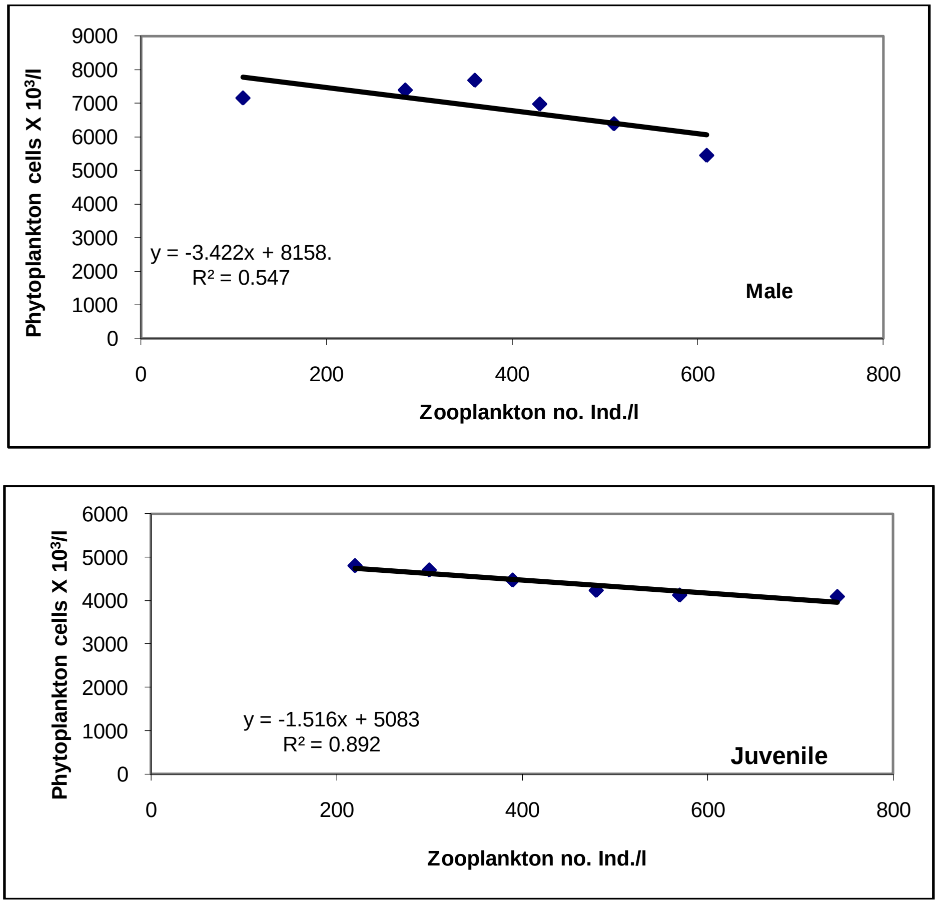
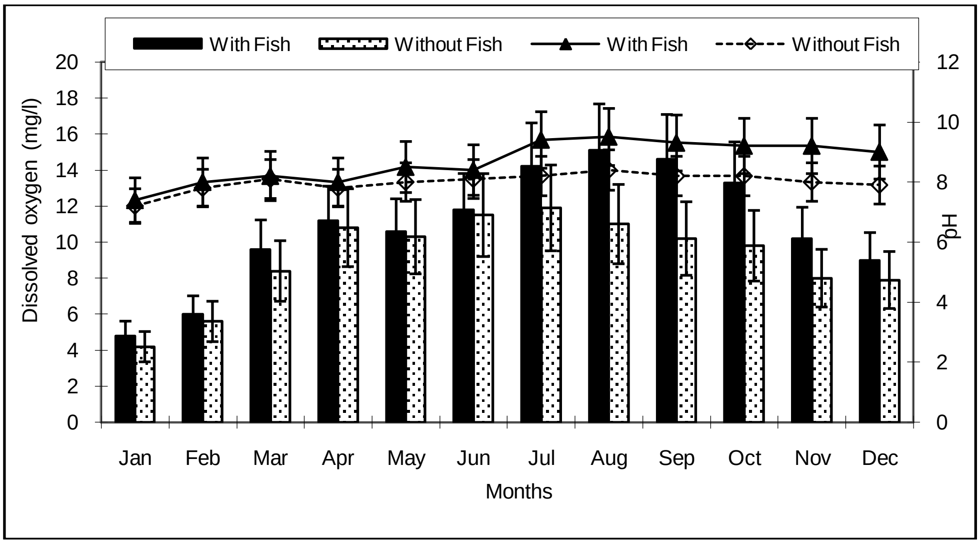


Figure 2: Correlation between phytoplankton and zooplankton density in experimental ponds with female, male and juvenile fish.

Interestingly the higher values of dissolved oxygen (DO) and pH were recorded in all the ponds with fish (after fish introduction) in comparison to control pond (Figure 4). The values of DO ranged from 4.8 mg/l (January) to 15.1 mg/l (August) in pond with fish while it varied from 4.2 mg/l (January) to 11.9 mg/l (July) in control pond. The pH values in control pond remained low (7.2-8.4) in comparison to experimental pond (7.4-9.5) during the 12 months study period (Figure 4). The high water transparency values were noted before fish introduction in both control and experimental ponds but water transparency values were significantly ($p<0.05$) reduced at the end of experiments in ponds with fish due to excessive growth of phytoplankton. Water temperature remained almost same in all the ponds (Figure 4). No mortality of fish occurred in any of the pond during the experiment.



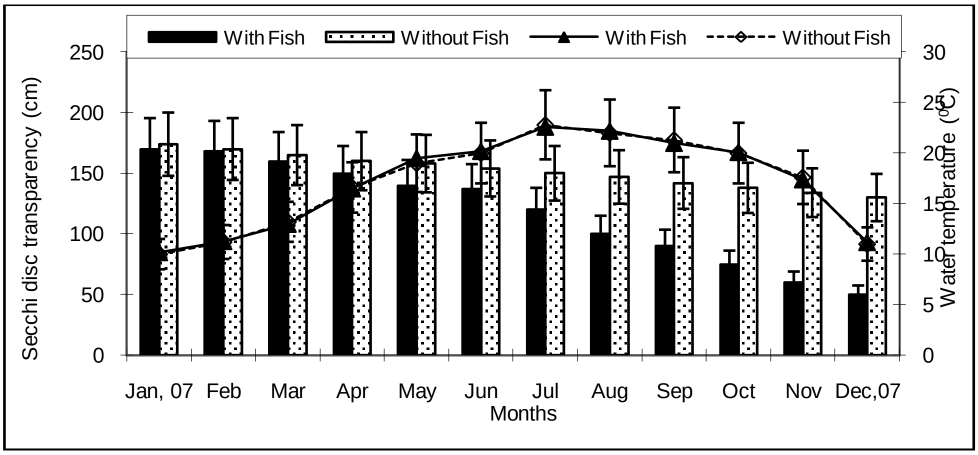


Figure 4: Seasonal variation in Dissolved oxygen (DO), pH, Secchi disc transparency and water temperature during the 12 months study period. (In all experimental ponds, almost similar trend of DO, pH, Secchi disc transparency and water temperature was found i.e. why single mean value is shown for convenience).

Discussion

The result of the present study shows that *G. holbrooki* has high grazing pressure on zooplankton community structure which leads to excessive growth of phytoplankton (Fig.1, Table 2). A significant reduction in zooplankton number in ponds with fish suggested that this fish had cascading effect on zooplankton community structure and abundance. However, in control pond (without fish) significant ($p<0.01$) increase (60 %) in zooplankton number was observed. Earlier studies both in laboratory and field by Hurlbert *et al.* 1972; Hurlbert and Mulla 1981; Margaritora *et al.* 2001; Nagdali and Gupta 2002; Ning *et al.* 2010; Susie *et al.* 2011; Gkenos *et al.* 2012 suggested that *G.*

holbrooki had top-down control on zooplankton community structure which in turn had cascading effect on phytoplankton density.

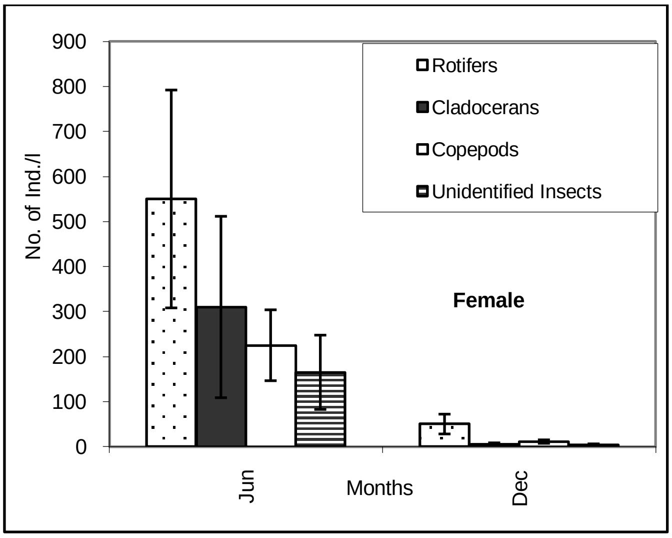
The strong inverse relationship between phytoplankton and zooplankton in the present study in experiments with fish (female, male and juvenile) supported the view that zooplankton grazing has substantial effect on phytoplankton community (Figure 2). The results of studies by Porter 1973; Gliwicz 1977; Lynch and Shapiro 1981; Sommer and Sommer 2005; Low *et al.* 2010; Symons *et al.* 2012 supported the present investigation.

The interesting results obtained in experiments with different categories of fish (female, male and juvenile) suggested that the feeding by fish is size-specific, i.e. large fish fed on large sized zooplankton while the smaller one on small sized zooplankton. Size specific predation by *Gambusia* was demonstrated by Bence and Murdoch 1986; Arthington 1989 and Mansfield and McArdle 1998 in their respective studies.

The decrease in zooplankton number in the experimental pond with female *G. holbrooki* was maximum while minimum with juvenile and remained moderate in experiment with male. Interestingly, maximum growth of phytoplankton was noticed in experiments with female fish while least with juveniles and moderate with male, which is exactly in reverse order with the reduction of zooplankton in these experiments (Figure 1). Intensive feeding of female on all the groups of zooplanktons seems to be reasonable for its viviparous nature (Vargas and de Sostoa 1996). Female and male has reduced the cladocerans (*Daphnia* sp. and *Ceriodaphnia* sp.) number, drastically while juvenile mainly fed on rotifers (Figure 3). Predation of *Gambusia* on herbivorous zooplanktons (*Daphnia* sp. and *Ceriodaphnia* sp.) in experimental ponds seems to be the one reasonable explanation for the increase phytoplankton community. Many investigations indicated that *Daphnia* is the most powerful grazer within the group of filter feeding-feeding zooplankton in lakes (e.g. Knisley and Geller 1986; Kasprzak *et al.* 1999). In the present study the maximum phytoplankton growth was observed in experiment with female fish and least in experiment with juvenile fish because large sized zooplankton are more effective in controlling the phytoplankton growth than small sized zooplanktons (Lynch and Shapiro 1981; Schoenberg and Carlson 1984).

A significant ($p<001$) decline (74 %) in phytoplankton density in control pond was due to grazing of zooplanktons while increase in phytoplankton density in experimental ponds may be due to direct grazing of fish on zooplankton as well as due to indirect effects such as nutrient inputs from fish excretion (unpublished data) (Figure 1).

pH and dissolved oxygen values were higher in fish ponds than in control ponds after the introduction of fish. This might be due to difference in greater abundance of phytoplankton density in fish ponds than in control pond. However the exact reason for increase in pH and dissolved oxygen in all ponds with fish is not known. Low water transparency values in all the ponds with fish were due to higher phytoplankton density in comparison to control ponds. Similar results were found by Hurlbert *et al.* 1972 and Hurlbert and Mulla 1981.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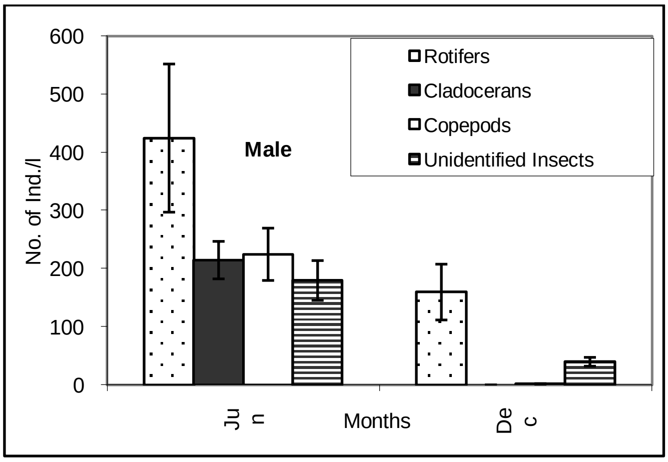
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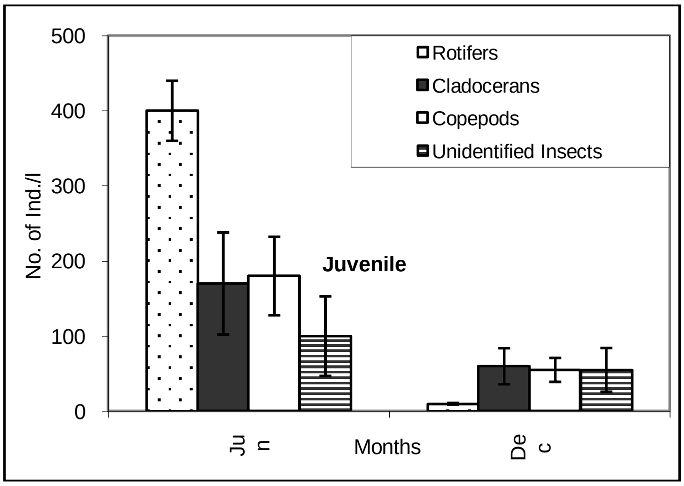
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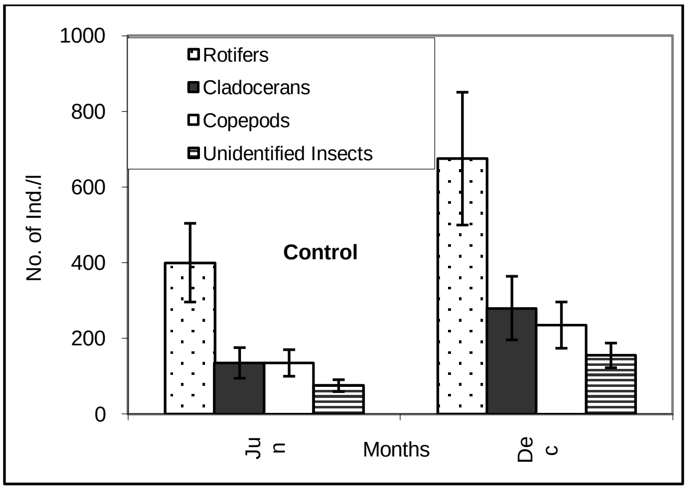

Figure 3: Comparison of Rotifers, Cladocerans, Copepods and Unidentified insects number before the introduction of the fish (Initial reading of June month) and after the six months of fish introduction (Final reading of December).

Conclusion

The present study clearly reveals that all the three categories of *G. holbrooki* (viz. female, male and juvenile) have suppressed zooplankton community structure and abundance in general. The selective predation pressure of *G. holbrooki,* lead to the change in food web structure, resulting in ecosystem alteration. Study also suggested that there was a strong controlling effect of zooplankton on phytoplankton and thinning of zooplankton by *G. holbrooki* increases the phytoplankton density.

In this context, I strongly recommended the removal of *Gambusia* from Lake Nainital which may be a key factor for conservation and management of the lake.

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