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RESEARCH ARTICLE

Excretion of nitrogen and phosphorus by *Gambusia holbrooki*

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Abstract

Excretion experiments were performed to assess the rates of Nitrogen and Phosphorus excretion by male and female *Gambusia holbrooki*. The effects of feeding (fed and unfed fish) and time since feeding (0, 4, 8 and 24 hours) on the N and P excretion rates and N:P ratio excreted were also examined. Results showed that N and P excretion rates significantly increased after feeding and declined thereafter for both male and female *Gambusia*. The P excretion rate decreased more rapidly after feeding than did the N excretion rate, thereby increasing the excreted N: P ratio with time since feeding. Males excreted more N and P in comparison to female son mass specific basis. The high density of *Gambusia holbrooki* in Lake Nainital is strongly correlated with nitrogen and phosphorus concentrations in the surface water of the Lake Nainital. It was concluded that nutrients excreted by *Gambusia* could be an important source of nutrients (bottom up control) for the phytoplankton community of Lake Nainital.

INTRODUCTION

Consumers in higher trophic levels can play important roles in controlling the primary producers through both top-down (Carpenter *et al*. 1987; Wooton and Power 1993) and bottom up effects (Andersson *et al*., 1988; Carpenter *et al*., 1992). The cascading effect on primary producers through grazing on zooplankton by fish has been well documented in literature (Carpenter *et al*., 1987; Spiller and Schoener, 1995). However, nutrient excretion by fish is also recognized asa potentially important mechanism in controlling the productivity of lakes (Carpenter *et al*., 1992; Schindler *et al*., 1993). Nutrients excreted by fishes have been shown to affect phytoplankton, abundance, biomass and community composition (Vanni and Findlay, 1990; Vanni and Layne, 1997). Depending upon feeding habits and movement patterns of fishes, this effect can increase nutrient recycling rates or can add a new source of nutrients to phytoplankton (Hurlbert *et al*., 1972; Vanni, 2002).

Gambusia holbrooki (Girard 1859), the Eastern mosquitofish, is a small viviparous fish, native to the Eastern U.S.A. which has been introduced to various water-bodies worldwide as a mosquito control agent (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 the Malaria Control Department in the 1990s (Nagdali and Gupta, 2002). Although the fish was supposed to be a useful biological control agent (Wilson, 1960), recent studies have indicated its potential negative impacts on aquatic biodiversity (Pyke, 2005; Reynolds, 2009; Stanback, 2010; Buttermore *et al*., 2011; Geoffery and Christopher, 2012; Singh, 2013).

Gambusia is highly carnivorous fish which feeds primarily on zooplankton (Singh and Gupta, 2010). It is presumed to impact the phytoplankton indirectly by affecting the structure and size composition of zooplankton and directly by excreting nutrients in forms usable for phytoplankton growth. Many studies (i.e., Hurlbert *et al*., 1972; Hurlbert and Mulla, 1981; Nagdali and Gupta, 2002) have reported the top-down effect shown by *Gambusia* on phytoplankton community structure and abundance due to grazing on zooplankton. However, there has apparently been little investigation of the role of nutrient excretion by *Gambusia holbrooki*. This prompted the authors to carry out the present investigation, which compared excretion by male and female *Gambusia* and looked at changes in excretion after the cessation of feeding.

Materials and Methods

Experimental setup for Excretion Measurements of Field-collected *Gambusia*

The methodology of the present investigation was modeled after Mather *et al*., 1995. Experiments were performed with female and male *Gambusia* to quantify the nitrogen and phosphorus excreted by the two sexes of the fish separately, due to the substantial differences in mass (Table I).Effects of feeding and time since feeding on nutrient excretion were also taken into consideration. Series of excretion experiments were performed with fed *Gambusia* (3^{rd}, 7^{th} and 9^{th} May, 2007) and unfed *Gambusia* (4^{th}, 8^{th} and 10^{th} May, 2007). The experiments were performed with three glass aquaria (60 X 30 X 30 cm) each time. Out of three glass tanks, two were kept as experimental tanks (with fed or unfed fish) one as control (without fish).

Measurement of excretion rates for Fed *Gambusia*

Gambusia was collected from Lake Nainital using a long handled hand net. Collection was made between 10 a.m. and 1 p.m., which are the peak feeding hours (Crivelli and Boy, 1987). Thus, field-collected fish were considered as fed fish. Each glass aquarium was filled with 4L of pre-filtered lake water. The lake water was filtered through a 1 µm pore size glass fiber filter to remove algae. Fish were separated as male and female on the basis of sexual dimorphism (Singh and Gupta, 2010).In the field, fifteen females and fifteen males were immediately transferred to separate plastic jars, each of which contained 1 L of pre-filtered lake water, so that excretion during transportation to the laboratory could also be included. The fish along with lake water were put into the experimental aquaria. One litre of pre-filtered lake water was also added to the control aquarium (without fish), bringing the final volume of water in each aquarium to 5 L. A sample of 20 mL water was taken from each aquarium for analysis of NH_3-N and PO_4-P at time intervals of 0, 4, 8 and 24 hours. Water samples for N and P were analyzed by using a YSI- Photometer-9100, following A.P.H.A., 1989.Changes in N and P concentrations over time were converted to excretion rates per hour, correcting for the decrease in volume following each sampling interval. Values in micrograms were converted to molar units and both units are shown in the results.

Measurement of excretion rates for Unfed *Gambusia*

For experimentation with unfed *Gambusia,* fishes were collected from the lake one day before the experimental dates and were brought to the laboratory. Field collected *Gambusia* were separated as male and female and kept for 24 hours without food so that their guts would be empty. The remainder of the experimental procedure was same as for the fed fish. Thus excretion measures were conducted on a total of 180 individuals, with 15 in each tank and 45 in each treatment. The average excretion values of 45 females and 45 males for N and P are shown in the results.

Abiotic factors such as pH, water temperature and dissolved oxygen were recorded directly using a multi-parameter YSI 600 XL probe during each experiment. No mortality of fish occurred in any of the aquaria during experimentation. The fish were sacrificed at the end of the experiment for length and weight measurements.

For both female and male *Gambusia*, a two way analysis of variance (ANOVA) using SPSS-12.0 software was used to check feeding effect and time, because feeding can influence N and P excretion rate and the N: P ratio excreted.

Seasonal variation in total abundance of *Gambusia holbrooki* and concentrations of NH_3-N and PO_4-P in Lake Nainital

To evaluate whether there is any relationship between seasonal variation in fish density of *G. holbrooki* and concentration of NH_3-N and PO_4-Pin Lake Nainital, fish and water samples were collected from three sampling sites within the lake from January, 2005 to December, 2005. Locations of the sites, viz. 'Tallital' (near pump house), 'Tallital' (near sluice gate) and 'Mallital' (near Naina Devi temple) are shown in Figure 1.

Fish were collected monthly using along handled net of 28 cm diameter with 1mm mesh size. The hauling was done horizontally from the shore of the lake for a distance of about 2-3 m. Collected fish were preserved in 4% formalin and were brought to the laboratory. In the laboratory, fish were washed thoroughly with water and their number was recorded.

Water samples were collected in acid cleaned broad mouth bottles from the three sampling sites and brought into the laboratory for analysis of NH_3-N and PO_4-Pusingthe previously mentioned method.

RESULTS

Nitrogen excretion rates for female and male *Gambusia*

Mean N excretion rates ranged from 2.1 to 3.6 μ mol $g^{-1}h^{-1}$for fed females, and from 0.4 to 0.7 μ mol $g^{-1}h^{-1}$ for unfed females (Fig. 2). Fed females excreted more N than unfed fish at all times (Fig. 2, Feeding effect, $P<0.01$, Table II). Overall, N excreted by fed females decreased through time because fish excreted more N immediately after eating (0-4, 4-8 h) than during the 8-24 h interval(Fig. 2, Time effect, $P<0.01$, Table II). Feeding and time had a significant interaction effect(Interaction effect, $P<0.01$, Table II) N excretion rates remained low throughout the experiment for unfed fish but were consistently higher for fed fish (Fig. 2).

Mean N excretion rates ranged from 2.8 to 4.7 μ mol $g^{-1}h^{-1}$for fed males and from 0.7 to 1.3 μ mol $g^{-1}h^{-1}$for unfed male (Fig. 2). The nitrogen excretion rate of unfed fish remained almost constant through time but for fed fish, the N excretion rate was high immediately after feeding and decreased with time (Fig. 2, $P<0.01$, Table II). The N excretion rates of fed males differed significantly ($P<0.05$, Table II) from the low and constant excretion rates of unfed male.

Phosphorus excretion rates for female and male *Gambusia*

Mean P excretion rates ranged from 0.06 to 0.24 μ mol $g^{-1}h^{-1}$for fed females, and from 0.01 to 0.05 μ mol $g^{-1}h^{-1}$for unfed females (Fig. 3). Overall, fed fish had higher P excretion rates than unfed fish (Fig. 2; Feeding effect, $P<0.01$, Table II). Phosphorus excretion was highest immediately after eating (0-4 h) and declined through time (Fig. 3; Time effect, $P<0.01$, Table II). The effect of time varied with feeding regime, i.e., for fed fish, P excretion rates declined markedly through 24 h, whereas for unfed fish, P excretion rates remained low throughout the experiment (Fig. 3, Interaction effect, $P<0.01$, Table II).

Mean P excretion rates ranged from 0.07 to 0.27 μ mol $g^{-1}h^{-1}$ for fed male and from 0.02 to 0.08 μ mol $g^{-1}h^{-1}$ for unfed males (Fig. 3). In total, fed males had higher P excretion rates than unfed fish (Fig. 3, Feeding effect, $P<0.01$, Table II). Phosphorus excretion was highest immediately after feeding (0-4 h) and declined through time (Fig. 3; Time effect, $P<0.01$, Table II). Unfed males excreted low levels of P throughout the experiment (Fig. 3).

N: P ratio excreted for female and male *Gambusia*

The mean molar ratio of excreted N to P ranged from 15.7: 1 to 35: 1 molar for fed females and 14: 1 to 25: 1 molar for unfed female *Gambusia* (Fig. 4). Fed fish excreted a higher N: P ratio than unfed fish (Fig. 4, Feeding effect, $P<0.01$, Table II). For both fed and unfed female, the N: P ratio increased through time (Fig. 4; Time effect, $P<0.01$, Table II).

The mean molar ratio of excreted N : P ranged from 17: 1 to 40 : 1 molar for fed males and 16.2 : 1 to 35 : 1 molar for unfed males. Fed males excreted a higher N: P ratio than unfed fish (Fig. 4, Feeding effect, $P<0.01$, Table II). For both fed and unfed fish, the N: P ratio increased through time (Fig. 4, $P<0.01$, Table II).

Abiotic factors

There was very little variation among the values of pH, water temperature and DO among different experiments performed with fed and unfed *Gambusia*. Therefore mean values ± standard deviations were shown in the Table I.

Seasonal trends in total abundance of *G. holbrooki*, NH_3-N and PO_4-P in Lake Nainital

The population abundance of the fish varied from 14 indiv./haul (December) to 70 indiv./haul (April) during the 12 months of the study (Fig. 5). Annual mean values were 41 indiv./haul. In general, the abundance was greater during March to September and lower during October to February (Fig. 5).

The value of NH_3-N in surface water of Lake Nainital varied from 160 µg/l (October) to 1500 µg/l (August) and that of PO_4-Pfrom 70 µg/l (January) to 324 µg/l (August) during the 12 months of the study (Fig. 5). The values of NH_3-N were generally remained high from April to September and PO_4-P from March to June and low during rest of the months (Fig. 5).

The population density of the fish in Lake Nainital showed a significant positive correlation with NH_3-N (r^2= 0.77, P< 0.01) and PO_4-P (r^2= 0.65, P<0.01) (Fig. 6).

Table 1. Biotic and abiotic factors recorded during the experiment. Mean values were shown for abiotic factors because value did not vary substantially during the study period.

Biotic factors			
S. No	**Fish Category**	**Size Range (mm)**	**Weight Range (mg)**
1	Female *Gambusia*	40 - 60	630-2630
2	Male *Gambusia*	25 - 35	230-280
Abiotic factors			
S.No.	**Parameters**	**Mean values ± S.D**	
1	Water temperature (^{o} C)	19.7 ± 0.22	
2	Water pH	7.8 ± 0.13	
3	Dissolved oxygen (mg/l)	7.5 ± 0.53	

Table 2. The results of an two way ANOVA testing the effects of feeding and time since feeding on the excretion rates of NH_3–N (µmol N .g $fish^{-1}$ $.h^{-1}$), PO_4-P (µmol N .g $fish^{-1}$ $.h^{-1}$) and N:P ratio excreted by female and male *Gambusia* (ns means Non-significant).

Fish category	**N excretion rate**	**P excretion rate**	**N:P ratio**
Female			
P value			
Feeding (Fed, Unfed)	<0.01	<0.01	<0.01
Time (0-4, 4-8, 8-24)	<0.01	<0.01	<0.01
Interaction	<0.01	<0.01	<0.05
Male			
P value			
Feeding (Fed, Unfed)	<0.01	<0.01	<0.01
Time (0-4, 4-8, 8-24)	<0.01	<0.01	<0.01
Interaction	<0.05	<0.01	ns

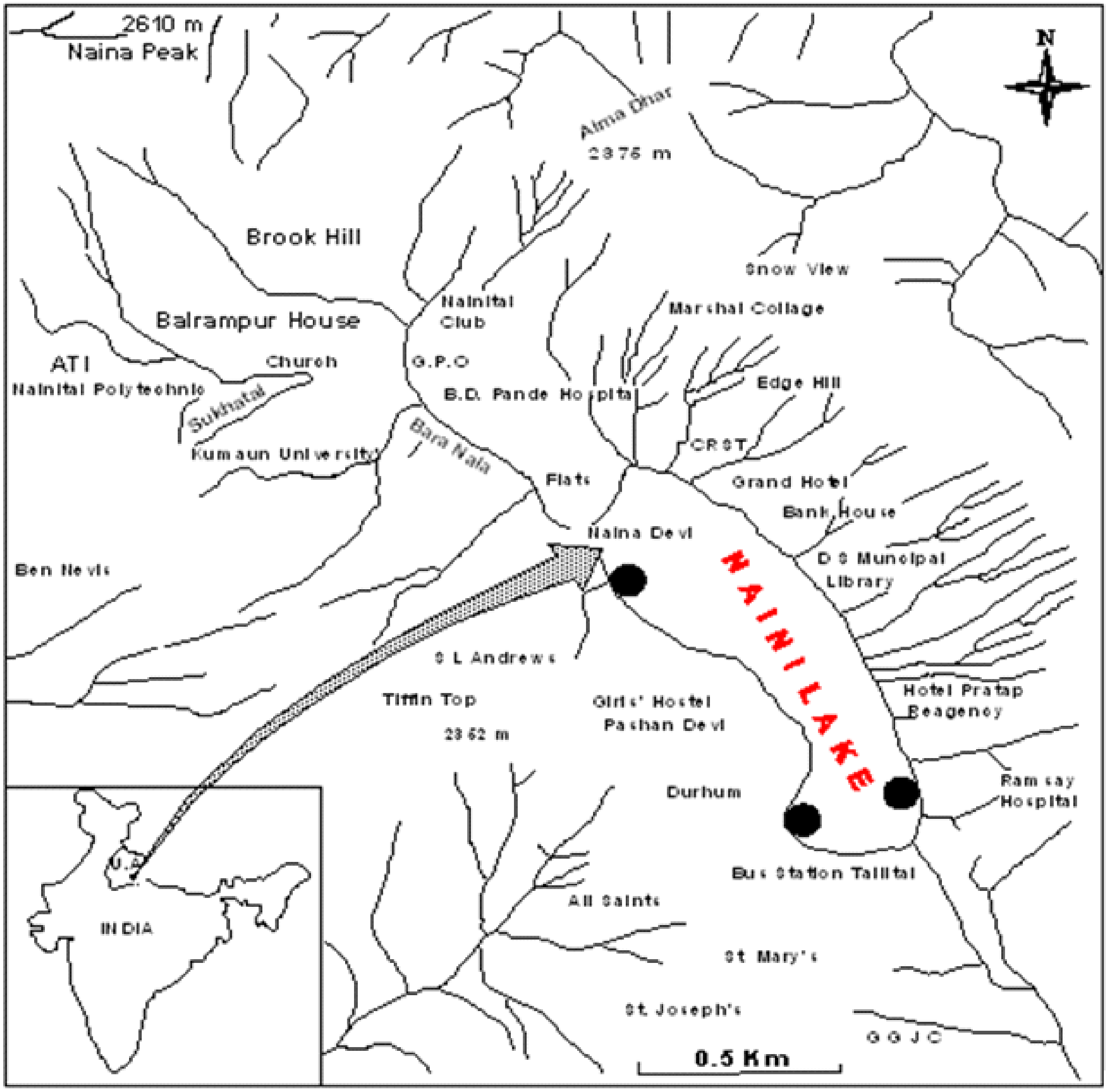

Fig. 1. Map of Lake Nainital (29°24' N latitude and 79°28' E longitude) showing sampling stations for collection of *Gambusia* and water samples for analysis of NH_3-N and Po_4-P (adapted from Valdiya, 1988**)**

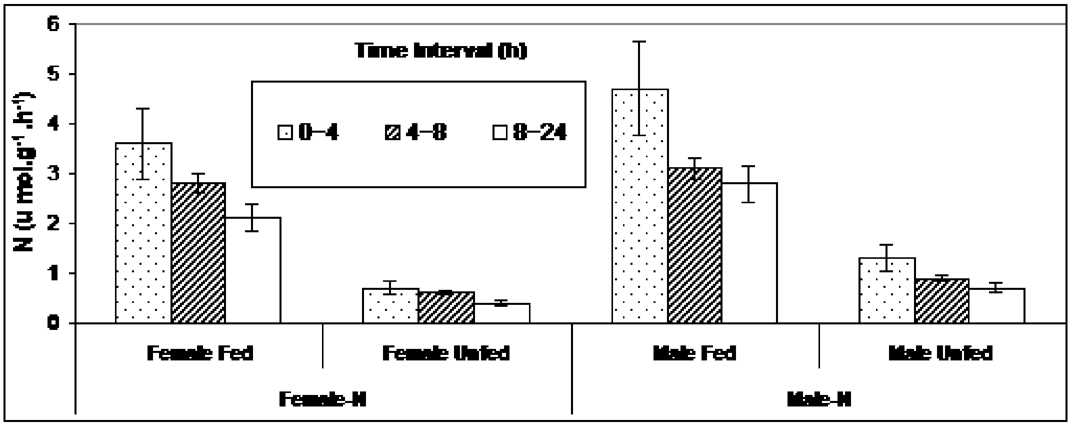

Fig. 2. Excretion rates of female and male *Gambusia holbrooki*: ammonia-N (µ mol.N.g fish $^{-1}$.h^{-1}), through time in treatments with fed fish and unfed fish. Vertical bars indicate standard error (±S.E).

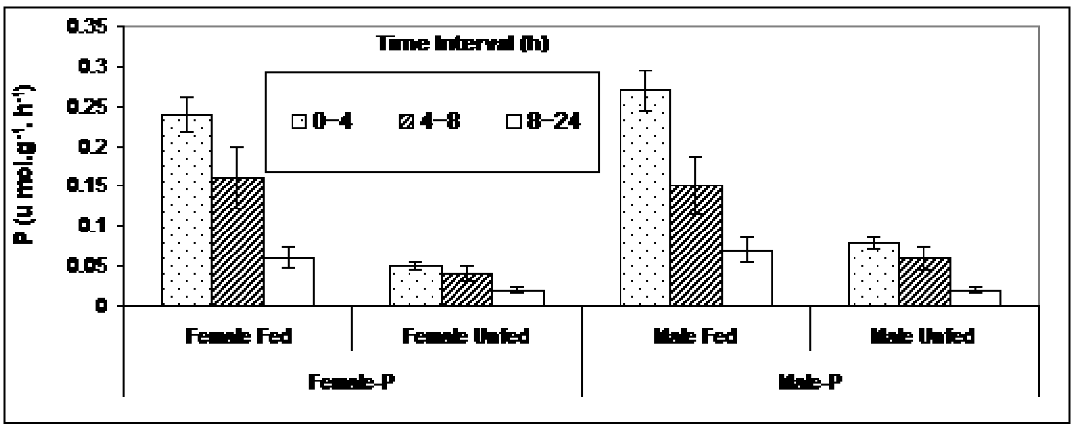

Fig. 3. Excretion rates of female and male *Gambusia holbrooki*: soluble reactive phosphorus (µ mol.N.g fish $^{-1}$.h^{-1}), through time in treatments with fed fish and unfed fish. Vertical bars indicate standard error (±S.E).

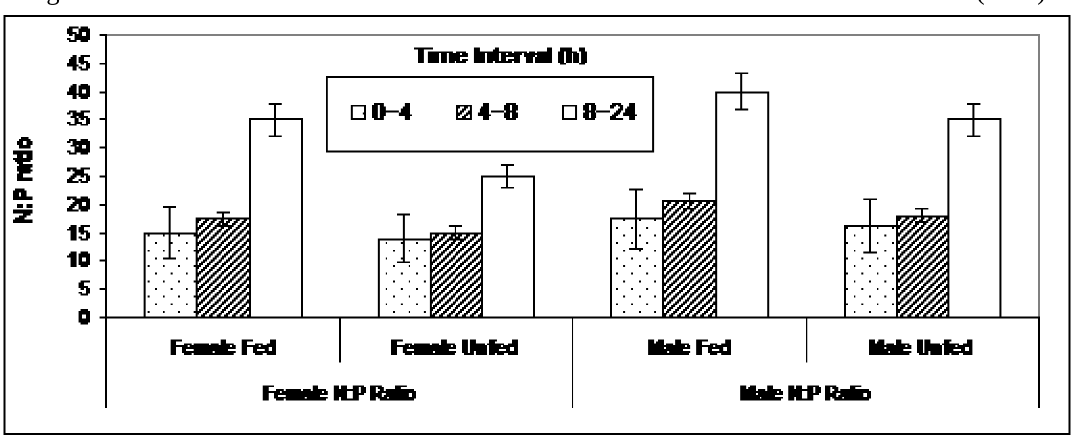

Fig. 4. Excretion rates of female and male *Gambusia holbrooki*: the ration of excreted nitrogen to phosphorus (molar), through time in treatments with fed fish and unfed fish. Vertical bars indicate standard error (±S.E).

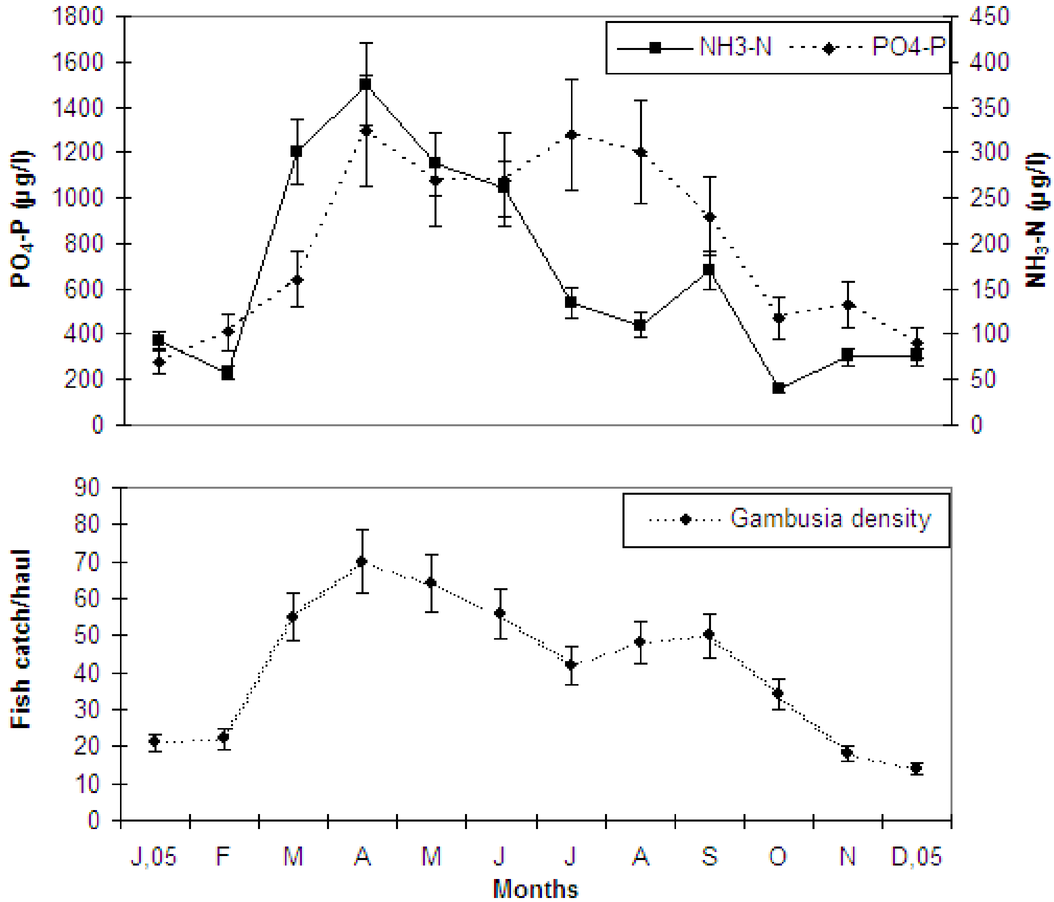

Fig. 5. Seasonal variations in population abundance of *Gambusia holbrooki* and NH_3-N (µ g/l) and PO_4-P (µ g/l) in Lake Nainital. Vertical bars indicate standard error (±S.E).

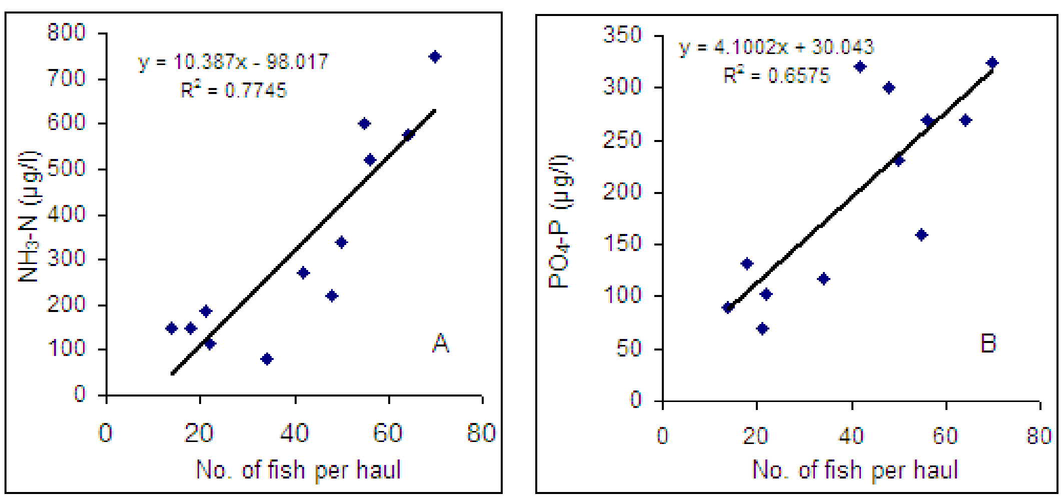

Fig. 6. Correlation between population density of *Gambusia holbrooki* and (A) NH_3-N and (B) PO_4-P of surface water of lake Nainital.

Discussion

About two and half decades after the introduction to Lake Nainital, *Gambusia* has become extremely abundant and occupies almost the entire littoral zone of the Lake. Because this fish is abundant, a prolific breeder and highly zooplanktivorous, we hypothesized it would have a strong effect on the ecological processes of the Lake. In addition to the effect of high fish biomass, others have found that factors such as diet, time since feeding and fish mass can impact the role of fish in lake nutrient cycles (Vanni, 2002). This study sought to examine the impact of these factors on excretion of N and P by *Gambusia*, in order to better understand the role of this species and consider ways to manage populations to improve water quality.

In the present study, feeding by *Gambusia* significantly increased the amount of N and P excreted by both sexes in comparison to unfed fish (Fig. 2 and 3). Nitrogen and Phosphorus excretion rates for both sexes were highest immediately following feeding (0-4 hr, 4-8 hr) and declined significantly over time (8-24 hr), especially for P (Fig. 2 and 3). This resulted in an excreted N: P ratio that was low (<20 by mass) during the first 8 hours after feeding, but increased significantly after that point. (Fig.4). Mather *et al*., (1995) observed similar trends in N and P excretion by bluegill and gizzard shad over time, with more rapid declines in P excretion following the cessation of feeding. Similar declines in excretion during longer term incubations have been noted for zooplankton (Lehman, 1980) and benthic invertebrate taxa (Arnott and Vanni, 1996), indicating that shorter term incubations are more appropriate in approximating excretion rates in the field. Organisms that show diel fluctuations in feeding activity may likewise experience daily fluctuations in nutrient release (Schaus *et al*., 1997), which has been poorly documented in the literature. Male *Gambusia* excreted more N and P than female *Gambusia* on a mass specific basis, most likely due to the substantially smaller size of males (Table 1). Over a 24 hour period, fed males excreted 28% more N and 9% more P on average per gram of fish, which is similar to the trends seen in other fish species (Lamarra, 1975; Brabrand *et al.,* 1990; Schindler *et al.,* 1993; Schaus *et al.,* 2010).

The importance of nutrients released by fish also can be impacted by the diet consumed. When fish feed primarily on pelagic zooplankton, fish mainly recycle nutrients already present in the water column (Vanni, 2002). However, when the consumed diet contains food from littoral and bottom areas (i.e. plants, benthic animals or sediments) the released nutrients can represent a net addition to the water column (Schindler *et al.,* 1996; Vanni, 2002). As a result, other investigators have observed that benthivores can have a stronger impact than planktivores on phytoplankton (i.e., Drenner and Hambright, 1999; Akhurst *et al.,* 2012). Consumption of littoral food items combined with water movements can transport nutrients from near shore to pelagic areas (Schindler *et al.,* 1996).Hurlbert *et al.,* (1972) reported that *Gambusia affinis* enhanced the cycling of P in the artificial ponds by preying on zooplankton and benthic animals. *Gambusia* is a small surface zooplanktivore (Crivelli and Boy, 1987; Singh and Gupta, 2010) that occupies the whole littoral zone of Lake Nainital (Nagdali and Gupta, 2002). During a gut analysis study of *G. holbrooki* from Lake Nainital, Singh and Gupta, 2010 reported that zooplankton form the major portion of its gut contents whereas chironomids and littoral cladocerans were observed regularly, but less frequently in the gut contents. On this basis, *Gambusia* may influence phytoplankton density in several different ways: (1) by modifying the abundance of zooplankton grazers, (2) by altering the nutrient recycling rates to provide nutrients in available forms (PO_4 and NH_3), (3) by excreting at a low N: P, potentially favoring the abundance of blue-greens, and/or (4) by releasing a “new” source of P to the epilimnion through consumption of either benthic or littoral sources.

Fish can impact the phytoplankton abundance and community structure through an interaction of top-down and bottom-up effects (Schaus *et al.,* 2002). Planktivores and omnivores can suppress zooplankton when fish are abundant and recycle nutrients to reduce the degree of nutrient limitation, which can impact phytoplankton when grazers are rare. Declines in planktivore abundance simultaneously increase top-down grazing pressure and reduce nutrient recycling through excretion by fish, most likely shifting algae toward grazer limitation (Schaus *et al.,* 2002). Some experimental studies have demonstrated the strong top-down effect of *Gambusia* on zooplankton, with corresponding increases in phytoplankton (Hurlbert *et al.,* 1972; Hurlbert and Mulla, 1981; Singh, 2013). Hurlbert *et al*., (1972) documented that with increased *Gambusia*, zooplankton decreased, phytoplankton increased, and water column P shifted to increased particulate forms with substantially decreased inorganic P availability. Thus algae shifted from top-down control in the absence of fish with abundant zooplankton to bottom-up control in the presence of fish, with low zooplankton and little dissolved P available (Hurlbert *et al.,* 1972; Schaus *et al*., 2002). In this system, Nagdali and Gupta, (2002) reported strong impacts of a mass fish mortality on phytoplankton abundance and water quality. During the three months when *Gambusia* declined by ~75% the zooplankton abundance doubled, phytoplankton abundance was reduced by ~50%, and soluble phosphorus declined (Nagdali and Gupta, 2002). While the shifts in zooplankton and phytoplankton can be clearly attributed to top-down effects, the decline in soluble phosphorus suggests that there was a simultaneous reduction in the recycling of nutrients, most likely due to a reduction in fish excretion.

In the present study a strong positive correlation was observed between two nutrients (NH_3-N and PO_4-P) and fish density (Fig. 5-6), indicating that fish biomass is likely more important than other factors, such as diet, fish size distribution, or sex. In a study of Venezuelan streams, excretion by fishes was shown to cause biogeochemical hotspots of enhanced nutrients, which primarily correlated with increased fish density (McIntyre *et al.* 2008). These authors also observed predictable differences in excretion among species due to body size and body N and P composition (Vanni *et al.,* 2002; McIntyre *et al.,* 2008), but among sections of the stream, drastic differences in fish biomass appeared to explain the majority of the variation in excretion rates (McIntyre *et al*., 2008). In a study of nutrient recycling by benthic invertebrates, invertebrate taxa did not differ significantly in their rates of nutrient cycling, but the largest differences were due to shifts in invertebrate biomass and lake temperature (Devine and Vanni, 2002). In a study of fish harvests to improve water in three Florida lakes, the largest reduction in nutrient cycling by fish was observed during years with the largest fish harvest (Schaus *et al.*, 2013). Although this study did observe differences in excretion by *Gambusia* due to size and time since feeding, at the lake scale the largest difference in nutrient effects was due to differences in fish density among months (Fig. 5-6).

Because fish can impact phytoplankton and water quality through top-down and bottom-up effects, many investigators have recommended large-scale fish removals to improve water quality (i.e., papers reviewed by Drenner and Hambright, 1999; Meijer *et al*., 1999; Søndergaard *et al.*, 2008). The removal of *Gambusia* from Lake Nainital could be considered a key mechanism in improving the water quality of lake (Nagali and Gupta, 2002) and its removal could be considered as important tool for the proper management of lakes. During 2007-2011 a team from Govind Ballabh Pant University (Pantnagar, Uttarakhand, India) removed 5.1 million *Gambusia* , 2.05 million *Puntius* (9212 kg total, or 191 kg ha^{-1}) from Lake Nainital, as well as 1100 Big Head Carp (6517 kg, or 135 kg ha^{-1}) (per. Communication with Nainital Lake Region Special Area Development Authority, Nainital Uttarakhand, India). The removal totaled 326 kg ha^{-1} of undesirable fish harvested during just over 4 years, and this project also added 125,000 juvenile silver carp (*Hypophthalmichthysmolitrix*, a phytoplanktivore) and 60,000 juvenile Golden Mahaseer (*Tor putitora*, a piscivore). These large harvests occurred after the conclusion of this study, whereas the large fluctuations in biomass we observed were due to natural variation in abundance. Other investigators recommend that biomanipulation efforts remove >75% of the fish biomass and/or >200 kg ha^{-1} in order to increase the likelihood of success (Hansson *et al.* 1998; Meijer *et al.*, 1999). The combination of different management strategies (fish removal, piscivore and phytoplanktivore stocking) during this time makes it more difficult to tie any water quality improvements to a specific management effort. However, biomanipulation efforts remain a useful option that can be combined with reductions to external nutrient loading, to seek to improve water quality.

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