Indian Journal of Environmental Sciences 21(2) 2017, pp. 71-93 ISSN 0971-8958
Green Earth Foundation

BIOMANIPULATION STUDIES IN THE MICROCOSMS AND MESOCOSM FOR IMPROVING WATER QUALITIES IN EUTROPHIC WATER BODIES

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ABSTRACT

Eutrophication deteriorates water qualities and trophic structure in the water bodies. Phosphorus is the vital nutrient for accelerating eutrophication. Various methods for controlling internal loading of phosphorus in the water body are; liming, aeration and desilting whereas reduction of nutrients in the agricultural runoff, reestablishment of wetlands and littoral zones, and restoration of channelized stream beds control external loading. Biomanipulation is a simple biological method involving food chain manipulation for controlling algal blooms as well as internal loading of phosphorus. The introduction of submerged macrophytes (4 species) decreased phytoplankton counts and their chlorophyll a content, and improved water qualities in the microcosms raised using sediment and water from eutrophic Mansagar lake in the Botanical garden. Similar observations were made when *Ceratophyllum demersum* (submerged) was introduced along with *Lemna aequinoctialis* (free floating) in a 7acre eutrophic water body (Check dam) in August 2007. Based on chlorophyll a concentrations, microcosms continued to be eutrophic after almost one year of submerged macrophytes introduction whereas Check dam transformed from eutrophic into a mesotrophic water body in almost 10years. Based on our findings, we recommend introduction of macrophytes (*Lemna* + *Ceratophyllum*) in combination with plantivorus, herbivorous and predatory fish for controlling eutrophication of water bodies.

KEY WORDS: Biomanipulation, *Ceratophyllum*, *Hydrilla*, *Lemna*, *Najas*, *Potamogeton*, *Gambusia*, phytoplankton, zooplankton, periphyton

INTRODUCTION

Eutrophication, a slow natural process, is on rise globally because of increasing human activities in the catchment of water bodies (Khan and Ansari 2005). About 54% of lakes in Asia, 53% in Europe, 48% in North America, 41% in South America, and 28% in Africa are in eutrophic state (Colin et al. 2007). The visible effects of cultural eutrophication are poor water clarity and foul odor. The increase in water turbidity shifts submerged macrophyte dominance to phytoplankton dominance, decreased biomass of large bodied zooplankton, increased biomass of planktivorous fish but reduced that of piscivores (Whillans 1996, Chow-fraser et al. 1998, Alvarez- cobeals et al. 2001) and net outcome is cyanobacterial blooms (Chislock et al. 2013).

Phosphorus is the vital nutrient for accelerating eutrophication and therefore reducing its input in the water bodies may lead to its control (Carpenter 2005). Various methods for controlling internal loading of phosphorus in the water body are; liming, aeration, desilting and macrophyte harvest (Cooke et al. 1993, Keto et al. 2004) whereas measures for controlling external loading include reduction of nutrients in the agricultural runoff, reestablishment of wetlands and littoral zones, and restoration of channelized stream beds (Jeppesen et al. 1999).

Simple biological methods (biomanipulation) for transforming turbid shallow lakes into clear water state are; promotion of macrophyte growth and fish manipulation (Lammens 1999, Tátrai et al. 2009). Submerged vegetation control algal blooms through nutrient competition (Lauridsen et al. 2003), allelopathic substances (Mulderiji et al. 2003, Jang et al. 2007, Zhang et al. 2014) and refugia for herbivorous zooplankton (Cerbin et al. 2003) for which macrophyte coverage should be at least 15-20% (Schriver et al. 1995). Submerged

vegetation also stabilizes sediment limiting phosphorus release (Jeppesen et al. 1997). Periphytic algae growing over submerged hydrophytes compete with phytoplankton for nutrient. Hydrophytes oxygenate water that favor microbial nitrification and denitrification at the sediment-water interface to complete natural nitrogen cycle and makes N: P ratio unfavorable for phytoplankton multiplication.

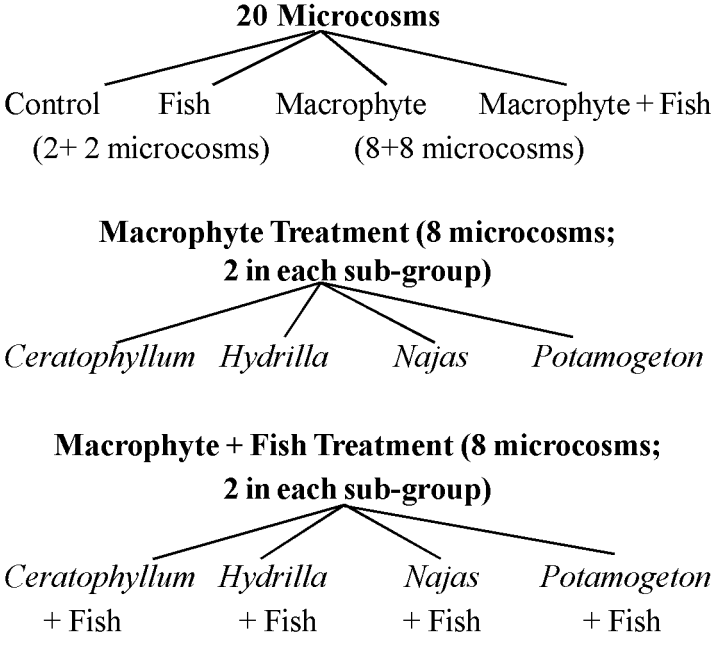
Piscivorous predator's removal transformed lake from a low to a high algal phase while vice versa had an opposite effect (Elser et al. 2000). Zooplanktivorous fish removal/reduction also controlled algal blooms in the lakes having three trophic levels (Meijer et al. 1994, Das and Naik 2017).

Biomanipulation is thus a simple method for controlling eutrophication in the water bodies. Most of findings on biomanipulation are from North America and Europe (temperate lakes) while there are only few reports from tropics and sub-tropics (Rohilla 2008, Das and Naik 2017) and present study is an attempt to generate more information on this subject.

MATERIALS & METHODS: Microcosm Study

The effects of introduction of plantivore fish (*Gambusia affinis*) and 4 species of submerged hydrophytes (*Ceratophyllum demersum* L., *Hydrilla verticillata* (L.f.) Royle, *Najas minor* All. and *Potamogeton pectinatus* L.) were assessed separately as well as in combination on plankton populations, physicochemical characteristics of water and phytoremediation of nutrients in the microcosms raised in 30L sized plastic buckets (buried 2/3rd into earthen floor) in the Botanical garden using sediment and water of Mansagar lake. After spreading 5cm thick layer of air dried sediment over the floor, 20L of lake water was filled in the bucket causing minimum disturbance to the sediment. After settling of suspended particles, floating materials were removed with a sieve from the water. The evaporative losses were compensated adding lake water in the microcosms.

After one week of stabilization, 20 microcosms were divided in to 4 groups. Group-1 and 2 had 2 microcosms each whereas group 3 and 4 had 8 microcosms each. Group -1 microcosms having only sediment and lake water served as control whereas 10 Gambusia fish were introduced in each microcosms of group 2 (fish treatment). Group 3 & 4 were divided in to 4 sub-groups having 2 micocosms each. Healthy young shoots (fresh weight = 5g) of *Potamogeton*, *Ceratophyllum, Hydrilla* and *Najas* initially grown for 7 days in the plastic tubs (3L sized) were transferred separately in group -3 microcosms whereas macrophyte and *Gambusia* fish (10) in group-4 microcosms. The schematic outlay of experimental set up is given below.



About 100mL water sample from a microcosm was centrifuged (3000 rpm) for 20 minutes and concentrate was fixed in Lugol's solution. Phytoplankton and zooplankton were identified (Smith 1950, Pentecost 1984, Tonapi 1980, Battish 1992) and counted using Hemocytometer and Sedgwick- Rafter (Trivedi and Goel 1984) respectively.

Periphytons attached over an artificial substrate were studied by hanging six microscopic glass slides (26 × 76 mm) just below the water surface in each microcosm. Three slides were removed after 7 and 15days of exposure and periphyton were removed carefully with a razor blade. Scrapings were dispersed in 15mL of distilled water containing 1-2 drops of Lugol's solution. After vigorous shaking, periphytons were counted similar to phytoplankton and zooplankton.

Shannon-Weaver index, Shannon equitability index and Simpson Reciprocal Index (RSR) of phytoplankton, zooplankton and periphyton were calculated using Online Biodiversity calculator (http://www.alyoung.com/ labs biodiversity_calculator.html).

MPN counts and physico-chemical characteristics of water were analysed according to Trivedy and Goel (1984).

Both algal (control) and macrophyte biomasses harvested at the termination of study were dried on the blotter paper for 48 hrs in a hot air oven at 60°C and weighed. Dried biomass was then powdered for analysis of total kjeldahl nitrogen (Bremner and Mulvaney 1982) and phosphorus by $SnCl_2$- ammonium molybdate method (Trivedi and Goel 1984). By multiplying algal and macrophyte biomasses with tissue concentrations of TKN and TP, standing crops of nutrients were calculated in the control and treatments.

Mesocosm Study

Road construction around Mansagar lake carved out a small water body (area: 7acre) named Check dam in the year 2004 (Fig.1). It receives run off from the surrounding Nahargarh hills in the rainy season. Because of connection with Mansagar lake through a tunnel underneath road, check dam also receives water from the lake when it is at full tank level in the rainy season. The drying of Check dam for two consecutive years (2006 and 2007) in the summer season resulted in dominance of *Clarias batrachus* commonly known Magur. It is primarily a carnivorous fish feeding on aquatic insects, insect larvae, small fish, fish eggs and larvae, but occasionally plant material also (Courtenay 1970, Courtenay and Woodard 1975). In August 2007 (rainy season), we introduced *Ceratophyllum demersum* and *Lemna aequinoctialis* Welw. in the Check dam. Physicochemical

Fig. 1. Google map showing Check dam and Mansagar lake

characteristics of Check dam water and chlorophyll a in the phytoplankton were analyzed as described earlier.

RESULTS: Microcosm Study

Phytoplankton

Twenty algal species included nine species of chlorophyceae (*Chlamydomonas, Chlorella, Cosmarium, Gloeocystis, Monoraphidium, Oedogonium, Pandorina, Scenedesmus, Stigeoclonium*), 6 of cyanophyceace (*Anabaena, Gloeothece, Oscillatoria, Merismopedia, Microcystis, Spirulina*), 4 of bacilariophyceae (*Cyclotella, Cymbella, Diatoma, Navicula*) and 1 of cryptophyceae (*Cryptomonas*).

Phytoplankton species richness was usually higher (11-17) in the cooler months (March & December) than (6-12) the relatively warmer month (August) because of increase in number of species of chlorophyceae and bacillariophyceae (Table 1). Similar seasonal variations in species richness were not evident in cyanophyceae. Species richness was found little higher than control in the treatments. Among treatments, species richness was lower in the macrophyte treatments in comparison to fish and macrophyte + fish treatments particularly in August (Table 1).

Phytoplankton counts followed trend similar to species richness. These were higher in the cooler months than warmer month because of build up in populations of chlorophyceae, bacillariophyceae and crytophyceae (Table 2). Unlike cyanophyceae counts increased throughout the study period, with the exception of macrophyte and macrophyte + fish treatments having lower counts in August possibly due to competition with macrophytes for light and nutrients (Table 2).

Compared with control, chlorophyceae counts were higher at three occasions in the fish treatment (3-8folds) but at 1-2 occasions in the macrophyte (2-4folds) and macrophyte +

Table 1. Species richness and diversity indices of phytoplankton in the control and treatments during warmer and cooler months (M = Macrophyte, F = Fish).

	Month	Control	Fish	*Ceratophyllum*		*Hydrilla*		*Najas*		*Potamogeton*	
				M	M+F	M	M+F	M	M+F	M	M+F
Total species	Dec.	12	13	13	15	15	13	13	13	16	12
	March	14	15	13	14	12	17	10	13	9	12
	Aug	9	12	9	9	7	6	11	10	8	6
	Dec.	11	12	12	9	15	11	10	13	8	12
Mean ± SD		11 ± 2	13 ± 1	12 ± 2	12 ± 3	12 ± 3	12 ± 5	11±1	12±2	10 ± 4	11 ± 3
RSI	Dec.	2.37	1.08	1.58	3.23	1.33	2.79	2.72	2.34	2.45	2.88
	March	2.85	0.66	2.9	1.50	0.83	1.48	1.76	1.01	2.07	2.65
	Aug.	2.51	1.79	2.11	1.83	1.57	1.81	2.26	1.71	1.39	1.07
	Dec.	2.44	2.90	2.99	2.01	3.28	2.05	2.20	3.23	2.45	2.88
Mean ± SD		2.54 ± 0.21	1.61 ± 0.98 (-37)	2.39 ± 0.67 (-6)	2.14 ± 0.76 (-16)	1.75± 1.06 (-31)	2.03 ± 0.56 (-20)	2.24 0.39 (-12)	2.07± 0.94 (-18)	2.09± 0.50 (-18)	2.37 ± 0.87 (-8)
Sha. Index	Dec.	2.72	1.86	2.32	3.07	0.65	3.01	2.95	2.62	2.85	2.89
	March	2.88	0.99	2.86	2.33	3.04	2.34	2.27	1.79	2.42	2.75
	Aug.	2.55	2.35	2.48	2.27	2.05	2.13	2.57	2.22	2.07	1.58
	Dec.	2.75	2.91	2.78	2.32	3.02	2.47	2.56	3.12	2.54	2.48
Mean ± SD		2.72 ± 0.14	2.03 ± 0.81 (-25)	2.61 ± 0.25 (-4)	2.50 ± 0.38 (-8)	2.19 ± 1.13 (-19)	2.49 ± 0.38 (-8)	2.59± 0.28 (-5)	2.44± 0.57 (-10)	2.47 ± 0.32 (-9)	2.43 ± 0.59 (-11)
Equit. Index	Dec.	0.76	0.50	0.63	0.785	0.59	0.79	0.79	0.71	0.71	0.81
	March	0.75	0.25	0.77	0.62	0.85	0.57	0.68	0.48	0.76	0.77
	Aug.	0.80	0.65	0.78	0.72	0.73	0.82	0.74	0.67	0.69	0.61
	Dec.	0.79	0.81	0.78	0.73	0.77	0.71	0.77	0.84	0.85	0.69
Mean ± SD		0.78 ± 0.2	0.55± 0.24 (-29)	0.74± 0.07 (-5)	0.71 ± 0.07 (-9)	0.74 ± 0.11 (-5)	0.72 ± 0.11 (-8)	0.75 ±0.05 (-4)	0.67 ±0.15	0.75 ± 0.07 (-4)	0.72 ± 0.09 (-8)

Data in parenthesis indicate percentage change in values in comparison to control, f = folds

Table 2. Phytoplankton counts (L^{-}) in control, fish , macrophyte and macrophyte + fish treatments.

	Month	Control	Fish	*Ceratophyllum*	*Ceratophyllum* + Fish	*Hydrilla*	*Hydrilla* + Fish	*Najas*	*Najas* +Fish	*Potamogeton*	*Potamogeton* +Fish
Total Counts	Dec.	41000±2725	92000±17300 2folds	59125±12350 (44)	30900±3750 (-25)	30225±1475 (-26)	76825±1250 (87)	25825±2575 (-37)	46000±5325 (12)	39725±6000 (-5)	60250±11650 (47)
	March	53250± 5750	105500±23750 (2F)	35650±5300 (-33)	40225±3500 (-24)	22250±3075 (-58)	78075±12900 (47)	9800±1150 (-82)	80025±10475 (50)	20575±1250 (-61)	105150±10500 (97)
	Aug.	31000±800	25425±2600 (-18)	4275±600 (-86)	8175±1600 (-74)	12000±1750 (-61)	2100±550 (-93)	14475±2100 (-53)	32750±5250 (6)	21900±1575 (-29)	9000±725 (-71)
	Dec.	30225±1475	76825±1250 (2.5F)	38175±3150 (26)	58767±3400 (94)	46225±8675 (53)	67000±2150 (122)	38275±4700 (27)	42075±2075 (39)	22775±1000 (-25)	34575±1375 (14)
	Mean ± SD	38868±10770	74937 ± 35024 (93)	34306 ± 22617 (-12)	34516 ± 21036 (-11)	34762±21394 (-11)	47606±33636 (22)	22093±12713 (-43)	50212±0637 (29)	26243±9032 (-32)	52243±41009 (34)
Cyanophyceae	Dec.	7800	4275 (-45)	6925 (-11)	2600 (-67)	5000 (-36)	5900 (-24)	2500 (-68)	1075 (-86)	8650 (11)	2825 (-64)
	March	17800	5740 (-68)	12975 (-27)	7750 (-56)	13000 (-27)	8575 (-52)	6725 (-62)	11925 (-33)	15250 (-14)	16425 (-8)
	Aug.	18075	19675 (9)	1600 (-91)	5425 (-70)	9250 (-49)	1000 (-94)	6800 (-62)	30350 (68)	17225 (-5)	7175 (-60)
	Dec.	19150	42375 (121)	17175 (-10)	21575(13)	12725(-34)	17325(-10)	12100(-37)	15750(-18)	7150 (-63)	12175(-36)
	Mean ± SD	15706± 5302	18016±17660 (15)	9668± 6829 (-38)	9337±8425 (-40)	9993 ±3741 (-36)	8200±6844 (-48)	7031±3931 (-55)	14775±12101 (-6)	12068±4918 (-23)	9650± 5915 (-38)
Chlorophyceae	Dec.	21000	66075 (3f)	42850 (2f)	19025 (-9)	13825 (-34)	12850(-39)	9900 (-53)	25175 (20)	9425 (-55)	30575 (46)
	March	11825	95665 (8f)	2575 (-78)	26575 (2f)	4850 (-59)	46800 (4f)	2350 (-80)	56925 (5f)	1150 (-90)	30900 (2.6f)
	Aug.	11350	1850 (-84)	75 (-99)	2675 (-76)	1000 (-91)	350 (-97)	4250 (-63)	1825 (-84)	2425 (-79)	1000 (-91)
	Dec.	4725	12700 (2.7f)	10675 (2.2f)	13192 (2.8f)	16825 (3.6f)	6500 (38)	9675 (2f)	12400 (2.6f)	6250 (32)	5900 (25)
	Mean ± SD	12225±6687	44072±44395 (3.6f)	14043± 19729 (15)	15366± 10079 (26)	9125± 7431 (-25)	16625± 20753 (36)	6543± 3826 (-46)	24081±23886 (97)	4812±3761 (61)	17093± 15881 (40)
Bacillariophyceae	Dec.	13550	16225 (20)	8925 (-34)	5350 (-61)	8050(-41)	20158 (49)	11750(-13)	4825 (-64)	20900 (54)	22175 (64)
	March	23150	4175 (-82)	20050 (-13)	5900(-75)	3975 (-83)	22325 (-4)	725 (-97)	11175(-52)	4175 (-82)	33325 (44)
	Aug.	925	2825 (3f)	2600 (2.8f)	75 (-92)	1075 (16)	750(-19)	2600 (2.8f)	500 (-46)	2250 (2.4f)	825 (-11)
	Dec.	5675	11750 (2f)	10000 (1.8f)	24000(4f)	15850 (2.8f)	42850 (7.6f)	16500 (2.9f)	9750 (72)	9575 (69)	15575 (2.7f)
	Mean ± SD	10825±9727	8743±6348 (-19)	10393±7217 (-4)	8831± 10447 (-18)	7237± 6414 (-33)	21520± 17213 (2f)	7893± 7491 (-27)	6562± 4871 (-39)	9225±8378 (-15)	17975± 13578 (66)
Cryptophyceae	Dec.	Absent	5425	425	3925	31675	4175	1675	14925	750	4675
	March	250	Absent	Absent	Absent	Absent	325(30)	Absent	Absent	Absent	24500 (98f)
	August	675	1075 (59)	Absent	Absent	675	Absent	825 (22)	75(-89)	Absent	Absent
	Dec.	675	10000 (15f)	325 (-52)	Absent	825 (22)	325 (-52)	Absent	4175 (6f)	Absent	925 (37)
	Mean ± SD	400± 333	4125± 4565 (10f)	187± 220 (-53)	981± 1962 (2f)	8293± 15591 (20f)	1206± 1985 (3f)	625± 800 (56)	4793± 7030 (12f)	187± 375 (-53)	7525± 11495 (19f)

Data in parenthesis indicate percentage change in values in comparison to control, f = folds

fish treatments (2-5folds) due to outburst in population of *Chlamydomonas* and *Scenedesmus* whereas *Monoraphidium, Cosmarium* and *Gleocystis* were the sub-dominant species (Table 2). The overall mean counts of chlorophyceae were also higher than control in the fish (3.6folds) and macrophyte + fish treatments (26-97%). These were however lower (25-61%) in the macrophyte treatments with the exception of *Ceratophyllum* treatment having counts more (15%) than control.

Bacillariophyceae counts were higher than control at three occasions in the fish treatment (almost 2-3folds) but often at two occasions in the macrophyte (2-3folds) and macrophyte + fish (3-8folds) treatments because of outburst in the population of *Navicula* while of *Cymbella* at one occasion in *Potamogeton* + fish treatment. The overall counts of bacillariophyceae were lower than control in the treatments (fish = 19%, macrophytes =15-33% and *Ceratophyllum* + fish =18% and *Najas* + fish = 39%) with the exception of *Hydrilla* + fish (2folds) and *Potamogeton* + fish (66%) treatments having higher counts.

Cyanophyceae counts were higher (almost 2 folds) than control at the termination of the study (one occasion) in the fish treatment because of outburst in population of *Microcystis*. *Oscillatoria* and *Gloeothece* were the sub-dominant species. Cyanophyceae counts were often lower than control in the macrophyte and macrophyte + fish treatments (Table 2). The overall mean counts of cyanophyceae were little higher than control in the fish treatment (15%) but lower in the macrophyte (23-55%) and macrophyte + fish (6-48%) treatments.

Cryptomonas, the only genus of family cryptophyceae, was often absent in the macrophyte and macrophyte + fish treatments. Compared with members of other algal classes, *Cryptomonas* counts were low in the control (250-675) as well as in the treatments (325-1075), though increased at few occasions in the latter (Table 2).

Total algal counts in fish treatment were almost 2- 2.5folds higher than control except in the rainy season. These were also higher (26-53%) in macrophyte treatments in winter (Dec. 2009 and 2010) due to increase in the population of bacillariophyceae but low (33-86%) in the intervening period. *Potamogeton* was the only macrophyte having plankton counts lower (5-61%) than control throughout the study period.

Phytoplankton counts in macrophyte + fish treatments were often higher (10-120%) than control, except in August due to significant reduction in the population of chlorophyceae (Table 2). The buildup of bacillariophyceae caused significant rise in December 2010.

The overall effect of fish introduction was marked increase in algal counts (93%) in the fish treatment which was low-moderate in macrophyte + fish treatments (22-34%) when compared with control. Only introduction of macrophytes decreased algal counts in comparison to control [(11-12% in *Ceratophyllum* and *Hydrilla* treatments but higher (32-43%) in *Najas* and *Potamogeton* treatments].

Periphyton – Algae

Species composition and their richness were almost similar to phytoplankton (Table 3). Species richness followed trend similar to phytoplankton being higher in the cooler months (7-18 species) than the warmer month (5-10 species). Species richness of cyanophyceae (2-4) differed little while that of bacillariophyceae (4-5) and chlorophyceae (6-9) was higher in the beginning but lower afterward (bacillariophyceae = 1-2 species, chlorophyceae = 3-4 species).

Chlorophyceae and bacillariophyceae counts were maximum in the beginning of the study but of cyanophyceae at the termination of study (Table 4).

Compared with control, counts of cyanophyceae were often lower in the treatments, except at termination of study. *Microcystis* was the dominant taxon while *Anabaena*, *Gloeothece* and *Oscillatoria* were subdominant taxa. Compared with control, *Microcystis* counts were lower in the beginning of study in the fish and macrophyte + fish treatments, as also noted for phytoplankton. The overall mean counts were lower in the fish (38%), macrophyte (11-36%)

Table 3 . Species richness and diversity indices of periphytic phytoplankton in the control and treatments during warmer and cooler months (M = Macrophyte, F = Fish).

	Month	Control	Fish	*Ceratophyllum*		*Hydrilla*		*Najas*		*Potamogeton*	
				M	M+F	M	M+F	M	M+F	M	M+F
Species Richness	December	15	15	15	14	15	18	17	16	14	14
	March	12	12	10	14	13	13	14	14	15	13
	August	8	8	6	7	7	5	5	10	6	5
	December	9	11	10	7	15	9	7	13	10	7
Mean ± SD		11 ± 3	11 ± 3	11 ± 4	10 ± 4	12 ± 4	11±5	11 ± 6	13 ± 2	11 ±4	10 ± 4
RSI	December	6.07	2.42	3.33	1.38	1.62	2.07	4.06	1.84	2.73	1.15
	March	2.36	1.65	1.93	0.94	3.88	1.59	1.75	2.99	3.63	1.73
	August	1.61	1.28	2.17	1.88	1.97	1.32	3.41	2.24	3.20	2.93
	December	1.47	2.29	2.21	2.27	3.09	2.59	2.23	2.65	2.484	1.43
Mean ± SD		2.88± 2.16	1.91± 0.54 (-34)	2.41 ± 0.63 (-16)	1.62 ± 0.58 (-44)	2.64 ± 1.04 (-8)	1.89± 0.56 (-34)	2.86± 1.06	2.43 ± 0.5 (-16)	3.01 ± 0.57 (5)	1.81± 0.78 (-37)
Shannon & Weiner Index	December	2.10	1.94	3.19	2.23	1.65	1.86	3.46	2.66	2.96	1.95
	March	2.77	2.27	2.35	1.6	3.16	2.20	2.49	3.04	3.24	2.44
	August	2.07	1.96	2.34	2.33	2.29	1.65	2.01	2.52	1.38	1.69
	December	2.08	2.67	2.35	2.43	3.03	2.55	2.37	2.81	2.59	1.86
Mean ± SD		2.26 ± 0.35	2.21 ± 0.34	2.56 ± 0.42 (13)	1.15 ± 0.37 (-49)	2.53 ± 0.7 (12)	2.06± 0.39 (-9)	2.58 ± 0.62 (14)	2.76 ± 0.22 (22)	2.54 ± 0.82 (12)	1.98 ± 0.32 (-12)
Equitability Index	December	0.78	0.72	0.82	0.99	0.61	0.64	0.85	0.67	.77	0.51
	March	0.77	0.63	0.71	0.42	0.85	0.59	0.65	0.80	0.83	0.66
	August	0.69	0.65	0.91	0.83	0.82	0.71	0.84	0.76	0.77	0.73
	December	0.66	0.77	0.71	0.87	0.78	0.80	0.84	0.76	0.78	0.66
Mean ± SD		0.73 ± 0.1	0.69 ± 0.06 (-5)	0.79 ± 0.1 (8)	0.78 ± 0.25 (8)	0.77 ± 0.11 (5)	0.68± 0.09 (7)	0.79 ± 0.1 (8)	0.75 ± 0.05 (3)	0.79 ± 0.03 (8)	0.64 ± 0.09 (-12)

Data in parenthesis indicate percentage change in values in comparison to control, f = folds

and macrophyte + fish (29-51%) treatments. This may be ascribed to competition for light and nutrients with macrophytes including their allelopathic effects and fish feeding.

Compared with control, counts of chlorophyceae were often higher (2-3folds) in the macrophyte, and macrophyte + fish treatments in winter (December 2009 and 2010) due to build up in the population of *Chlorella, Cosmarium, Gloeocystis, Pandorina* and *Scenedesmus* but lower in the intervening period (March and August) (Table 4). The overall mean counts were lower than control in fish treatment (19%) but significantly higher (about two folds) in the macrophyte and macrophyte + fish treatments.

Compared with control, bacillariophyceae counts in the treatments were often higher in winter (December 2009 and 2010) particularly in the macrophyte + fish treatments due to build up in the population of *Cymbella* and *Navicula* but lower in the intervening period (March and August) (Table 4). Their overall mean counts were lower than control in the fish (15%) and macrophyte treatments (11-44%) but higher in the macrophyte + fish treatments (almost 2-3folds in *Ceratophyllum* + fish and *Hydrilla* + fish treatments, 36% in *Potamogeton* + fish treatment), with the exception of *Najas* + fish treatment having lower counts (12%).

Cryptomonas found absent in the control throughout the study period was rarely sighted in the treatments (Table 4).

Periphytic algal counts (mean) were almost similar to control in macrophyte treatments, lower than control in fish (18%) but higher (12-74%) in the macrophyte + fish treatments (Table 4).

Table 4. Periphyton (phytoplankton) counts /10cm^2 in control, fish , macrophyte and macrophyte + fish treatments.

Class	Month	Control	Fish	*Ceratophyllum*	*Ceratophyllum* + Fish	*Hydrilla*	*Hydrilla* + Fish	*Najas*	*Najas* + Fish	*Potamogeton*	*Potamogeton* +Fish
Total Counts	Dec.	23762± 2277	41276± 7401(73)	27837± 1929(17)	36808± 5693(55)	62375± 9584(162)	77933 ± 15056(228)	36341± 3954(53)	51468± 6516(117)	23310± 2720(-2)	59969± 9472(152)
	March	66749± 6484	22171± 2014(-67)	27422± 3195(-59)	77113± 13538(16)	44575± 2562(-33)	77493± 10216(16)	51768± 53138(-22)	42509± 2435(-36)	17870± 1550(-73)	42384± 2594(-37)
	Aug.	20654± 3511	12240± 1645(-41)	2741± 506(-87)	3447± 569(-83)	11703± 1518(-43)	9604± 980(-54)	2625± 538(-87)	40265± 2024(95)	28118± 269(36)	11070± 2309(-46)
	Dec.	24039± 2372	35426± 5124 (47)	62943± 5377 (162)	45326± 4302 (89)	64936± 2341 (170)	70630± 6674 (194)	32601± 2499 (36)	56427± 5251 (135)	70537± 7275 (193)	37735± 3701 (57)
	Mean ± SD	33801± 22018	27778±13083 (-18)	30235 ± 24761(-11)	40673 ± 30277(20)	45897 ± 24528 (36)	58915± 33043(74)	30833± 20554(-9)	47667± 7584 (41)	34958± 24085 (3)	37789 ± 20223(12)
Cyanophyceae	Dec.	3700	4016	3384	2404	8193	2751	6009	2846	1265	1265
	March	16236	411	4017	2942	13158	12874	6990	13854	8318	12778
	Aug.	15277	9394	1455	2309	7148	5914	2214	21160	12963	10849
	Dec.	14803	17334	35457	17080	29416	28656	16881	24671	20022	10533
	Mean ± SD	12504 ± 5899	7788 ± 7356 (-38)	11078± 16288 (-11)	6183 ± 7269 (-51)	14478± 10297 (16)	12548 ± 11540	8023 ± 6253 (-36)	15632 ± 9642 (25)	10642 ± 7888 (-15)	8856 ± 5157 (-29)
Chlorophyceae	Dec.	14654	15528	19071	9764	46687	40801	20180	44723	14359	45420
	March	13917	6831	12462	15877	10247	5376	34349	8824	4522	4081
	Aug.	1676	1803	316	917	2847	3374	411	16542	13790	800
	Dec.	6389	5567	17586	13380	18282	8667	7813	15277	28151	411
	Mean ± SD	9159± 6231	7432 ± 5804 (-19)	12358± 8513 (35)	9984± 6545(9)	19515± 19179 (2f)	14554± 17633 (1.6f)	15688± 14875 (1.7f)	21341 ± 15949 (2.3f)	15205 ± 9737 (1.7f)	12478± 22037(1.4f)
Bacillariophyceae	Dec.	5408	21730	5282	24640	7495	34381	10152	3899	7686	13284
	March	36596	14929	10943	58294	17501	59053	14329	19831	5030	25525
	Aug.	3701	1043	970	221	1487	175	210	2530	1360	221
	Dec.	2720	3479	9900	14866	16733	32484	7907	16258	21920	26794
	Mean ± SD	12106± 16364	10295± 9734 (-15)	6773± 4584 (-44)	24505± 24659 (2f)	10804± 7697(-11)	31479± 24225 (2.6f)	8097± 6018 (-33)	10629± 8703 (-12)	8999± 8995 (-26)	16456± 12419 (36)
Cryptophyceae	Dec.	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	March	Absent	Absent	Absent	Absent	4302	Absent	Absent	Absent	Absent	Absent
	Aug.	Absent	Absent	Absent	Absent	221	316	Absent	Absent	Absent	Absent
	Dec.	Absent	8951	Absent	Absent	411	Absent	Absent	221	411	Absent

Data in parenthesis indicate percentage change in values in comparison to control, f = fold

Key Stone Species

The algal species contributing maximum to the counts were similar in planktonic and periphytic algae. *Microcystis*, *Scenedesmus*, *Cyclotella* and *Navicula* were the dominant taxa while *Anabaena*, *Gloeothece*, *Chlorella*, *Chlamydomonas*, *Gloeocystis*, *Monoraphidium*, *Diatoma* and *Cymbella* were the sub-dominant taxa.

Chlorophyll a

Mansagar water when filled in the microcosms on the first week of Nov. 2009 contained 150mg/m^3. In comparison to lake water, its content rose sharply in different microcosms (236-386 mg/m^3) in the beginning of study (3rd week of Nov. 2009) possibly due to release of nutrients particularly phosphorus from the sediment (Table 5).

Chlorophyll a contents in the fish treatment were either higher or almost similar to control but decreased markedly in the macrophyte (August = 26-85%, September = 77-94%) and macrophyte + fish treatments (August = 20-85%, September = 23-95%) because of significant reduction in plankton counts.

Table 5. Chlorophyll a contents (mg/m^3) in control and treatments

	Initial study	Aug. 2010	Sep. 2010
Control	236±108	602±266	432±349
Fish	243±43 (3)	865±296 (44)	399±234 (-8)
Ceratophyllum	223±81 (-5)	97±13 (-83)	16± (-94)
Ceratophyllum + Fish	155±51 (-34)	89±19 (-85)	32±15 (-93)
Hydrilla	327±97 (38)	87±6 (-85)	37±19 (-91)
Hydrilla + Fish	270±69 (14)	95±30 (-84)	11±2 (-95)
Najas	179±17 (-24)	124±62 (-79)	32±10 (-93)
Najas + Fish	386±114 (63)	479±243 (-20)	199±128 (-54)
Potamogeton	127±3 (-46)	447±248 (-26)	101±49 (-77)
Potamogeton + Fish	319±116 (35)	310±237 (-48)	332±160 (-23)

Data in parenthesis indicate percentage change in values in comparison to control

Zooplankton: Plankton

Zooplankton had 13 species; one species each of protozoa (*Amoeba*), euglenozoa *(Euglena*) and gastrotricha (*Chaetonotus*), 3 each of ciliophora (*Holophrya, Paramecium, Vorticella*) and arthropoda (*Cyclops, Nauplius, Moina*) and 6 of rotifera (*Monostyla, Lepadella, Philodina, Brachionus, Polyarthra, Testudinella*) (Table 6). Their richness differed little between control (10-11species) and fish treatment (9-12 species) but fluctuated in the macrophyte (8-15 species) and macrophyte + fish treatments (7-13 species).

Amoeba populations were low in both control (20-193) and treatments (13-260) with the exception of *Ceratophyllum*, and macrophyte + fish treatments having outbreaks (2920-14,400) in March and August (Table 7).

Ciliophora populations fluctuated in both control (166-14006) and treatments (46-134439) being often higher in December and March and low in August (Table 7). The outbreaks in *Paramecium* and *Vorticella* populations raised counts, particularly in the fish and macrophyte + fish treatments. An exceptional build up in *Holophrya* population (133666±50918) was observed in the *Hydrilla* + fish treatment in March. The overall mean population of ciliophora was higher in the fish (67%) and macrophyte + fish treatments (2-8folds) but lower (37-83%) in the macrophyte treatments.

Rotifers populations were often lower in winter (December). Compared with control, rotifer populations were higher in the treatments in December and March due to increase in counts of *Brachionus, Lepadella, Monostyla, Philodina* and *Polyarthra*. An exceptional breakout was observed in the population of *Brachionus* during August in control and fish treatment, particularly in the former. As a result overall

Table 6. Species richness and diversity indices of zooplankton in the control and fish, macrophyte and macrophyte + fish treatments

Parameters	Month	Control	Fish Tret.	*Ceratophyllum*		*Hydrilla*		*Najas*		*Potamogeton*	
				M	M+F	M	M+F	M	M+F	M	M+F
Species Richness	December	11	9	9	12	9	11	10	7	8	9
	March	11	12	15	9	12	13	11	10	12	11
	August	10	11	11	7	10	11	12	10	11	10
	December	11	11	12	12	11	9	12	12	12	13
Mean ± SD		11 ± 1	11± 1	12 ± 3	10 ± 3	11 ± 1	11 ± 2	11 ± 1	10 ± 3	11 ± 2	11 ± 2
RSI	December	2.13	1.72	1.16	0.53	2.51	2.55	2.488	2.06	1.64	2.24
	March	0.52	0.83	3.13	0.60	0.52	1.26	0.6352	0.53	0.54	1.16
	August	0.66	0.55	1.55	0.72	0.67	2.8	2.735	1.09	1.92	0.81
	December	1.07	1.13	1.75	1.79	1.40	1.37	1.956	1.81	1.98	2.34
Mean ± SD		1.09 ± 0.73	1.06 ± 0.5	1.90 ± 0.86 (74)	0.91 ± 0.59 (-16)	1.27 ± 0.91 (16)	1.99 ± 0.79 (82)	1.95 ± 0.94 (79)	1.37 ± 0.70 (26)	1.52 ± 0.67 (33)	1.64 ± 0.77 (50)
Sha. Index	December	2.52	2.21	1.82	0.28	2.60	2.67	2.555	2.30	2.09	2.54
	March	0.16	1.31	3.13	0.63	0.20	1.68	0.7663	0.23	0.33	1.72
	August	0.61	0.30	2.12	1.02	0.87	2.7	2.837	1.42	2.28	1.19
	December	1.73	1.42	2.25	2.19	1.99	1.77	2.352	2.42	2.46	2.73
Mean ± SD		1.25 ± 1.07	1.31 ± 0.78 (5)	2.33 ± 0.56 2folds	1.03 ± 0.83 (-18)	1.41 ± 1.08 (13)	2.20 ± 0.56 (76)	2.13 ± 0.93 (70)	1.59 ± 1.01 (27)	1.79 ± 0.98 (43)	2.00 ± 0.74 (60)
Equit. Index	December	0.73	0.70	0.57	0.079	0.82	0.77	0.769	0.82	0.69	0.80
	March	0.047	0.37	0.82	0.20	0.06	0.45	0.2215	0.07	0.093	0.50
	August	0.18	0.087	0.63	0.36	0.26	0.80	0.7914	0.43	0.66	0.36
	December	0.50	0.42	0.64	0.61	0.57	0.56	0.6561	0.676	0.69	0.74
Mean ± SD		0.36 ± 0.31	0.39 ± 0.25	0.66 ± 0.11 2folds	0.31 ± 0.23	0.43 ± 0.33 (19)	0.65 ± 0.17 (81)	0.61 ± 0.26 (69)	0.50 ± 0.33 (39)	0.53 ± 0.29 (47)	0.60 ± 0.21 (67)

Data in parenthesis indicate percentage change in values in comparison to control, f = folds

mean populations of rotifers were lower than control in the treatments (36-94%), particularly in the macrophyte and macrophyte + fish treatments (Table 7).

Chaetonotus, the only genus of gastrotricha, was often absent in March and August in both control and treatments, particularly in the macrophyte and macrophyte + fish treatments. Its counts varied (6-906) in the control as well as in treatments though it was exceptionally high (2046) in August in the control. Compared with control, overall mean population of *Chaetonotus* was lower in the treatments (37-96%), particularly in the fish and macrophyte + fish treatments.

Arthropods were either absent or had very low populations in the beginning of the study (December 2009, March 2010) in fish treatment and macrophyte + fish treatments possibly due to fish feeding but this trend reversed afterward and *Moina* and *Nauplius* larvae were the dominant arthropods (Table 7). Arthropods counts were however almost similar to control in the macrophyte treatments with exception of *Potamogeton* having higher counts. This explains reason for low algal counts in *Potamogeton* treatment. Compared with control, overall mean population of arthropods were lower in the treatments, particularly in the fish (40%) and macrophyte + fish treatments (41-68%).

Euglena was the only genus of euglenozoa having often low populations in winter (December 2009 & 2010) but outbreaks were observed in March and August in the control as well as in the treatments particularly in the fish treatment (Table 7). As a result, overall mean population of euglenoids

Table 7. Zooplankton counts (L^{-}) in control, fish , macrophyte and macrophyte + fish treatments.

Class	Month	Control	Fish	*Ceratophyllum*	*Ceratophyllum* + Fish	*Hydrilla*	*Hydrilla* + Fish	*Najas*	*Najas* + Fish	*Potamogeton*	*Potamogeton* +Fish
Total	Dec.	6789± 320	7563 ± 825 (11)	2450± 405 (-64)	182581± 67197 27folds	6371± 1060 (-6)	12242± 1469 2folds	7069± 414 (4)	4538± 490 (-33)	5471± 953 (-19)	8430± 1410 (24)
Counts	March	138263± 49659	1553920± 262760 11folds	10222± 1305 (-92)	612724± 141796 4folds	379949 ± 138880 3folds	318775 ± 69966 2folds	19142± 6209 (-86)	1486270 ± 438417 11folds	249263 ±37129 2folds	153910± 45861 (11)
	Aug.	1112123± 47671	1339371± 472786 12folds	15669± 4701 (-98.6)	25365± 8711 (-97.7)	11956± 3489 (98.9)	2176± 2566 (99.8)	3164± 573 (99.7)	8530± 1414 (99.2)	9982± 1465 (99.1)	11063± 3071 (99.0)
	Dec.	21100± 1191	31000± 1813 (47)	15000 ± 2150 (-29)	23623± 2691 (12)	48966± 10312 2folds	27786± 7409 (32)	10915± 2843 (-48)	5289± 892 (-75)	12436± 573 (-41)	6762± 486 (-68)
	Mean ± SD	319568 ± 513641	732963 ± 828785 2folds	10835± 6093 (-97)	211073± 277945 (-34)	111810± 179755 (-65)	90244 ± 152712 (-72)	10072 ± 6824 (-97)	376156± 740077 (18)	69288 ± 120018 (-78)	37789 ± 20223 (-88)
Protozoa	Dec.	193	Absent	Absent	186	Absent	Absent	Absent	Absent	Absent	Absent
	March	20	26	260	7413	Absent	4840	Absent	2920	Absent	14400
	Aug.	Absent	66	8253	Absent	Absent	Absent	100	13	226	86
	Dec.	153	Absent	91	1226	113	373	Absent	Absent	Absent	120
	Mean	91	23 (-75)	2151 (24f)	2206 (24f)	28 (-69)	1303 (14f)	25 (-73)	733(8f)	56 (-38)	3651(40f)
Ciliophora	Dec.	2566	3273	1553	1226	1180	3206	1540	553	2493	3220
	March	166	8459	420	31573	3713	134439	Absent	4813	5906	30333
	Aug.	Absent	353	6	Absent	80	46	612	Absent	Absent	Absent
	Dec.	14006	15873	800	4100	5506	2060	3400	1073	686	580
	Mean	4184	6989 (67)	694 (-83)	9224(2.2f)	2619 (-37)	34937 (8.3f)	1388 (-67)	1609(-62)	2271 (-46)	8533(2f)
Rotifera	Dec.	1732	3772	465	3471	2366	6779	2906	2392	1813	3378
	March	346	36098	4079	12505	3305	28939	131	24312	1692	13086
	Aug.	151626	59926	2506	23266	98	325	206	5073	105	8952
	Dec.	2732	732	18475	17732	39052	23079	6091	3491	8752	4618
	Mean	39109	25132 (-36)	6381 (-84)	14243 (-64)	11205 (-71)	14780 (-62)	2333 (-94)	8817 (-77)	3090(-92)	7508(-81)
Gastro-tricha	Dec.	93	173	60	306	586	906	806	73	373	346
	March	Absent	46	113	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Aug.	2046	Absent	20	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Dec.	6	86	12	73	766	113	126	20	133	53
	Mean	536	76 (-86)	51 (-90)	94 (-82)	338 (-37)	254 (-53)	233 (-57)	23 (-96)	126 (-76)	99(-82)
Arthropoda	Dec.	1946	Absent	352	26	2093	532	1651	Absent	646	Absent
	March	1199	26	1931	Absent	985	1059	1905	86	2452	86
	August	1752	2966	3198	86	1432	1292	1313	3424	5272	1985
	Dec.	2233	1258	1100	172	3012	259	1205	672	2585	1065
	Mean	1782	1062 (-40)	1645 (-8)	574 (-68)	1880(5)	785 (-56)	1518 (-15)	1052(-41)	2738 (54)	822 (-54)
Euglenozoa	Dec.	153	86	Absent	177000	Absent	566	Absent	1520	Absent	993
	March	136086	1508167	2886	558333	86	148646	86	1449426	86	95153
	Aug.	956467	1275667	1620	Absent	10273	500	840	Absent	4053	Absent
	Dec.	1966	13046	910	86	453	1900	93	86	280	86
	Mean	273668	699241(2.6f)	1354 (-99)	183913 (-33)	95598(-65)	37903(-86)	4468(-98)	362744(33)	60863 (-78)	24118(-91)

Data in parenthesis indicate percentage change in values in comparison to control, f = fold

Table 8. Species richness and diversity indices of periphytic zooplankton in control, and treatments of fish, macrophyte and macrophyte + fish

Parameters	Month	Control	Fish	*Ceratophyllum*		*Hydrilla*		*Najas*		*Potamogeton*	
				M	M+F	M	M+F	M	M+F	M	M+F
Species Richness	December	9	10	8	9	10	10	9	9	8	8
	March	12	8	9	9	13	10	11	9	9	10
	August	9	8	8	7	7	6	8	9	8	10
	December	7	8	8	9	9	9	12	12	10	9
Mean ± SD		9 ± 2	8 ± 1	8 ± 1	9 ± 1	10 ± 3	9 ± 2	10 ± 2	10 ± 2	9 ± 1	9 ± 1
RSI	December	0.54	0.53	1.21	0.64	1.12	2.14	0.57	0.74	0.56	0.58
	March	0.54	2.39	1.22	0.52	1.42	0.59	0.53	1.18	0.99	0.56
	August	1.94	1.39	0.74	1.15	0.84	1.36	1.47	0.50	0.89	1.11
	December	2.56	0.79	1.01	1.44	1.67	1.62	2.06	2.13	2.54	2.92
		1.39 ± 1.02	1.27 ± 0.83 (-9)	1.04 ± 0.22 (-25)	0.94 ± 0.43 (-32)	1.26 ± 0.36 (-9)	1.43 ± 0.65	1.16 ± 0.74 (-17)	1.14 ± 0.72 (-18)	1.24 ± 0.88 (-11)	1.29 ± 1.11 (-7)
Sha. Index	December	0.35	0.27	1.66	0.77	1.77	2.43	0.521	1.09	0.43	0.55
	March	0.36	2.48	1.66	0.18	2.12	0.48	0.26	1.45	1.10	0.48
	August	2.19	1.92	1.14	1.76	1.27	1.67	2.00	0.044	1.21	1.87
	December	2.35	1.22	1.76	1.83	2.11	2.00	2.48	2.54	2.65	2.71
Mean ± SD		1.31 ± 1.08	1.47 ± 0.95 (12)	1.56 ± 0.28 (19)	1.13 ± 0.80 (-14)	1.82 ± 0.40 (39)	1.64 ± 0.84 (25)	1.32 ± 1.09	1.28 ± 1.03	1.35 ± 0.93	1.40 ± 1.08 (7)
Equit. Index	December	0.11	0.080	0.55	0.24	0.53	0.73	0.16	0.35	0.14	0.18
	March	0.10	0.83	0.53	0.056	0.57	0.14	0.075	0.46	0.35	0.14
	August	0.69	0.64	0.38	0.63	0.45	0.65	0.67	0.014	0.40	0.56
	December	0.84	0.41	0.59	0.58	0.67	0.63	0.69	0.71	0.79	0.85
Mean ± SD		0.43 ± 0.39	0.49 ± 0.32 (14)	0.51 ± 0.09 (19)	0.38 ± 0.28 (-12)	0.56 ± 0.09 (30)	0.54 ± 0.27 (26)	0.40 ± 0.33 (-7)	0.38 ± 0.29 (-12)	0.42 ± 0.27	0.43 ± 0.34

Data in parenthesis indicate percentage change in values in comparison to control, f = folds

were almost three folds higher than control in the fish treatment but low in others, particularly in the macrophyte treatments (65-99%).

Zooplankton counts were low in control and treatments in the winter (Dec. 09, 10) but were high in the relatively higher in March and August 2010 due to outbreaks in populations of *Paramecium*, *Vorticella* and *Euglena* (Table 7). The intensity of outburst was lower than control in the macrophyte, and macrophyte + fish treatments but higher in the fish treatment. Zooplankton counts were therefore more than two folds higher in fish treatment while these were lower (65-97%) in the macrophyte, and macrophyte + fish (34-88%) treatments when compared with control.

Euglena (Euglenozoa) was the dominant species contributing maximum to zooplankton population whereas *Vorticella* (Ciliophora), *Filinia*, *Lepadella*, *Monostyla*, *Polyarthra*, *Philodina* and *Brachionus* (Rotifers) were the subdominant species in the community.

Periphytic Zooplankton

Species composition of periphytic zooplankton was almost similar to zooplankton. Their species richness was higher in the cooler months (8-13 species) in comparison to warmer month (6-10 species). The overall species richness was similar to control in the treatments though it decreased a little in the fish treatment (Table 8).

The counts of protozoan (1-78), gastrotricha (1-28) and arthropods (nil -5) were lower (1-78) in both control and treatments in comparison to ciliophora, rotifers and euglenoids (Table 9). Single spike in ciliophora population was observed in the control, fish, *Najas* + fish and *Potamogeton* treatments due to build up in the population of *Vorticella*.

Rotifer populations were low (4-77) in the control as well in treatments, except at one occasion (207-464) in the fish and macrophyte + fish treatments because of higher population of *Brachionus* and *Philodina* (Table 9).

Table 9. Periphytic zooplankton counts /10cm^2 in control, fish , macrophyte and macrophyte + fish treatments.

Class	Month	Control	Fish	*Ceratophyllum*	*Ceratophyllum* + Fish	*Hydrilla*	*Hydrilla* + Fish	*Najas*	*Najas* + Fish	*Potamogeton*	*Potamogeton* +Fish
Total	December	353± 111	4136± 1248 12folds	134±35 (-62)	292±98 (-17)	170±14 (-52)	116±35 (-67)	144±26 (-59)	529±146 (50)	333±69 (-6)	934±314 2.6fold
	March	1704± 65	577±528 (-66)	151±146 (-91)	16927±2856 10folds	292±29 (-83)	7724± 3065 4.5folds	3948± 1127 2.3folds	5118±3111 3folds	3392±820 2folds	1103±450 (-35)
	August	52±10	95±27 (83)	106±36 2folds	37±9 (-29)	67±14 (29)	81±15 (56)	35±6 (-33)	7768±2916 149folds	145±51 3folds	45±6 (-13)
	December	21±3	166±49 8folds	141±13 7folds	219±39 10folds	158±29 7.5folds	367±63 17folds	101±20 4.8folds	44±14 2folds	83±15 4folds	34±5 (62)
	Mean ± SD	532 ± 795	1243 ± 1940 2folds	133 ± 19 (-75)	4368 ± 8372 8folds	171 ± 91 (-68)	2072 ± 3770 4folds	1057 ± 1927 2folds	3364 ± 3720 6folds	988 ± 1606 (86)	526 ± 569 Nil
Protozoa	December	Absent	12	Absent	1	Absent	1	1	1	Absent	Absent
	March	Absent	Absent	Absent	66	3	3	78	8	Absent	25
	August	Absent	Absent	2	1	8	29	8	Absent	Absent	5
	December	7	11	4	56	5	10	Absent	1	Absent	3
	Mean	2	6	2	31	4	11	22	3	Absent	8
Ciliophora	December	339	4021	80	257	109	44	135	430	315	25
	March	1633	110	83	9	57	507	5	1965	1979	8
	August	Absent	Absent	Absent	3	Absent	2	Absent	7739	34	2
	December	5	131	93	109	17	105	41	16	29	5
	Mean	494	1065 (2f)	64 (-87)	94 (-81)	45 (-91)	164 (-67)	45(-91)	2537 (5f)	589 (19)	10 (-98)
Rotifera	December	9	67	48	30	32	63	4	36	12	30
	March	37	378	11	207	60	77	37	464	49	19
	August	14	33	11	5	6	11	5	16	4	4
	December	7	14	27	52	131	240	49	24	42	23
	Mean	17	123	24	73	57	98	24	135	27	19
Gastrotricha	December	Absent	9	1	1	1	2	1	1	1	2
	March	4	28	1	1	2	7	1	6	2	10
	August	Absent	Absent	5	Absent	2	Absent	Absent	1	Absent	Absent
	December	Absent	2	13	2	1	7	5	Absent	2	2
	Mean	1	10	5	1	2	4	2	2	1	4
Arthropoda	December	2	Absent	1	Absent	2	Absent	1	Absent	1	Absent
	March	3	Absent	Absent	Absent	2	Absent	1	Absent	Absent	Absent
	August	4	5	Absent	1	2	Absent	2	2	3	3
	December	2	2	3	Absent	4	1	5	4	5	1
Euglenozoa	December	1	7	1	1	20	1	1	51	2	866
	March	22	25	50	16606	160	7121	3823	2649	1360	1038
	August	19	51	87	24	51	40	19	5	103	30
	December	0	6	1	Absent	Absent	Absent	1	Absent	5	Absent
	Mean	10	22	35	4158	58	1790	961	676	368	483

Data in parenthesis indicate percentage change in values in comparison to control, f = folds

Euglenoid populations were low in control and treatments, with the exception of macrophyte + fish treatments having higher counts in March (Table 9).

Periphytic zooplankton counts were often low in both control and treatments except March 10 (spring) having higher counts particularly in the treatments. Periphytic counts varying little in *Ceratophyllum* and *Hydrilla* treatments were less (68-75%) than control but almost 2-8folds higher than control in other treatments, particularly in fish and macrophyte + fishe treatments.

Vorticella (Ciliophora) was the only key stone species in periphytic community.

Indices

Since species richness of the habitats is not very informative and therefore, diversity indices were calculated to measure proportional abundance of species in the microcosms.

Phytoplankton

RSI, Shannon index and Equitability index fluctuated little during study period in the control and their values were on higher side in comparison to treatments because of higher species richness and evenness in the phytoplankton community (Table 1). In contrast, their values fluctuated in the treatments, particularly in the first half of the study possibly due to unevenness in the community because of fostering of population of more opportunistic species. The fluctuations in indices were maximum in fish and macrophyte + fish treatments.

Periphyton- Algae

Shannon index and Equitability index varied little in comparison to RSI in the control during the study period (Table 3). In comparison to control, Shannon and Equitability indices fluctuated in treatments because of greater heterogeneity in the community of periphytic algae. RSI values were lower than control in the treatments, particularly in the beginning of study in the fish and macrophyte + fish treatments due to reduction in number of dominant species in the community.

Zooplankton

Diversity indices of zooplankton varied in control and treatments because of heterogeneity in their communities ascribed to outburst in population of various zooplankton species stated in the preceding section (Table 6).

Periphytic Zooplankton

Diversity indices of periphytic zooplankton increased with time in the control, macrophyte and macrophyte + fish treatments due to increase in homogeneity of community but fluctuated in fish treatment due to heterogeneity (Table 8).

Macrophyte

Macrophytes growth found poor in the beginning was possibly because of excessive growth of *Oedogonium* and *Stigeoclonium* around their shoots and low water temperature (December – February). However, removal of filamentous algae at 2-3 occasions (Nov. 2009- Feb. 2010) and rise in water temperature improved macrophytes growth which was maximum in the rainy season.

I. Biomass

In macrophytes treatments, *Potamogeton* biomass was maximum followed by *Hydrilla, Najas* and *Ceratophyllum* (Table 10). In macrophyte + fish treatments, *Ceratophyllum* and *Hydrilla* biomasses were higher than other macrophytes.

II. Standing Crops of Nutrients

Tissue concentrations of TKN and TP differed little between microphyte and macrophytes (Table 10) though standing crops were higher in the macrophyte treatments (Table 10). Among macrophytes, locking of nutrients was found maximum in *Hydrilla* followed by *Potamogeton* and *Ceratophyllum* and was minimum in *Najas*.

Water Qualities

pH values of microcosms were in alkaline range (pH = 8.1-9.7). EC values (2.60-3.03 m mho/cm) increased in summer (5.4-6.8 m mho/cm) because of salts build up due to evaporative losses but declined sharply (0.9-1.17 mmho/cm) in the rainy season. It increased again in the post-rainy season (1.50-2.50 mmho/cm) and then decline again after winter rain (0.8-1.4 mmho/cm).

Dissolved oxygen (DO) concentrations were minimum (0.2-1.9 mg/L) in the morning (7 am) particularly in fish (0.6 mg/L) and macrophyte (0.2-1.9 mg/L) treatments because of utilization in respiration during night. Oxygen contents increased in control (8.1 mg/L) and treatments (8.1-22.7 mg/L) in the noon and were maximum in the evening (11.2-25.3 mg/L), particularly in macrophyte treatments (11.2-25.3mg/L) because of oxygenation of water.

COD values differed little between control (480-496 mg/L) and treatments (350-480mg/L) in the beginning of study but

Table 10. Dry weights (g), tissue concentrations (mg / g dry weight) and standing crops (mg) of TKN and TP in microphytes (control & fish treatment) and macrophytes (macrophyte and macrophyte + fish treatments)

Treatments	Dry weight (mean)	Tissue Concentration (mean)		Standing crops (mean)	
		TKN	TP	Nitrogen	Phosphorus
Control	1.316	10.5	2.2	13.7	2.8
Fish	2.99	15.6	1.8	46.6	5.4
Ceratophyllum	22.02	13.7	2.3	301.7	51.3
Hydrilla	27.36	11.6	2.6	318.0	70.9
Najas	24.19	10.4	2.5	251.6	60.0
Potamogeton	36.09	10.2	2.2	368.1	82.3
Ceratophyllum + Fish	31.99	10.1	1.5	322.4	47.7
Hydrilla + Fish	30.61	13.1	2.8	395.1	85.4
Najas + Fish	6.73	15.1	3.0	101.7	20.3
Potamogeton + Fish	27.28	11.2	2.5	305.5	68.2

Table 11. MPN (counts/100 mL) in Control and Treatments

Treatments	Dec. 2009	Sep. 2010	Oct.- 2010	Dec. 2010
Control	800	>16000	400	500
Fish	<200	>16000	1300	700
Ceratophyllum	400	3500	90	210
Hydrilla	800	16000	330	200
Najas	<200	5000	3000	1700
Potamogeton	<200	170	9000	400
Ceratophyllum + Fish	200	900	210	220
Hydrilla + Fish	<200	390	2800	1400
Najas +Fish	<200	5000	2200	3500
Potamogeton + Fish	200	230	800	390

decreased significantly afterwards in the treatments (211-258 mg/L) particularly in the macrophyte treatments (191-231 mg/L) when compared with control (400mg/L).

Compared with control (TP = 2.33 ± 0.31 mg/L), percentage reduction in the TP levels was higher in the macrophyte (30-71%) and macrophyte + fish (10-71%) treatments during active growing period of macrophytes (rainy season). The order of reduction in TP levels in the macrophyte treatments was; *Hydrilla* (56-71%) > *Ceratophyllum* (50-61%) H" *Najas* (35-71%) > *Potamogeton* (29-49%)

MPN counts decreased significantly in control (800/100mL) and treatments (<200-800/100mL) almost after two months of filling lake water (MPN=1700) in the microcosms. These increased significantly in control and fish treatment in warmer months (September and October-2010) but remained low in macrophyte treatments (Table11). MPN values however, decreased markedly in winter (Dec-2010) in both control and treatments and were almost similar to December-2009. It is evident that water temperature affected MPN counts in microcosms.

Check dam

Lemna multiplied fast and covered almost entire water body within 3 months (Fig. 2) while *Ceratophyllum* took more than 12 months for spread in the Check dam. Macrophytes

Table 12. Chlorophyll a and total phosphorus in Check dam water

Months	Chlorophyll a mg/m3	Total phosphorus mg/L
Aug. 2007- Dec. 2007	50- 380	2.6-3.5
Jan. 2008 – Dec. 2008	20-110	Not available
Feb. 2010	30	1.85
Oct. 2012	25.2	2.15
June 2017	65.6	2.64
Nov. 2017	11.08	Not available

Fig. 2. Check dam having good growth of *Lemna* (Year: 2008)

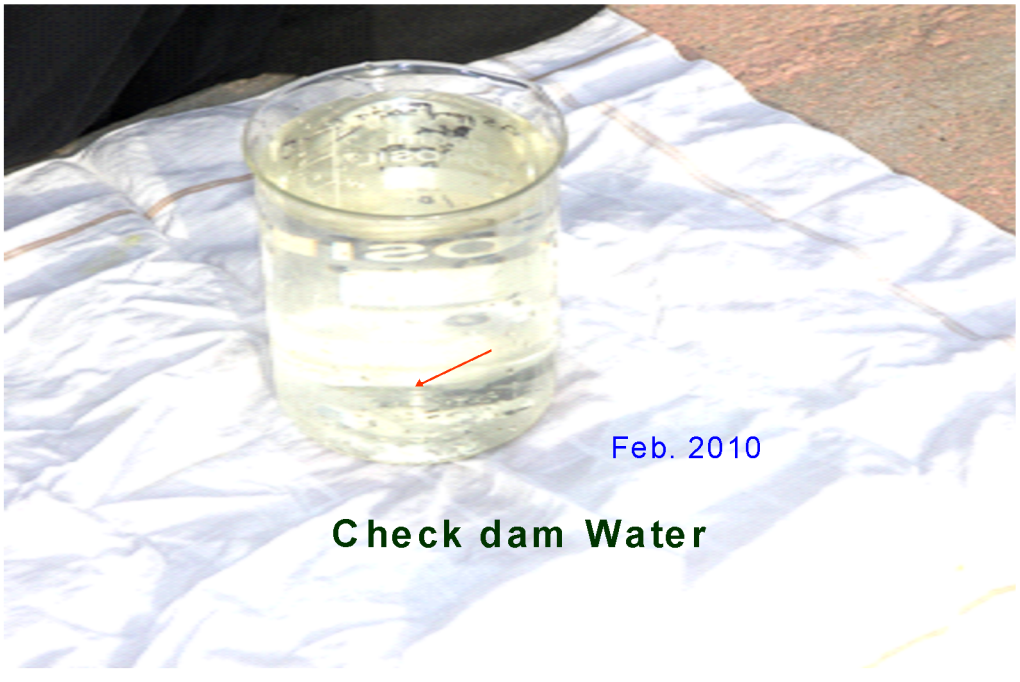


Fig.3. Check dam water (clean) with *Daphnia* population (marked with arrow)

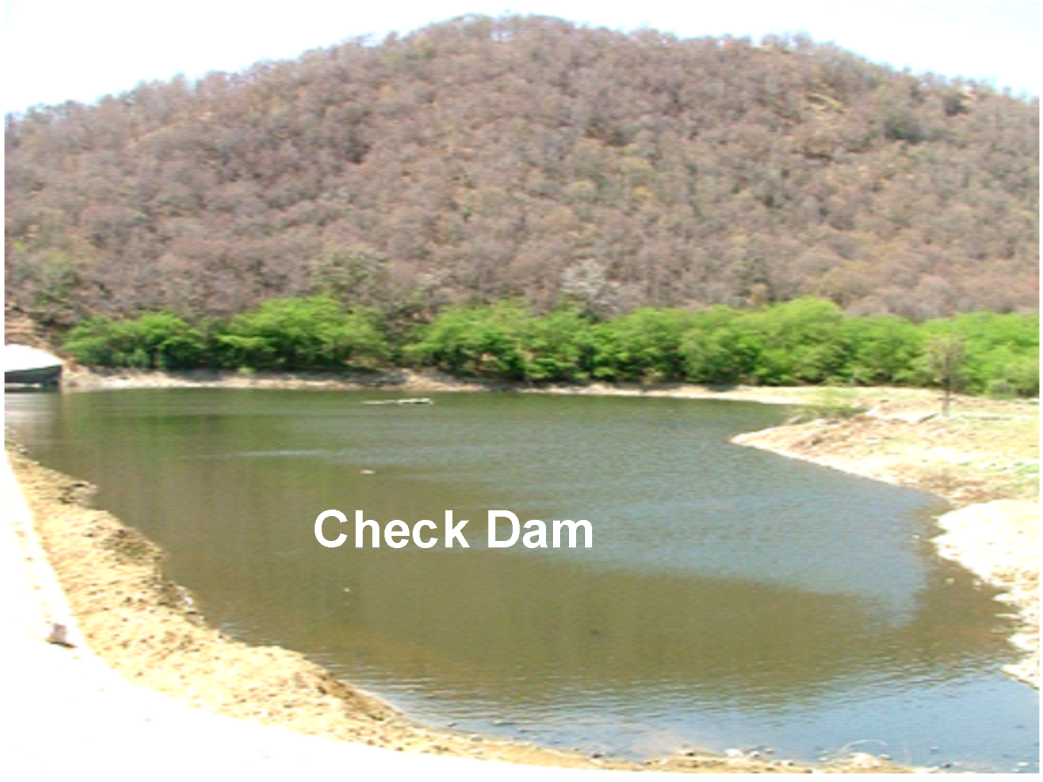


Fig. 4. Check dam without macrophyte (Year: 2012)

competed with phytoplankton for nutrient and light as evident by maximum reduction in the chlorophyll a and total phosphorus content in the water (Table 12). Zooplankton counts increased particularly of larger arthropods such as *Daphnia* grazing on phytoplankton (Fig. 3). Macrophytes growth however, declined from the year 2010 onward and were absent since 2012 (Fig. 4) possibly due to feeding by waterfowls and fish (*Clarius*) which led increase in chlorophyll a (Table 12).

DISCUSSION

Biomanipulation studies for controlling eutrophication of freshwater bodies initially drew considerable attention in North-West Europe and North America in 1970's are now gaining momentum in India because of pollution of majority of the urban water bodies on account of diversion of municipal and industrial sewage and Mansagar lake of Jaipur is not an exception though attempts have been made to control both *Ex Situ* and *In Situ* pollution during implementation of National Lake Conservation program (2002-2010). Although 2 wastewater drains entering Mansagar lake have been diverted but still storm water runoff (about 90% of total runoff from the catchment) from walled city of Jaipur enrich lake with nutrients. The possibility of recovery of this lake through biomanipulation has been studied in both bench scale and field studies and important findings are discussed below.

Macrophyte growth was poor in the beginning of study in the microcosms possibly because of luxuriant growth of periphytic algae such as *Oedogonium* and *Stigeoclonium* and low water temperature. *Lemna* and *Ceratophyllum* however, grew well in the Check dam following their introduction in the rainy season. Various authors however, reported good growth of submerged macrophytes in low nutrient enrichment condition while increase in periphyton biomass and algal turbidity in nutrient enrichment condition (Gerking 1962, Dvorac and Best 1982, Orth et al. 1984, Rabe and Gibson 1984, Gregg and Rose 1985). The poor growth of submerged macrophytes in the microcosms may possibly be related to allelopathic effects of algae (Inderjit and Dakshini 1994), unfavorable low water temperature (winter) and high nutrients favorable for algae.

Members of chlorophyceae and bacillariophyceae contributed maximum to phytoplankton and periphyton

counts in the beginning of study but of cyanophyceae afterwards particularly in the fish treatments (with and without macrophytes, Table 2,4) which may adversely affect biomanipulation because of their less palatability and poor nutritive value to zooplankton (Burns et al. 2011).

Microcystis, Gloeothece, Scenedesmus, Monoraphidium, Cyclotella and *Navicula* were the taxa contributing maximum to phytoplankton counts. *Oscillatoria, Chlamydomonas* and *Cryptomonas* were the other codominant taxa in the fish and macrophyte + fish treatments. Taxa contributing maximum to periphyton (algae) counts were similar to phytoplankton and were *Microcystis, Oscillatoria, Gloeocystis*, *Chlorella* and *Navicula*. According to Palmer (1969), abundance of *Scenedesmus, Oscillatoria, Microcystis, Navicula, Nitzschia* and *Euglena* indicate organic pollution in the water bodies. *Microcystis aeruginosa* and *Oscillatoria* are the best indicators of pollutants of biological origin (Nandan and Aher 2005, Gadag et al. 2005) whereas excessive growth of *Scenedesmus*, *Anabaena, Oscillatoria* and *Melosira* indicate nutrient enrichment of aquatic bodies (Zargar and Ghosh 2006). The occurrence of aforesaid algal species in the microcosms suggests eutrophic condition.

Euglena blooms were common in control and treatments (Table 7) though their intensity was mild in the macrophyte treatments (with and without fish). Rahman et al. (2007) also reported euglenophytes blooms in the fish ponds rich in nitrate and phosphorus. Mild blooms in the macrophyte treatments were therefore on account nutrient uptake.

Rotifers counts were higher at most of sampling occasions in fish and fish + macrophyte treatments particularly in the former while arthropods counts followed opposite trend (Table 7). Because of fish predation, small cladocerans, rotifers and their juveniles mostly dominate in the tropical and subtropical lakes (Dumont 1994, Lewis 1996, Branco et al. 2002, Garcia et al. 2002). Rotifers abundance followed by cladocerans is an indication of the eutrophic nature of the water bodies (George1966).

Arthropods such as *Cyclops* feed on protozoan (Wickham 1975, Czeczuga et al. 2000) and rotifers (Plaâmann et al. 1997) whereas *Daphnia* suppress rotifers by mechanical interference (Gilbert 1985 and 1998). Fish feeding on *Cyclops* reduced predatory pressure on *Euglena* and *Paramecium* which led significant build up in their populations (Table 7). Mild blooms of *Euglena* or even their absence in macrophyte treatments (with and without fish) may possibly be also on account of shelter of *Cyclops* in the macrophyte bed. Our findings on higher rotifer densities at most of sampling occasions but lower of arthropods (cladocerans and cyclopoids) in the fish treatments agree with other workers (Vanni 1987, Threlkeld 1988, Lazzaro et al. 1992, Mieiro et al. 2001, Romo et al. 2004, Singh 2013). Nathan et al. (2010) reported reduction in total abundance and diversity of zooplankton resting stages in the flood plain river having higher biomass of *Gambusia holbrooki* and *Hypseleotris* spp. which may in turn reduce active zooplankton community.

Fish introduction increased phytoplankton counts (93%) because of relaxing of grazing pressure of zooplankton due to their predation (Table 2, 6), as also reported in several studies (Proulx et al. 1996, Sosnovsky and Quiros 2009, Singh 2013). The increase in phytoplankton counts was however, comparatively poor (22-34%) when fish were introduced along with macrophytes (Table 2). It is possible only when most of larger zooplankton escape fish predation in the macrophyte bed. Similarly phytoplankton counts were lower than control in the macrophyte treatments; 11-12% in *Ceratophyllum* and *Hydrilla* but higher (32-43%) in *Najas* and *Potamogeton* possibly due to their alleopathic effects on phytoplankton (Feng et al. 2008, Wang et al. 2010a, b, Amsalu 2017). Besides *Potamogeton* and *Najas* having relatively longer leaves spreading on the water surface possibly reduced more light underneath water column than *Ceratophllum* and *Hydrilla* with smaller leaves attached closely to shoot.

Fish introduction decreased cyanophyceae counts (45-68%) in the beginning of study which increased afterwards (9-121%). *Gambusia* is an omnivorous fish feeding on zooplankton, aquatic and surface insects, snails, other fish species and algae (Crivelli and Boy 1987, Garcia- Berthou 1999). Xie and Liu (2001) reported build up of *Microcyctis* in fish free enclosures in a hyper-eutrophic lake that declined after fish introduction as also observed in the present study. The reduction in *Microcystis* population in the beginning of study may be attributed to *Gambusia* feeding while reversal may be ascribed to change in their diet pattern (Cabral et al. 1998). Spencer and King (2011) reported dense blue green algal blooms in the ponds having dense population of planktivorous fish and sparse cladoceran population but clear water in ponds without fish having abundant cladoceran and dense growth of submerged macrophytes. At the termination

of study, cyanophyaceae counts were in the following order.

Fish > Control > Macrophyte + fish > Macrophyte

The poor build up of cyanophyceae (*Microcystis*) in macrophyte and macrophyte + fish treatments may possibly be related to competition for nutrients with them and also with periphyton (Table 2, 4), their alleopathic effects and higher populations of larger arthropods as explained earlier.

Submerged vegetation thus affected microcosms through nutrient competition (Lauridsen et al. 2003), allelopathic substances (Mulderiji et al. 2003, Jang et al. 2007, Zhang et al. 2014), and habitat for herbivorous zooplankton (Cerbin et al. 2003). Submerged vegetation and zooplankton grazing were the major factors responsible for phytoplankton control in the ponds without fish (Spencer and King 1984, Søndergaard and Moss 1998, Blindow et al. 2000, Peretyatko et al. 2007a, b, 2009, 2012).

Submerged macrophytes absorb nutrients from the sediment and therefore, do not compete directly with phytoplankton for nutrients (Carigan and Kalff 1980) though nutrients locking in their biomass decreased availability from the sediment pool (Table 10). Their profuse growth however increases surface area for periphyton production which competes with phytoplankton for nutrients from the water (Cattaneo and Kalff 1979) and reduces phosphorus levels in both microcosms (macrophyte treatments) and Check dam as stated earlier though these were still higher than its threshold limit for oligotrophic water bodies (Xu et al. 2015). Submerged macrophytes also stabilize sediment which control phosphorus release (Jeppesen et al. 1997). Hydrophytes do oxygenate water as also observed in the present study that favor microbial nitrification and denitrification at the sediment–water interface to complete natural nitrogen cycle and makes N: P ratio unfavorable for phytoplankton multiplication. The increased oxygenation also reduces BOD and COD of water in the microcosms including desorption of phosphorus from the sediment. Thus submerged vegetation indirectly control nutrients availability to phytoplankton.

The diversity indices of phytoplankton fluctuated in the fish treatments (with and without macrophytes) due to build up in populations of opportunistic species (Table 1). Macrophyte introduction however reduced oscillations and increased stability of the community.

Chlorophyll a, a good indicator of trophic status of water body, decreased markedly in the macrophyte + fish and macrophyte treatments particularly in the latter but exceeded limit for eutrophic water body (Table 5, Wetzel 1983). Macrophytes introduction also reduced chlorophyll a content markedly in the Check dam and values were closer to the upper limit for mesotrophic water (Table 12). Such difference may be related to shorter period (about 1year) of study in the bench scale study which was almost 10 years in the Check dam.

Present study highlighted role of macrophytes in controlling algal blooms in the eutrophic water bodies. Free floating macrophyte (*Lemna*) in combination with submerged (*Ceratophyllum*) was found more effective in the Check dam because they compete effectively with phytoplankton for light as well as nutrients. *Ceratophyllum* grew well in mixed culture with *Lemna* in the Check dam possibly because of its ability to grow in low light intensity even at a depth of 4.5m (http://www.lakeandwetlandecosystems.com accessed on 4th Nov. 2017). We also observed its better growth in mixed culture with *Lemna* in the water tanks in the Department of Botany, University of Rajasthan, Jaipur (Personal observation).

Gambusia is important for transferring energy to higher trophic level. It also reduced *Microcystis* population in the beginning of study though overall algal counts were higher than control. The introduction of rohu, catla, silvercarp and grasscarp did not reduce algal biomass in the fish ponds (Rohilla 2008). However, introduction of grass carp and silver carp along with predatory fish (*Wallaga attu*) were effective in reducing algal blooms in the eutrophic water body (Das and Naik 2017). Among submerged macrophytes, *Ceratophyllum* attained higher biomass in the macrophyte + fish treatment and so also nutrient locking (Table 10). Being a rootless angiosperm, *Ceratophyllum* absorbs nutrients directly from water as does *Lemna* and compete with phytoplankton. Their periodic introduction may however be required to maintain good populations in the water body as observed in the Check dam. When these macrophytes grow profusely, their harvest alongwith fish may help in the faster recovering of eutrophic water body. Although *Potamogeton* was more effective in reducing phytoplankton counts in the microcosms but its harvest is difficult when compared with *Ceratophyllym*. In brief macrophytes (*Lemna* + *Ceratophyllum*) in combination with plantivorus, herbivorous and predatory fish shall be more effective in controlling eutrophication of water bodies.

ACKNOWLEGEMENTS

Thanks are due to UGC, New Delhi for awarding Emeritus Fellowships to Prof. K.P. Sharma, Prof. Subhasini Sharma & Post-Doctoral Fellowship to Dr. Shweta Sharma and CSIR-JRF to Dr. Aruna Rajawat.

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