Environmentalist (2010) 30:353–360
DOI 10.1007/s10669-010-9293-6

The impact of duckweed growth on water quality in sub-tropical ponds

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Published online: 7 December 2010

Abstract Field experiments on duckweeds, *Lemna aequinoctialis*, were carried out to determine the effect of plant characteristics on water-quality variables in ponds. In view of their rapid growth rates and ability to cover water surfaces very quickly, such studies are necessary, especially in sub-tropical countries, in order to develop viable management strategies. Morphological parameters like leaf length, leaf width, and root length of individual plants along with cover percent of *Lemna* were studied in each pond along with important water-quality parameters. The plant parameters were related to the studied water-quality variables using regression analysis. Equations showed that changes in root length and cover, both easily measurable growth responses of duckweeds, could be used to reflect changes in two important water-quality variables like DO and phosphorus (both total and soluble reactive) concentrations in pond waters and hence be used effectively for routine monitoring. This study also gives an indication that ponds with low cover of duckweeds could possibly be more effective for prediction purposes.

Keywords Duckweeds · Root length · Cover percent · Dissolved oxygen · Phosphorus

1 Introduction

Duckweeds, a gregarious free-floating form of aquatic macrophytes, belonging to the family Lemnaceae, are commonly found in fresh water ditches and ponds (Landolt 1986). Although eutrophication and unplanned urbanization is resulting in the decline and disappearance of many aquatic plants (Ghosh 2005), duckweeds continue to flourish unabated worldwide. These plants rapidly take up nutrients from water and form thick floating mats over the water surface that has detrimental effects on aquatic life (Cronk and Fennessy 2001). In India too, duckweeds grow luxuriantly in ditches and ponds throughout the country and often restrict the proper functioning of ponds, which are extensively used by the rural and peri-urban people for various anthropogenic purposes. The rampant use of ponds in these regions often necessitates periodic removal of thick mats of duckweeds that if left to rot release nutrients rapidly, thereby compounding the problem of eutrophication. Mukhopadhyay and Dewanji (2006) found excessive growths of duckweeds in two ponds, post-monsoons, and reported a sharp rise in phosphorus concentration of water, which was evident in the succeeding month post complete removal of duckweeds.

Duckweeds have been extensively studied under controlled conditions because of their wide use as experimental organisms in numerous physiological and ecotoxicological studies (Hillman 1961; Wang 1990; Al-Nozaily et al. 2000; Radić et al. 2010). Under field conditions, studies on the growth of duckweeds have mostly been reported from temperate regions (McLay 1974; Rejmánková 1982; Boyd and Tucker 1998). However, there are some studies on its habitat limnology from India and Bangladesh also (Pandit et al. 1978; Nurul Islam and Khondker 1991; Khondker et al. 1994). Apart from the influence of both nitrogen and phosphorus on growth of duckweeds (Rejmánková 1975; De Groot et al. 1987; Portielje and Roijackers 1995; Mukhopadhyay and Dewanji 2005), duckweed root lengths have been related to be indicative of changes in water quality as well (Lüönd 1980; Elster et al. 1995). The use of

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duckweeds in waste water treatment has also long attracted global attention due to its great capacity in absorbing nutrients (Vermaat and Hanif 1998; Körner et al. 2003; Cheng and Stomp 2009), but even then the plants have to be harvested regularly to maintain a healthy duckweed crop (Skillicorn et al. 1993).

Since duckweeds cannot be completely eliminated from waters, which are conducive to its growth and have to be harvested periodically to prevent matting, proper management strategies therefore become critical. This paper, therefore, reports the spatial growth and morphological changes in duckweeds under field conditions and tries to relate them to changes in water quality in an attempt to identify plant parameters capable of effectively predicting important nutrients present in the water. This would probably aid in developing a screening tool that could provide early warnings of potential water-quality problems. A secondary aim, which could be of significance later, was to create a baseline data for future comparisons and to document all associated flora in an effort to relate the effect of duckweed growth on plant biodiversity in ponds.

2 Materials and methods

2.1 Field sampling

Twenty ponds with varying degree of *Lemna* coverage located in and around Kolkata, India (22°37′–22°30′N and 88°23′–88°18′E) were selected for this study. All the ponds were generally small in size (17 ponds had an area <0.3 ha, while pond nos. 8, 9, and 20 were larger with an area >0.3 ha.), and their depth varied from 1 to 3 m. The ponds were located near residential areas and surface runoff was the major source of nutrients (Mukhopadhyay and Dewanji 2002).

Water and duckweeds were sampled on January 13, 2004, from 10 ponds (nos. 1–10) and on January 20, 2004, from the other 10 ponds (nos. 11–20). Water was sampled at 0.25 m depth using a Van Dorn horizontal bottle sampler from four stations where the species was observed, and a composite water sample was taken to the laboratory for chemical analysis. Water temperature, pH, specific conductance (Scon), and dissolved oxygen (DO) of water were measured in situ using a multiparameter calibrated probe (YSI 63 and YSI 550 DO).

Cover percentage of *Lemna* (*Lemna aequinoctialis* L. is the species most commonly found here) and other floating species in each pond was measured in the field using 1 m^2 quadrat (Srivastava et al. 1995). Plant samples were collected with a strainer from all the sampling points for morphological analysis. *Lemna* and its associated floral communities were also noted during the study period. Plant species were identified following Cook (1996), and reconfirmation was done from The Central National Herbaria, Indian Botanic Garden, Sibpur, Howrah.

2.2 Water parameters

Ammonia nitrogen (Ammonia N), total phosphorus (TP), and soluble reactive phosphorus (SRP) were analyzed following standard methods (APHA 1998), while nitrate nitrogen (Nitrate N) was measured following phenol sulphonic acid method (Trivedy and Goel 1986).

2.3 Plant parameters

Plants were washed thoroughly to remove debris. Thirty plants were randomly selected from the pooled plant samples of each pond. The root length, leaf length, and leaf width of each plant was measured with the help of a simple (dissecting) microscope.

2.4 Statistical analysis

Descriptive statistics, Spearman's Rank Correlation analysis and regression equations were fitted to the data following Snedecor and Cochran (1989) for prediction of water-quality parameters. Data were transformed to increase homogeneity of variance and normality, and the best transformations were used for model building during regression analysis.

For a particular water-quality variable, all the independent predictor variables that were significantly correlated with it were first chosen for model building. If the predictor variables were found to be correlated, then the one with higher correlation with water quality was retained. Following this strategy, a model with a fixed set of predictor variables was chosen for each water-quality variable using the stepwise regression method. All statistical analysis was done using SPSS version 12.0 (SPSS Inc., Chicago, USA).

3 Results and discussion

3.1 Associated flora and cover percent of *Lemna*

The distribution of aquatic plants in the 20 ponds along with the total number of species present in each pond is reported in Table 1. Among the twenty ponds studied, eleven ponds had ≥60% *Lemna* cover. Although 14 other species have been listed in Table 1, the maximum number of species occurring in any given pond was 5 (ponds 18 and 19), while the minimum was one (ponds 6 and 13). If only true aquatics (floating and submersed species) are considered, then a maximum number of four species was found in

Table 1 Distribution of aquatic plant species in 20 ponds

	Ponds																			
Plant species	1	2	3	4	5	6	7	**8**	**9**	10	11	12	13	14	15	16	17	18	19	**20**
Lemnaceae																				
Lemna aequinoctialis	●	●	●	●	●	○	●	○	○	●	○	○	●	●	○	●	●	○	○	○
Spirodela polyrhiza		+	+	+					+		+						+		+	+
Wolffia arrhiza																		+		
Other floating species:																				
Azolla pinnata																		+	+	+
Eicchornia crassipes			+		+															
Submersed:																				
Ceratophyllum demersum								+												
Vallisneria spirallis																			+	
Emergent:																				
Alternanthera philoxeroides	+			+	+		+			+	+	+		+	+	+	+	+		+
Cyperus rotundus	+																			
Commelina benghalensis		+																		
Amaranthus viridis				+																
Mikania scandens										+				+						
Colocasia esculenta										+										
Ipomoea aquatica																+		+		
Marsilea minuta																			+	
Total number of plant species	3	3	3	4	3	1	2	2	2	4	3	2	1	3	2	3	3	5	5	4

● *Lemna* cover ≥60%, ○ *Lemna* cover <0, + Presence

Pond numbers with bold face are larger in size (>0.3 ha) others are smaller (<0.3 ha)

1 pond, three species in 4 ponds, two in 6 ponds, and only *Lemna* itself in 9 ponds. Thus, maximum number of species found in most ponds was two or even less, signifying low plant species diversity associated with *Lemna* growths.

Members of *Lemnaceae* generally often grow together with other species of the same family, and in this study, *Lemna* was present together with *Spirodela* in 8 ponds and with *Wolffia* in just one. *Azolla* and *Eichhornia* were the only 2 other floating species present in 5 ponds. Hillman (1961) reported the association of *Lemna* with other *Lemnaceae* species, *Azolla, Eichorrnia,* and *Nympheacae.* Khondker et al. (1993) found an association of *Spirodela* with *Eichhornia, Lemna, Enhydra,* and *Pistia* in polluted shallow ditches and open drains in Bangladesh, while Mukhopadhyay et al. (2004) observed an association of *Lemna* with *Nymphoides* and *Vallisneria* throughout a 1-year period in a pond in India.

Cover percent merits some investigation in view of its wide variation between ponds (3–100%) as is evident from the bar graphs of *Lemna* cover of individual ponds shown in Fig. 2. Ponds with low cover could be associated with more number of plant species as seen from Table 1 (ponds 18 and 19 with cover <30%, as is evident from Fig. 2 had five species each). Eight of the 9 ponds with monospecific stands of *Lemna* (ponds 1, 7, 10, 12, to 16 see Table 1) had more than 40% cover (Fig. 2), thereby showing its ability to out compete other aquatics when *Lemna* had almost covered half of the pond. Thirteen ponds with >40% cover (Fig. 2) had no submersed plants in them (Table 1), thereby showing that submersed vegetation was highly affected by *Lemna* cover. At high covers, duckweeds often restrict the growth of submerged macrophytes by obstructing penetration of light for submerged species to develop (Sculthorpe 1967). Thus, the two ponds with submersed plants namely, pond 8 with *Ceratophyllum demersum* and pond 19 with *Vallisneria spiralis* had low cover values as can be seen from Fig. 2. The predominant emergent vegetation was *Alternanthera*, which was present in 13 out of 20 ponds, and its presence was unaffected by high cover of *Lemna* in 8 ponds (Table 1).

3.2 Morphological parameters

The variation in leaf length, leaf width, and root length of *Lemna* in 20 ponds is reported in Fig. 1. The maximum and minimum values were 5 mm and 2 mm for leaf length, 4 mm and 1.5 mm for leaf width, and 35 mm and 2 mm

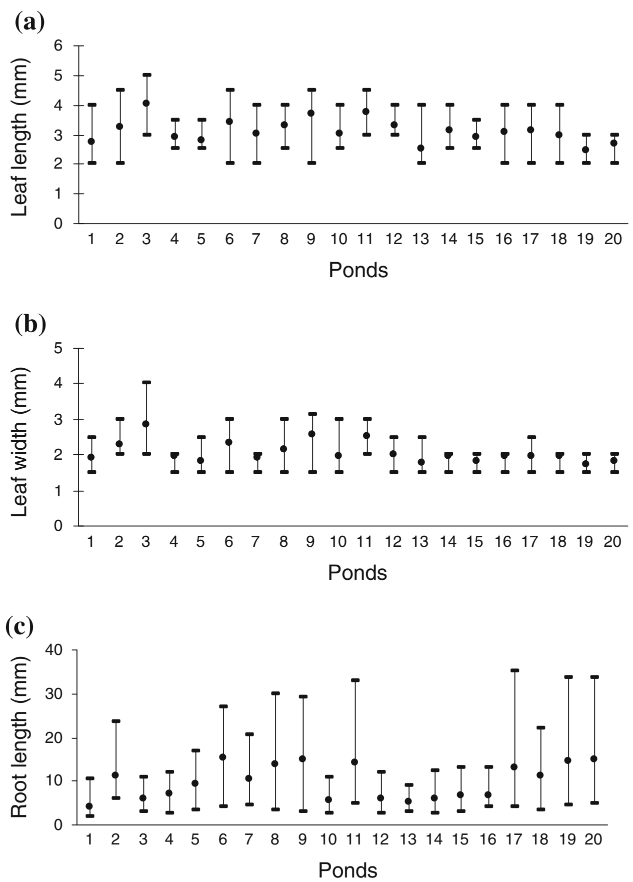

Fig. 1 Variation in leaf length, leaf width, and root length (values based on 30 individual plants) of *Lemna* in 20 ponds. **a** Leaf Length, **b** Leaf Width, **c** Root Length

for root length. Thus, the maximum variation observed in root lengths of *Lemna* could possibly be a reflection of differences in water quality between ponds. Root length in *Lemna* was reported to vary from 5.2 to 45.9 mm in field studies (Elster et al. 1995), while a maximum root length of 64 mm was observed when *Lemna* was grown in nutrient solutions (Lüönd 1980).

From Fig. 1c, it is also evident that root lengths appear to have larger variance with higher means (≥10 mm). It is interesting to note that among the 8 ponds that exhibited large variance in root length (ponds 6, 8, 9, 11, 17, 18, 19, and 20), seven ponds had low cover values (<30%—Fig. 2). On the other hand, 10 out of 12 ponds with very low variance in root length exhibited high cover values (≥60%— Fig. 2). The possible reason for low variance at high covers is probably because of the uniform spread of the plants at higher covers, sometimes leading to the formation of thick mats, which restrict movement of the plants. On the other hand, at low covers, the plants probably have enough room to move around due to wind action allowing them to resettle in another region of the pond with differing levels of nutrients, thereby showing more variance in root length.

3.3 Water quality in duckweed ponds

During the period of study, water temperature in the 20 ponds varied from 15.5 to 22.9°C. The variation in each of the water-quality variables studied with respect to the 20 ponds along with the variation in cover percent of *Lemna* is shown in Fig. 2, while the descriptive statistics for the water-quality variables is reported in Table 2. pH values were mostly alkaline in the ponds studied and varied between 6.9 and 9.1. The lower and upper limits of pH for growth of *Lemna* are reported to be 4 and 10 with an optimum at or below pH 7 (McLay 1976). The mean DO of the 20 ponds was low (2.93 mgl^{-1}), and the lowest value of 0.71 mgl^{-1} was found in pond 13 with 100% cover, thus reiterating the negative impact of high duckweed coverage. Similarly, pond 9 with the least *Lemna* cover had maximum DO (10.4 mgl^{-1}). There was a lot of variation in specific conductance values between the ponds as has been reported for waters where *Lemna* can be found (Landolt 1975). TP values in the 20 ponds varied from 0.27 to 4.0 mgl^{-1}, while the readily absorbable form, SRP, ranged from 0.04 to 1.78 mgl^{-1}. Higher SRP values (0.1 to 3.94 mgl^{-1}) have been reported in duckweed-infested ditches from Bangladesh (Khondker et al. 1994) that had an inflow of organic wastes and domestic effluents. Different forms of nitrogen (ammonia N and nitrate N) are known to be important for plant growth, but nitrogen values found in this study were very low when compared to those reported in waters with organic sources of nutrient inputs (Khondker et al. 1994; Driever et al. 2005). From Fig. 2, it can also be seen that pond 13 (with highest cover) had high values of both nutrients (N and P), while pond 9 (with least cover) exhibited highest values for both DO and pH.

3.4 Water quality and its prediction using plant variables

The correlation coefficients between the water-quality variables, plant morphological parameters and *Lemna* cover are reported in Table 3. Among the plant variables, leaf length and leaf width were highly correlated (0.908). Root length and cover percent showed a significant negative correlation (−0.636), which is probably a reflection of the higher means observed for root lengths at low cover (Fig. 1). Both cover percent and root length were significantly correlated with pH and DO. pH, DO, SRP, and TP were the four water-quality variables that showed significant correlation with root length. However, neither forms of N (ammoniaN or nitrateN) appeared to be correlated with root length unlike preferences for nitrogen reported in

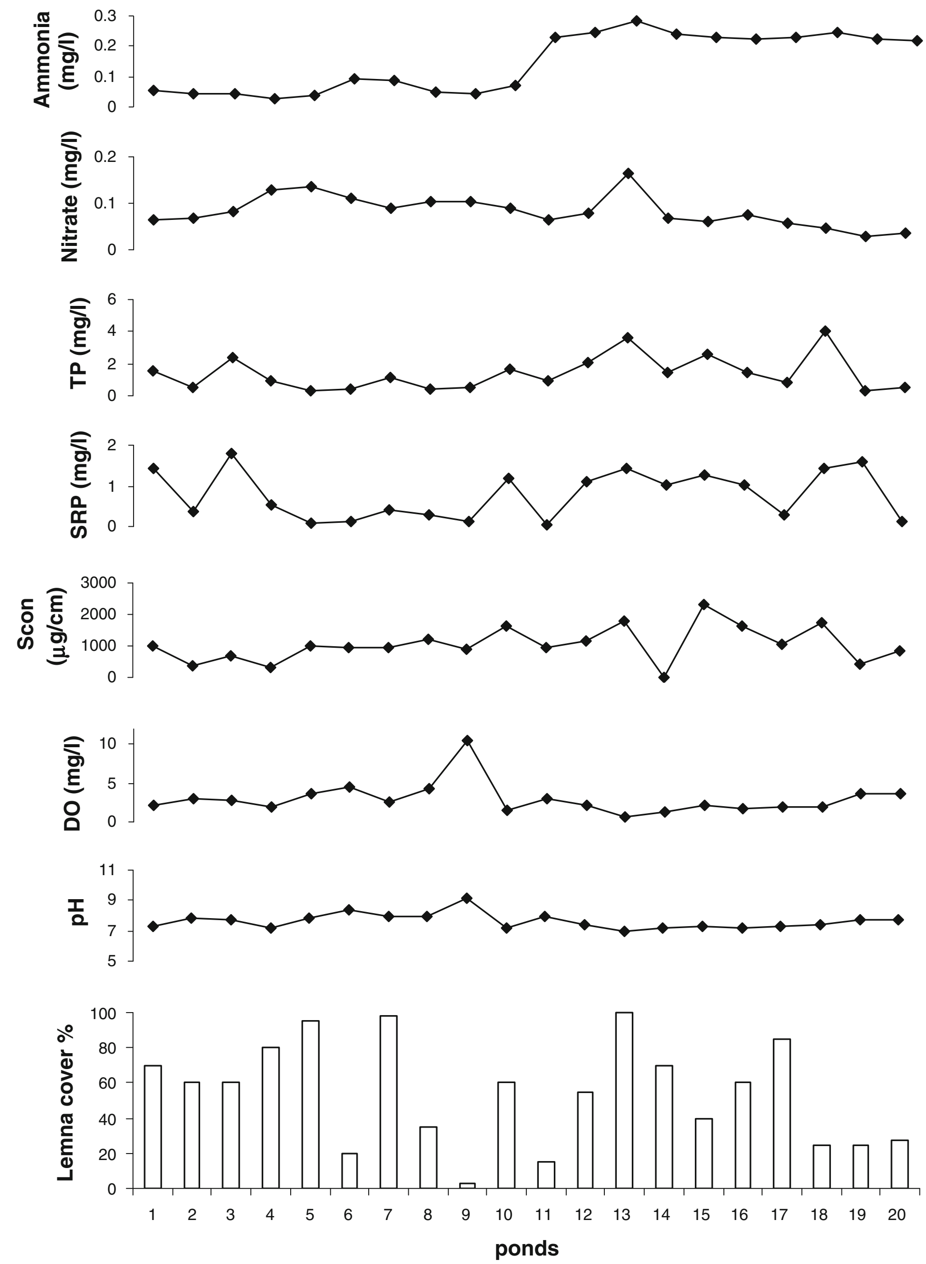

Fig. 2 Variations in different water-quality parameters and cover of *Lemna* in 20 ponds

Table 2 Descriptive statistics of water-quality variables in 20 ponds

	Minimum	Maximum	Mean	Standard deviation (±)
Water-quality parameters				
pH	6.90	9.10	7.63	0.50
DO (mgl^{-1})	0.71	10.40	2.93	2.03
Specific conductance (μScm^{-1})	328.40	2331.00	1105.60	522.40
Soluble Reactive Phosphorus (mgl^{-1})	0.04	1.78	0.78	0.60
Total Phosphorus (mgl^{-1})	0.27	4.00	1.37	1.08
Ammonia Nitrogen (mgl^{-1})	0.03	0.28	0.15	0.10
Nitrate Nitrogen (mgl^{-1})	0.03	0.16	0.08	0.03

temperate countries (Körner et al. 2001; Rubio et al. 2003). This could probably be due to the presence of very low concentrations of N in pond waters. As runoff was the main source of nutrient enrichment in the ponds studied, it probably could not contribute significantly to N uptake by the plants.

Table 3 Spearmans's Rank Correlation between different water-quality and plant morphological parameters ($n = 20$)

	Dependent water-quality variables							Independent plant variables			
	pH	DO	SCon	SRP	TP	Ammonia N	Nitrate N	Leaf L	Leaf W	Root L	Cover
pH	1.000	**0.931****	−0.432	**−0.564****	**−0.626****	−0.403	0.098	0.397	0.394	**0.721****	**−0.525***
DO		1.000	**−0.512***	**−0.468***	**−0.722****	**−0.495***	0.028	0.234	0.264	**0.731****	**−0.578****
Scon			1.000	0.241	**0.560***	**0.560***	0.039	−0.156	−0.246	−0.418	0.135
SRP				1.000	**0.640****	0.291	−0.211	−0.352	−0.276	**−0.611****	0.209
TP					1.000	**0.479***	−0.092	−0.036	0.002	**−0.704****	0.235
Ammonia N						1.000	−0.424	−0.128	−0.264	−0.136	−0.068
Nitrate N							1.000	0.188	0.222	−0.213	0.348
Leaf L								1.000	**0.908****	0.196	−0.371
Leaf W									1.000	0.209	−0.378
Root L										1.000	**−0.636****
Cover											1.000

Significant values are shown in bold

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

The regression equations for the best-fitted prediction models for water-quality variables using cover and root length of *Lemna* are given in Table 4. From the table, root length appears to the plant predictor variable of primary importance for both forms of phosphorus (TP and SRP) and showed a clear inverse trend with respect to phosphorus, which is consistent with other reports in literature of increased root elongation in nutrient deficient ponds (Lüönd 1980; Elster et al. 1995).

Although root length appears to be the most useful morphological feature of duckweeds and features in all the four models, nevertheless, inclusion of cover improves the prediction equations in case of pH and DO and seems to be a good fit as is evident from the R^2 values. This is probably because at low covers, a larger surface area is exposed for sunlight penetration that promotes higher photosynthetic activity within the water, thereby resulting in higher DO (Pokorny and Rejmankova 1983). The significant positive correlation between DO and root length (0.731) could probably be another explanation for the higher means for root length of duckweeds at low cover. The normal probability plots of the standardized residuals based on the fitted model are presented in Fig. 3. The normal PP plots (Fig. 3a) are quite close to the diagonal line showing evidence in favor of the normality assumptions. The scatter plots of standardized residuals against the residual values of the error variance (Fig. 3b) are similar over the ponds and scattered around zero.

This study also indicates that water-quality parameters of interest could be ascertained by studying root length of duckweeds in ponds with low covers because of the heterogeneity of variance observed in root lengths at low covers (Sect. 3.2). This observation could prove useful for management purposes, since as soon as there are indications of higher phosphorus levels in the ponds through measurement of root lengths, duckweeds could be manually removed before they assume exponential growths and start restricting water body use.

4 Conclusions

Reliable predictions for DO and phosphorus using the best-fitted models could be made during routine monitoring of duckweed ponds. DO happen to be a very important variable for aquaculture ponds and phosphorus is a nutrient directly related to eutrophication. However, the applicability of this model should be tested on more pond types, and the phenomenon of higher variability in duckweed root lengths at low covers should also be verified with

Table 4 The best-fitted prediction models for pH, dissolved oxygen, soluble reactive phosphorus, and total phosphorus

Variable	Fitted model	R^2
log pH	0.856 + [0.197* *reciprocal* cover] + [1.625/10000* *square* root length] + error	0.702
log DO	0.164 + [0.002* *square* root length] + [1.458* *reciprocal* cover] + error	0.657
log SRP	1.356 − [0.544* *square* root length] + error	0.488
log TP	0.351 − [0.033* *square* root length] + error	0.496

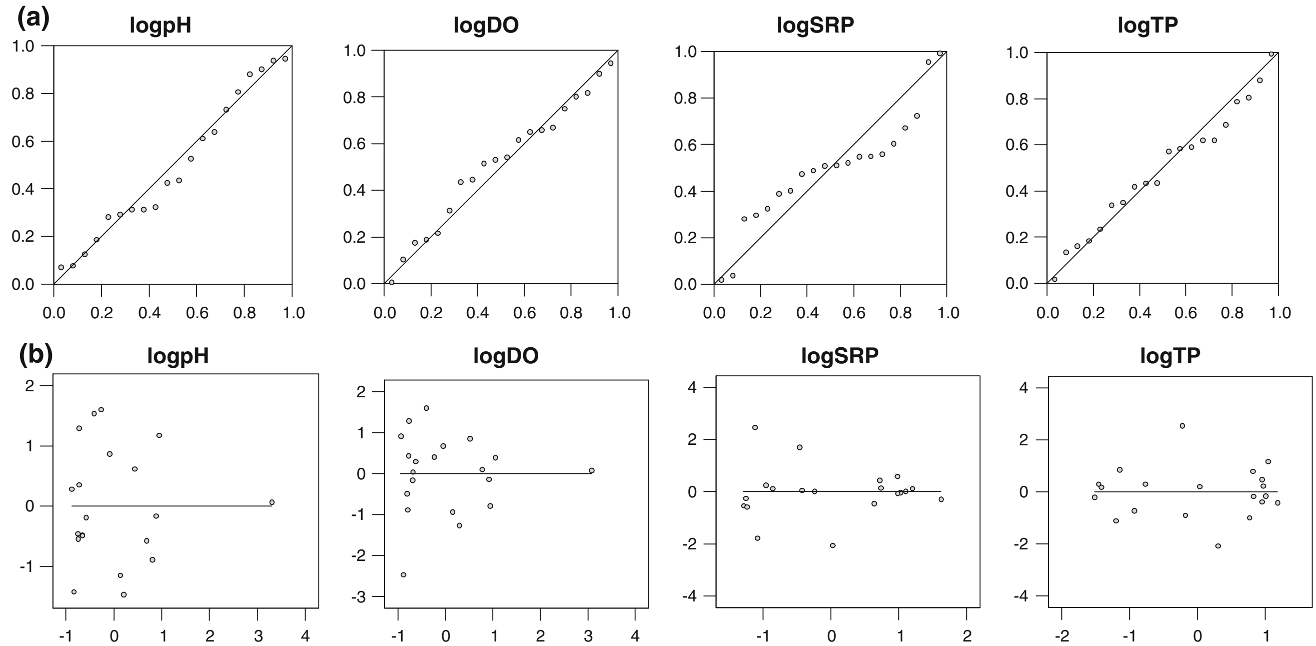

Fig. 3 **a** Normal P–P Plot of Regression Standardized Residual. **b** Scatter Plot of Standardized Residuals against predicted values

observations from more ponds. Moreover, the temporal influence on duckweed growth and morphology may be very important and needs immediate attention. Nevertheless, in India, where regular monitoring of water quality through sophisticated laboratory techniques is less practicable, easily measurable changes in morphological and cover characters of duckweeds could prove to be very promising for indicating changes in important water-quality variables.

Acknowledgments We gratefully acknowledge the help of Dr. Gautam Mukhopadhyay, Lachman Bahadur Magranti, Sandip Chatterjee and Susant Mahankur of Agricultural and Ecological Research Unit during field and laboratory work**.** Statistical guidance provided by Prof. Anup Dewanji, Applied Statistics Unit is also gratefully acknowledged.

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