

Research Article

***Gerris spinolae* Lethierry and Severin (Hemiptera: Gerridae) and *Brachydeutera longipes* Hendel (Diptera: Ephydriidae): Two Effective Insect Bioindicators to Monitor Pollution in Some Tropical Freshwater Ponds under Anthropogenic Stress**

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The abundance patterns of two insects, *Gerris spinolae* and *Brachydeutera longipes*, were found to be affected by abiotic aquatic factors including free carbon dioxide, dissolved oxygen, BOD, and phosphate concentrations prevailing in four tropical freshwater ponds, three of which being anthropogenically stressed. Regression analysis between each individual-independent water quality variable and insect abundance demonstrated a significant positive correlation in each case between *B. longipes* abundance and BOD, phosphate, free CO₂, and algae dry weight, while a significant negative correlation of each of these variables was found with *Gerris spinolae* abundance. Moreover, a significant negative correlation of *B. longipes* abundance was calculated with dissolved oxygen concentration, while *G. spinolae* abundance exhibited a positive correlation with the same. Thus, *G. spinolae* appears to be a pollution sensitive, effective bioindicator for healthy unpolluted ponds, while *B. longipes* has potential as a pollution-resistant insect species indicative of pollution occurrence.

1. Introduction

Freshwater bodies in urban ecosystems are under stress due to anthropogenic pressures. Pollution of inland water habitats, both *lotic* (running water) and *lentic* (lakes and ponds), impacts pollution of soil and ground water and thereby affects the essential basic (drinking water supply) and social requirements (aesthetic, religious, etc.) of human societies. Monitoring and maintaining the water quality of wetlands is also important since these recharge the groundwater and also affect the plant diversity in its vicinity. Environmental monitoring of inland freshwater bodies is an essential prerequisite for their management. Biological indication is, therefore, increasingly being advocated. Biological indication or bioindication is the process of using a species or group of species that readily reflects the abiotic and biotic states of an environment, represents the impact of environmental change on a habitat, community, or ecosystem, or is indicative of the diversity of a subset of taxa or of the entire diversity, within

an area [1, 2]. Bioindicators or ecological indicators are taxa or groups of animals that show signs that they are affected by environmental pressures due to human activities or the destruction of the biotic system [3]. Bioindicators also provide information about the cumulative impact of the various pollutants in an ecosystem [1, 4–6]. An ideal taxon must respond predictably, in ways that are readily observed and quantified to environmental disturbance [7]. Aquatic bioindicators used so far are plants [8–10] including diatoms [4, 11]; vertebrates, mainly fish [5, 12, 13] and macroinvertebrates [1, 6, 7, 14, 15]. Among invertebrates, insects are good candidates [16–21]. However, not all insects respond in a predictable manner to environmental pollution. Insects widely utilized as bioindicators include larval Chironomids (Diptera: Chironomiidae) [22] and water striders (Hemiptera: Gerridae), the latter being particularly well known for indicating heavy metal pollution, [18, 23–26] which is also characterized by oxygen stress. Insects offer a number of advantages as bioindicators. These include the

availability of a wide range of insects from various insect orders which (i) exhibit high sensitivity and the degree of sensitivity gives a series of choice of bioindicators depending upon the needed resolution, (ii) involve the entire trophic levels, thus ecosystems can be monitored from functional point of view, (iii) exhibit high fecundity, greater breeding potential reduces the chances of the potential bioindicator getting destroyed entirely from the ecosystem, and finally (iv) involve less ethical problems.

While some studies have been carried out on bioindicators of lotic ecosystems [19, 27–29] studies of potential insect bioindicators of lentic ecosystems are scanty [30, 31], especially those of the tropical regions [15]. The present study focuses on assessing the potential of two insect species: the water strider, *Gerris spinolae* (Lethierry and Severin) and the shorefly, a semi-aquatic dipteran, *Brachydeutera longipes* (Hendel). Studies pertaining to the genus *Brachydeutera* are scanty although its occurrence is reported from lentic habitats [32]. While water striders, common in freshwater water bodies of temperate and tropical water bodies, are predaceous [33, 34], the genus, *Brachydeutera*, is documented to include species such as *B. hebes*, *B. argentata*, and *B. neotropica* the larval stages of which scavenge upon dead and decaying plant and animal tissues and also consume algae [32]. Since *B. longipes* is reported to be an algal feeder [35] and algal blooms are characteristic feature of polluted ponds, this species merits further investigation to examine its potential as a bioindicator. The impact of abiotic aquatic factors on the ecological response of the two focal insect species was investigated and the relationship between the abundance pattern of each species and the degree of pollution was determined.

2. Materials and Methods

2.1. Study Sites. The investigations were carried out from January to March, 2011 (3 months), in Varanasi, Uttar Pradesh, India. Four man-made ponds presently under anthropogenic stress were selected for the present study. While the pond located in the Botanical garden of Banaras Hindu University (not being under any anthropogenic stress) was considered as the control and the three ancient ponds, about 200 years old [36] under anthropogenic stress (due to human activities such as bathing, washing clothes, dumping organic wastes in the form of flowers, and so forth, in the ponds, particularly during religious ceremonies and festivals) located in a thickly populated urban ecosystem, were taken as the experimental.

The Kurukshetra (Krk), Sankuldhara (Skd), Durgakund (Dgk), and Botanical garden (Btg) ponds are located in Assi, Khajwa, Durgakund, and Banaras Hindu University Campus areas, respectively. All the ponds except Btg have 1-2 old temples around them. The dimensions of Krk pond are about 20 m × 25 m × 6 m. Its four banks are bounded by stone tiles

from all around. Due to dense human inhabitation around it, there is heavy anthropogenic pressure on it. The dimensions of Skd pond are about 30 m × 30 m × 7 m. Its parapets are also bounded by stone tiles and, in addition it is surrounded by iron grid fencing. The dimensions of Dgk pond are about 40 m × 40 m × 10 m. Its parapets are also bounded by stone tiles and in addition, it is surrounded by an iron grid fencing. Human activities including occasional bathing, washing of clothes, and dumping of organic wastes in the form of flowers, and so forth during religious and social ceremonies occur in all these three ponds. The dimensions of Btg pond are about 10 m × 8 m × 2 m. It was constructed for the purpose of watering garden plants. It is free from all anthropogenic pressures.

2.2. Water Quality Assessment. Transparency was determined for each pond by using the Secchi disc method while total solids were assessed by the standard dry weight method [37]. All the other physical and chemical parameters were monitored twice a week ($n = 15$) at each study site. DO and BOD (5 days, 20°C) were determined by the modified Winkler's method [37]. Free CO₂ level was assessed by titrating the samples with 0.05 N NaOH solution in the presence of phenolphthalein indicator. Phosphate ion concentration was determined by the standard spectroscopic method [37].

2.3. Insect Diversity of the Ponds and Selection of Insect Species for Investigation of Aquatic Bioindicator Potential. The study revealed that each of the ponds supported a variety of aquatic insects from different orders, including water striders, back swimmers, water bugs (Order: Hemiptera), flies, mosquito larvae (Order: Diptera), and damselfly, dragonfly (Order: Odonata). Among these, two insect species, namely, *Gerris spinolae*, Lethierry and Severin, (Hemiptera: Gerridae; det. NPIB) RRS No. 1116-1117/11 and *Brachydeutera longipes*, Hendel (Diptera: Ephydriidae; det. NPIB), and (RRS No. 1118-1124/11), were selected for further studies. These were identified by experts of the Network Project on Insect Biosystematics (NPIB), Division of Entomology, Indian Agricultural Research Institute, New Delhi. These two species were selected to study the impact of specific abiotic factors prevailing in the three anthropogenically stressed ponds, on the basis of the preliminary field observations regarding their differential habitat preferences.

2.4. Abundance of Adult Stages of the Two Insect Species: *Gerris spinolae* and *Brachydeutera longipes*. Insect abundance was monitored twice a week ($n = 15$) per pond. Quadrat sampling was done from sixteen different sites of each pond (four sites per side per pond), $n = 240$ quadrats per pond. The following formula was used to calculate the abundance:

$$\text{abundance} = \frac{\text{total number of individuals of the focal species in all the sampling units}}{\text{number of sampling units in which the species occurred}}. \quad (1)$$

2.5. Life Cycle of *B. longipes*. *Brachydeutera longipes* was cultured under laboratory conditions by carefully adding about 10 mg of fresh algae, *Microcystis* sp. (which was carefully layered on the water surface), to 1 liter pond water contained in a 5 liter glass jar ($n = 3$). Thereafter, 2 pairs of *B. longipes* were introduced in each jar. Small fractions of the algae were examined daily under the Stereobinocular microscope and the various life cycle stages and feeding behaviour of the larval stages were recorded.

2.6. Statistical Analysis. Variation in the abiotic factors, that is, temperature, pH, free CO₂, dissolved oxygen (DO), biological oxygen demand (BOD), phosphate ion concentration, and a biotic factor-concentration (dry weight/m²) of the algae, *Microcystis* sp. in each of the four ponds was analysed by using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test by using SPSS-PC software. Regression analysis for calculation of the correlation between the abundance of each of the two insect species, *Brachydeutera longipes* and *Gerris spinolae* with each of the above-mentioned seven water quality parameters considered individually in each case, was carried out by using SPSS-PC software.

3. Results

3.1. Life Cycle of *B. longipes*. Examination of the surface of the algal vegetation under laboratory conditions showed the presence of pale brown, cigar-shaped operculated eggs. Three larval instars were recorded, the duration of each stage was found to be approximately 2-3 days with that of the pupal stage being about 3-4 days. The larvae were observed to feed voraciously on *Microcystis* sp. Adults were recorded to have an approximate life span of 2-3 months.

3.2. Water Quality Assessment. A significant variation in water transparency was found in the four ponds: Btg pond (182.5 cm), Krk pond (63.8 cm), Skd pond (52.67 cm), and Dgk pond (29.67 cm). There was also variation in the amount of total solids present in each pond, with Btg pond having least amount of total solids including dissolved (275.8 mg/L) and suspended (3.9 mg/L) in comparison to the solids present in the other three ponds. The amount of dissolved solids were 321.7 mg/L, 537.2 mg/L, and 873.9 mg/L and suspended solids were 4.1 mg/L, 4.6 mg/L, 7.3 mg/L in Krk, Skd, and Dgk ponds, respectively (Figure 1).

Six abiotic parameters, namely, temperature, pH, free CO₂, dissolved oxygen (DO), BOD, phosphate ion concentration, and one biotic parameter, that is, food availability of *B. longipes* larvae in terms of the dry weight of *Microcystis* sp. per square meter, were monitored in all the four ponds. Significant variation was found in case of each parameter except temperature, for all the four ponds: ANOVA-temperature ($F_{3,56} = 0.01$; $P > 0.05$), pH ($F_{3,56} = 9.307$; $P < 0.001$), free CO₂ ($F_{3,56} = 41.667$; $P < 0.001$), dissolved oxygen ($F_{3,56} = 437.235$; $P < 0.001$), biological oxygen demand ($F_{3,56} = 188.284$; $P < 0.001$), concentration of phosphates ($F_{3,56} = 32.839$; $P < 0.001$), and food availability of *B.*

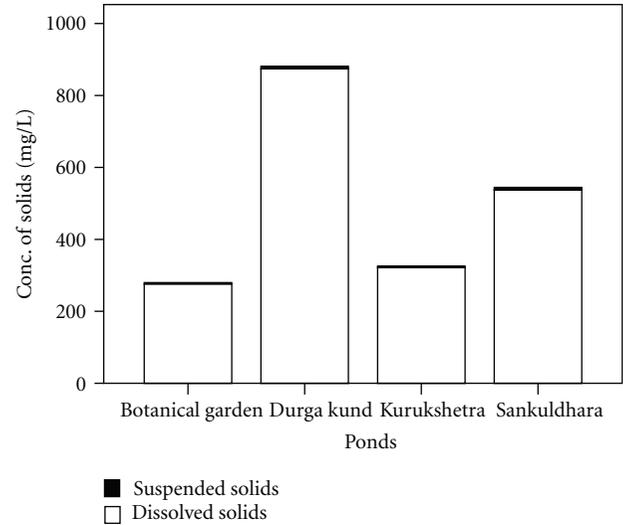


FIGURE 1: Concentration (mg/L) of total solids, suspended solids and dissolved solids in the control (Botanical garden) and anthropogenically stressed (Kurukshetra, Sankuldhara and Durgakund) ponds.

longipes larvae in terms of dry weight of *Microcystis* sp. per square meter ($F_{3,56} = 604.686$; $P < 0.001$) Table 1.

Post hoc tests revealed significant differences (Dunnett's test, $P < 0.001$) in case of each parameter under study (except free CO₂ level which was not found to be significantly different ($P > 0.05$ in the Krk pond), in all the three experimental ponds in comparison to the control.

3.3. Abundance of Adult Stages of Insects, *Gerris spinolae* and *Brachydeutera longipes*, in the Four Ponds. The abundance of adult stages of *Gerris spinolae* and *B. longipes* in the four ponds varied significantly: one-way ANOVA: $F_{3,56} = 11.124$; $P < 0.001$, for *G. spinolae*, and $F_{3,56} = 17.327$; $P < 0.001$, for *B. longipes* (Figures 2(a) and 2(b)).

Post hoc tests revealed significant differences in the abundance of *B. longipes* in all the three experimental ponds in comparison to the control pond, the lowest being in Dgk pond (Dunnett's test, $P < 0.001$), with abundance being in the increasing order in Skd and Krk ponds (Dunnett's test, $P < 0.001$, for both). The two experimental ponds Dgk and Skd differed significantly from the control (post hoc test: Dunnett's test, $P < 0.001$, for both) in exhibiting significantly lower abundance of the *G. spinolae*. However, Krk pond did not show significant deviation from the control pond in this respect (Dunnett's test, $P > 0.05$).

Regression analysis between each individual independent water quality variable: temperature, pH, BOD, DO, free CO₂, phosphate, dry weight of algae, with the abundance of adult stage of each of the two insect species, *Brachydeutera longipes* and *Gerris spinolae* (dependent variables) reveals the following: a significant positive correlation ($P < 0.001$) between *B. longipes* abundance and BOD ($r = 0.528$), PO₄ ($r = 0.587$), free CO₂ ($r = 0.473$), and dry weight of algae

TABLE 1: Physical, chemical, and biological parameters of water quality in the four ponds (Botanical garden—control; Kurukshetra, Sankuldhara and Durgakund—anthropogenically stressed) located in different parts of Varanasi, India.

Water parameters	In Botanical garden pond		In anthropogenically disturbed ponds		
	(Control)		Kurukshetra	Sankuldhara	Durgakund
Temperature ($^{\circ}\text{C}$) (Mean \pm SEM)	16.19 \pm 2		16.35 \pm 2.01 ^{ns}	16.53 \pm 2.02 ^{ns}	16.67 \pm 2.02 ^{ns}
pH (Mean \pm SEM)	6.89 \pm 0.11		9.16 \pm 0.015 ^{***}	8.85 \pm 0.013 ^{***}	6.402 \pm 0.016 ^{***}
Dissolved oxygen (mg/L) (Mean \pm SEM)	8.84 \pm 0.50		7.61 \pm 0.42 ^{***}	4.98 \pm 0.33 ^{***}	4.13 \pm 0.45 ^{***}
Free CO ₂ conc. (mg/L) (Mean \pm SEM)	1.8 \pm 0.25		1.07 \pm 0.16 ^{ns}	5.18 \pm 0.38 ^{***}	5.38 \pm 0.5 ^{***}
BOD (mg/L) (Mean \pm SEM)	5.68 \pm 0.42		6.49 \pm 0.47 ^{***}	8.03 \pm 0.53 ^{***}	8.47 \pm 0.62 ^{***}
Phosphate ion conc. (mg/L) (Mean \pm SEM)	0.16 \pm 0.02		0.43 \pm 0.05 ^{***}	0.47 \pm 0.05 ^{***}	0.63 \pm 0.09 ^{***}
Dry weight of algae, <i>Microcystis</i> sp.(g/sq m) (Mean \pm SEM)	00.00		10.81 \pm 0.35 ^{***}	10.94 \pm 0.29 ^{***}	30.58 \pm 0.36 ^{***}

Where * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, ns—not significant.

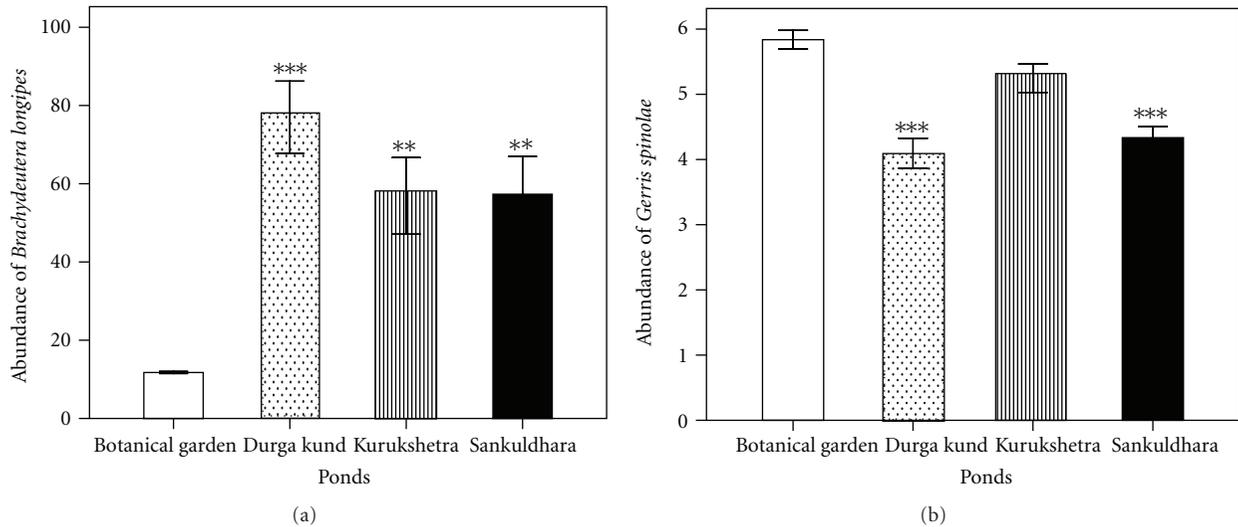


FIGURE 2: Abundance (No./sq. m) of adults of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) in the control (Botanical garden) and anthropogenically stressed (Kurukshetra, Sankuldhara and Durgakund) ponds, where * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, ns—not significant.

($r = 0.519$) and a significant negative correlation ($P < 0.001$) with DO ($r = 0.527$). On the other hand, *G. spinolae* abundance exhibited a significant positive correlation ($P < 0.001$) with DO ($r = 0.780$) and temperature ($r = 0.60$) and a significant negative correlation ($P < 0.001$) with BOD ($r = 0.686$), PO₄ ($r = 0.604$), free CO₂ ($r = 0.829$), and dry weight of algae ($r = 0.547$) Table 2.

Brachydeutera longipes abundance showed less significant positive correlation with pH ($r = 0.346$, $P < 0.01$) and a negative correlation with temperature ($r = 0.305$, $P < 0.05$) whereas *G. spinolae* abundance demonstrated no significant correlation with pH ($r = 0.115$, $P > 0.05$) Figures 3(a), 3(b); 4(a), 4(b); 5(a), 5(b); 6(a), 6(b); 7(a), 7(b); 8(a), 8(b); and 9(a), 9(b).

4. Discussion

Our study clearly reveals that the abundance of adult stages of the two insect species, *G. spinolae* and *B. longipes* in the three ponds under anthropogenic stress is affected (although in a contrasting manner) due to differences in the levels of organic pollution and the resulting impacts of abiotic and biotic aquatic components of the ponds. Durgakund, Sankuldhara, and Kurukshetra ponds exhibit pollution in a decreasing order with higher concentrations of total dissolved and suspended solids, free CO₂ levels, phosphate ion concentration, and amount of *Microcystis* sp. being more prevalent in the most polluted Durgakund pond and less in the remaining two anthropogenic stressed ponds.

TABLE 2: Regression analysis output obtained by correlating each variable (water quality parameter) independently with the abundance of each of the two insect species, *Brachydeutera longipes* and *Gerris spinolae*.

Parameters	Insect species	Regression coefficient (β)	r	Significance
BOD	<i>Brachydeutera longipes</i>	18.29	0.528	***
	<i>Gerris spinolae</i>	-0.613	0.686	***
DO	<i>Brachydeutera longipes</i>	-10.067	0.527	***
	<i>Gerris spinolae</i>	0.384	0.780	***
PO ₄	<i>Brachydeutera longipes</i>	134.317	0.587	***
	<i>Gerris spinolae</i>	-3.563	0.604	***
Free CO ₂	<i>Brachydeutera longipes</i>	7.946	0.473	***
	<i>Gerris spinolae</i>	-0.359	0.829	***
Dry weight of algae	<i>Brachydeutera longipes</i>	1.835	0.519	***
	<i>Gerris spinolae</i>	-0.050	0.547	***
Temperature	<i>Brachydeutera longipes</i>	-1.584	0.305	*
	<i>Gerris spinolae</i>	0.081	0.602	***
pH	<i>Brachydeutera longipes</i>	11.314	0.346	**
	<i>Gerris spinolae</i>	-0.97	0.115	ns

Where * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, ns—not significant.

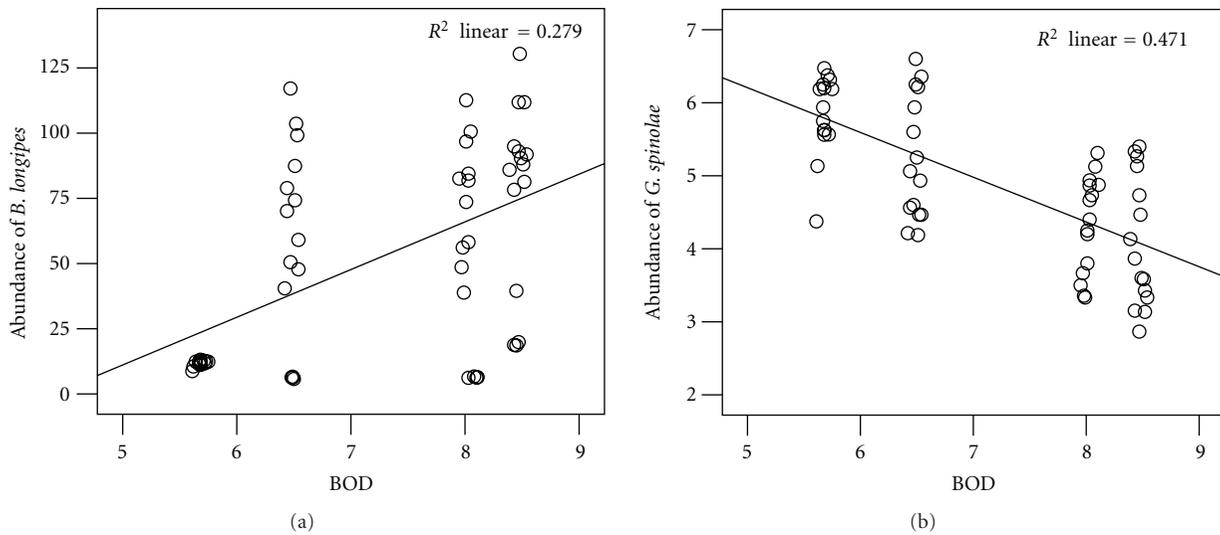


FIGURE 3: Relation between BOD and abundance of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) as shown by regression analysis.

Temperature and pH were higher in the polluted ponds in comparison to the control while transparency was much reduced. Thus, the greater the pollution level in the pond, the lesser is the abundance of *G. spinolae* as demonstrated by its low abundance in the Durgakund pond. Regression analysis between each individual independent water quality parameter with the abundance of *B. longipes* revealed a significant positive impact of BOD, free CO₂, phosphate concentration, and dry weight of algae (characteristic of polluted aquatic conditions) and a negative impact of DO concentrations. On the other hand, a significant positive influence of dissolved oxygen concentration (characteristic of unpolluted aquatic conditions) was found on *G. spinolae*

abundance with the correlation being negative with BOD, free CO₂, phosphate concentration, and dry weight of algae (characteristic of polluted aquatic conditions). Therefore, higher abundance of *B. longipes* appears to indicate greater aquatic pollution. Since the maintenance of integrity between the physico-chemical and biological components of an ecosystem determines its health status [5, 38], it is abundantly clear that *G. spinolae* prefers unpolluted, while the semiaquatic shore fly prefers polluted lotic water bodies.

Earlier studies demonstrate that physical, chemical, and biological parameters of an aquatic ecosystem are found to be correlated [39, 40]. Since each parameter in an aquatic ecosystem regulates the others, a freshwater pond supports

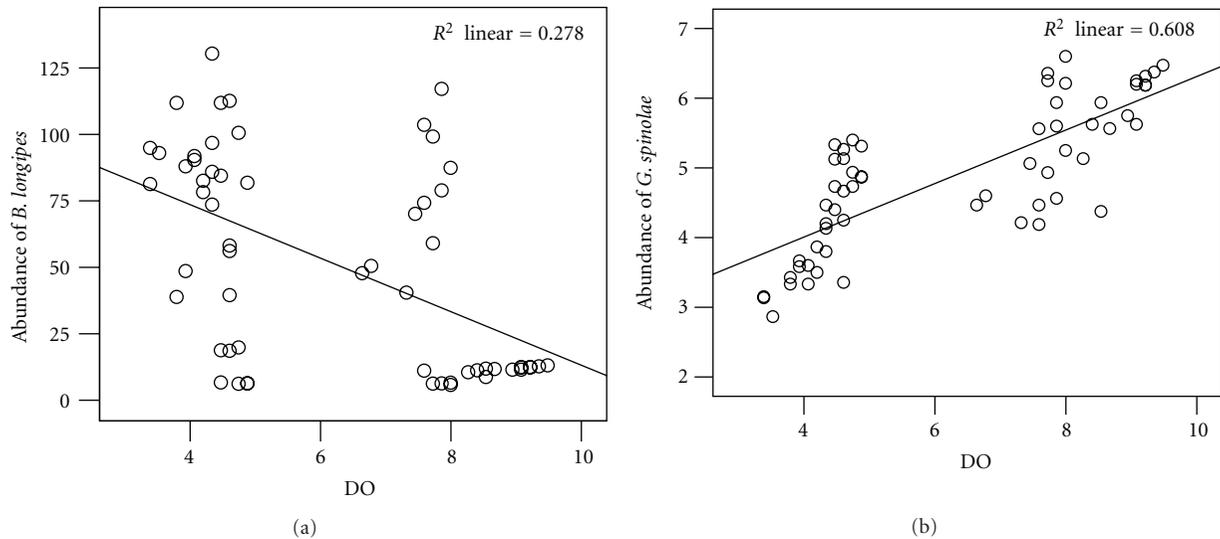


FIGURE 4: Relation between DO and abundance of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) as shown by regression analysis.

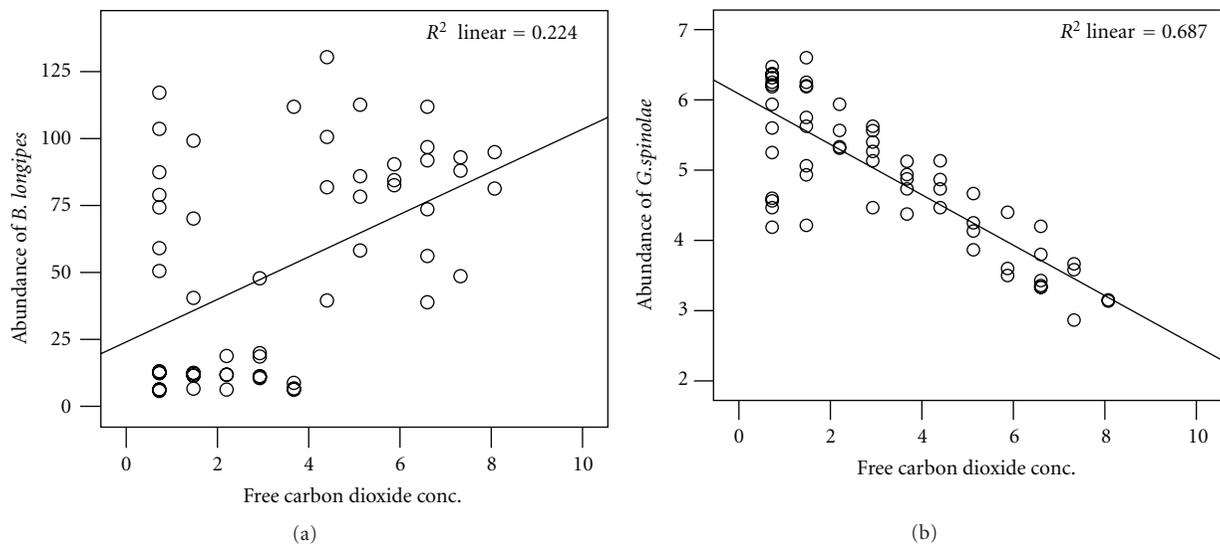


FIGURE 5: Relation between free carbon dioxide and abundance of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) as shown by regression analysis.

complex dynamics. It has been reported that pH decreases with the increase in temperature [41]. Moreover, increasing turbidity of water increases heat retention capability of water [42]. Hence, ponds having turbid water exhibit relatively high temperature and slightly low pH as is evident in the Durgakund pond. Physical parameters also regulate the concentration of several ions, content of free CO_2 , dissolved oxygen, even BOD [43]. However, anthropogenic stress in the three experimental ponds is apparently due to the dumping of organic wastes [44]. Increasing organic degradation initially results in nutrient enrichment and finally in colonization of the various algae and “algal bloom” formation resulting in “eutrophication.” The extent of organic pollution in terms of increase in BOD, free CO_2 , and heavy oxygen

stress can be monitored conveniently by using *G. spinolae* and *B. longipes* as bioindicators. The extent of pollution in ponds can be assessed by the abundance of *B. longipes* which may be predicted to increase and that of *G. spinolae* to decrease with increasing pollution levels. The reason behind the contradictory responses of the two insects under study is due to differences in the habitat requirements of their life-history stages. *Gerris spinolae* lays eggs on the submerged vegetation at depths of 2-3 meters from the surface [14]. This submerged oviposition is regulated by the level of dissolved oxygen and male presence [14]. After emergence, the nymphs respire using dissolved oxygen of the water, though the adults “skate” on the pond surface. This explains the negative correlation of their abundance with parameters indicative

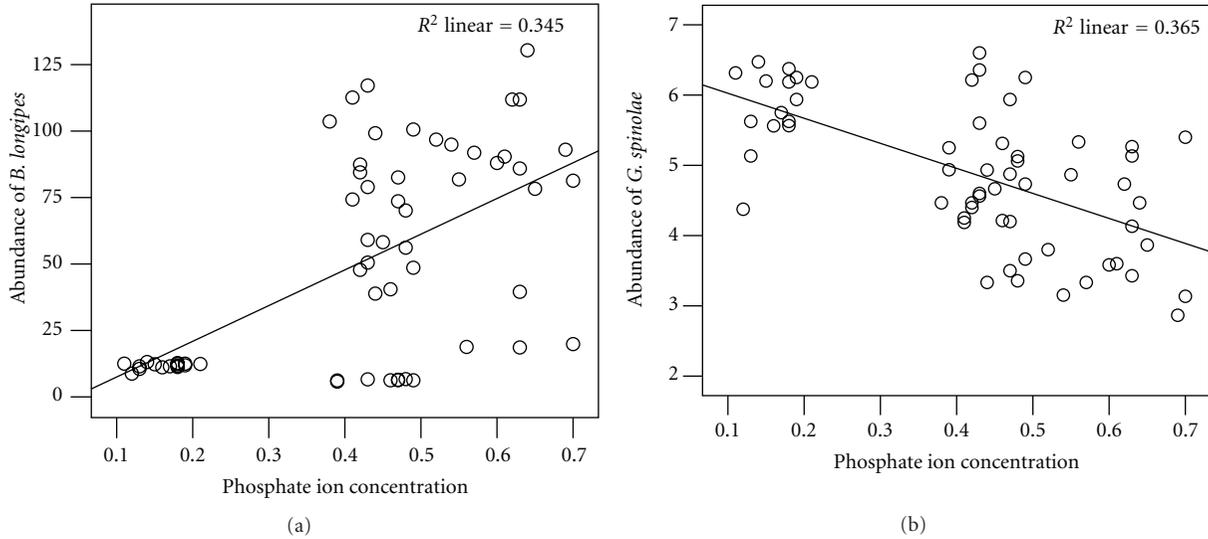


FIGURE 6: Relation between phosphate ion concentration and abundance of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) as shown by regression analysis.

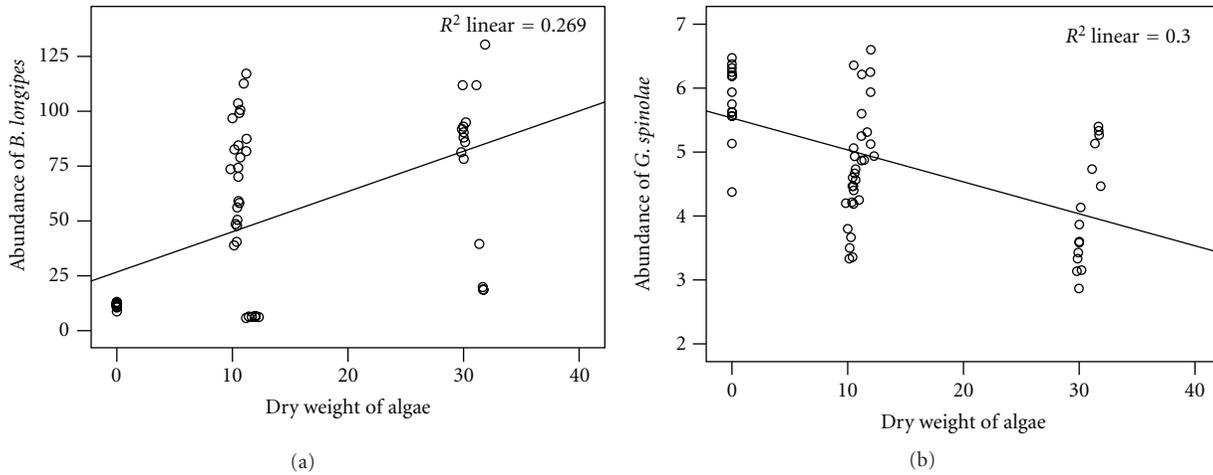


FIGURE 7: Relation between dry weight of algae (g/sq. m) and abundance of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) as shown by regression analysis.

of higher level of pollution. Consequently, reduction in the abundance of *G. spinolae* may be a good indication of oxygen deficiency of water. Contrastingly, *B. longipes* does not rely on dissolved oxygen for respiration. The surface-living maggots feed on some species of algae like *Microcystis* sp., while the adults are free flying and are reported to feed on particles floating on the pond surface by rapidly extending and retracting their proboscis [32], so their number increases with eutrophication. The study clearly demonstrates that *G. spinolae* and *B. longipes* are good positive and negative indicator taxa for healthy fresh water ponds. We, therefore, conclude that occurrence of higher *G. spinolae* population level indicates a positive correlation with healthy unpolluted

pond conditions while enhanced abundance of *B. longipes* indicates higher pollution level of the pond.

Since insects exhibit high fecundity, are fast breeding, easy to sample, and ethical constraints are not involved, *Gerris spinolae* (Lethierry and Severin) and *Brachydeutera longipes* (Hendel) appear to be suitable insect bioindicator candidates for assessing pollution in fresh water bodies. Utilisation of insect bioindicators would be an inexpensive method for monitoring pollution and for carrying out preliminary assessments of the water quality of inland ponds and lakes. This would avoid direct assessment of water quality involving expensive analytical methods, particularly at the preliminary stages. Integration of inexpensive

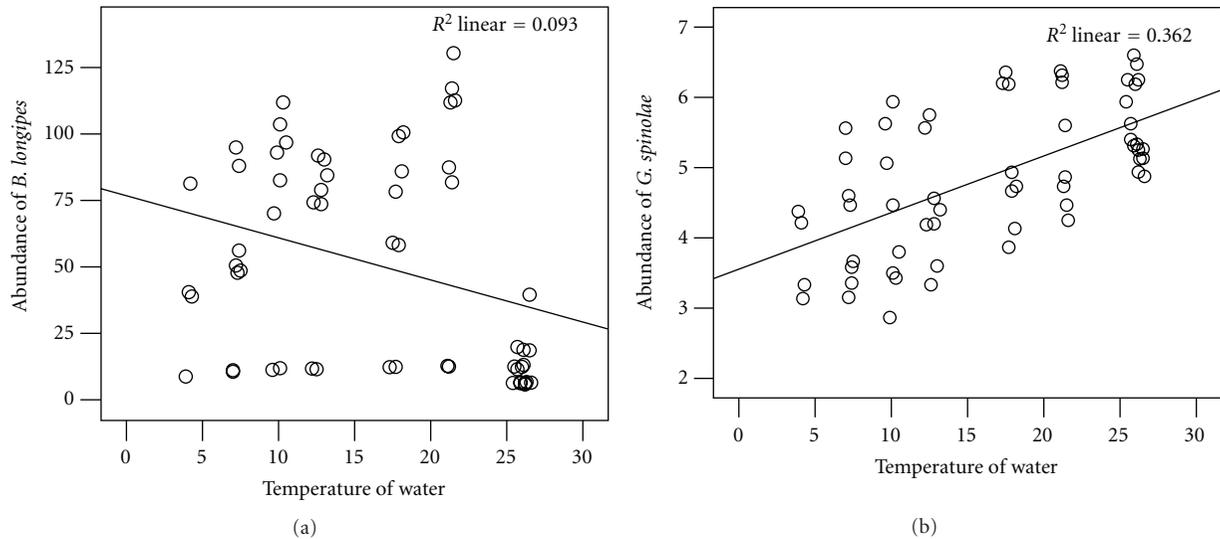


FIGURE 8: Relation between temperature of water and abundance of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) as shown by regression analysis.

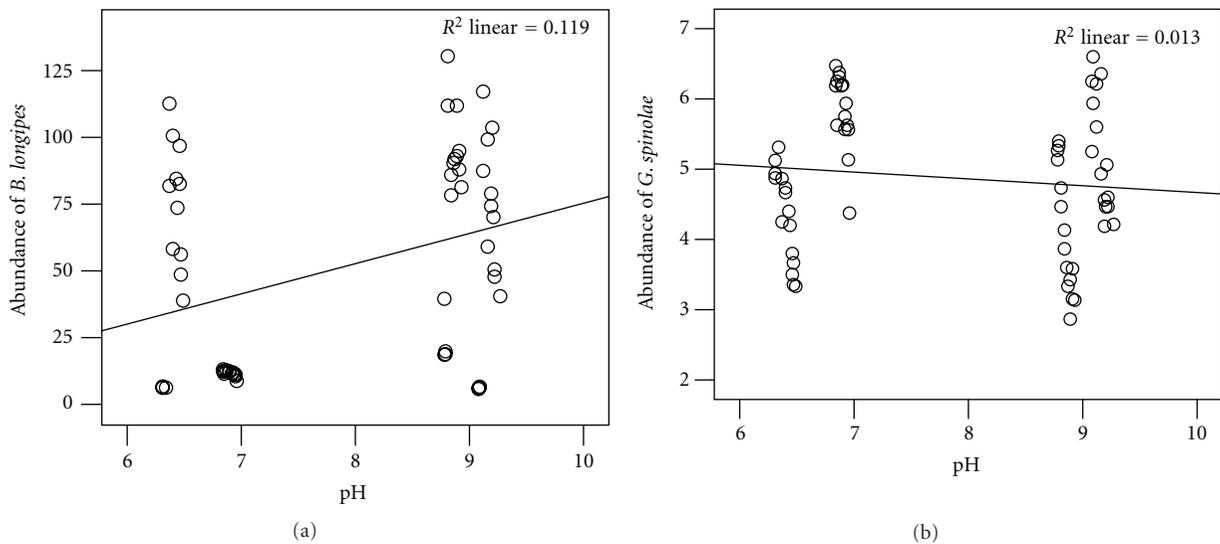


FIGURE 9: Relation between pH of water and abundance of (a) *Brachydeutera longipes* (Hendel) and (b) *Gerris spinolae* (Lethierry and Severin) as shown by regression analysis.

biomonitoring methods with chemical-specific assessment methods would facilitate the restoration of the biological integrity and ecological health of freshwater bodies.

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