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SYNERGETIC EFFECTS OF PHYSICOCHEMICAL PARAMETERS OF WATER ON THE PARASITIC EFFECTS OF *Trichodina* sp. SAMPLED FROM THE EPIDERMIS OF *Channa punctatus* IN RELATION TO ORGANIC POLLUTION OF RIVER SARASWATI (INDIA)

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ABSTRACT

The impact of water pollution on aquatic biota and ecosystem health are determined by the type and degree of water pollution. The present study was aimed to assess the impact of degraded physicochemical regime of river Saraswati in India on the parasitic load of *Trichodina* sp. obtained from C*hanna punctatus.* The overall water quality status of the polluted river was assessed by using ten physicochemical parameters of water. A total of 404 fish were examined for ciliate ectoparasite *Trichodina* sp. and prevalence, mean intensity and abundance of parasitic load for each month determined. The parasitic load of *Trichodina* sp. was significantly ($p < 0.001$) high in winter and negatively correlated with temperature, free CO_2, and biochemical oxygen demand BOD, but positively correlated with electrical conductivity (EC). Fish health was adversely affected by degrading water qualities and parasitic infection. A 'vicious cycle' develops because of interplay among deteriorating water quality, withering fish immunological defence, and amplifying parasitic infection.

Key Words: Parasitic load, pollution; riverine health; Saraswati river, *Trichodina* sp., water parameters

INTRODUCTION

Pollutants are a global environmental concern. Aquatic pollution is caused by a variety of anthropogenic activities such as discharge of domestic wastewater and industrial effluents, poor sanitation practices, use of fertilizers and pesticides in agriculture, inefficient irrigation practices, etc. which degrade water quality of the aquatic system and overall aquatic health (Yurova and Shirokova, 2020; Bashir *et al.*, 2020; Heneash *et al.*, 2021). The overall health of aquatic organisms is influenced by different physicochemical and biotic factors such as temperature, electrical conductivity (EC), total dissolved solids (TDS), turbidity, salinity, pH, alkalinity, dissolved oxygen (DO), free carbon dioxide (CO_2), biochemical oxygen demand (BOD), chemical oxygen demand (COD), etc. (Sarkar *et al.*, 2020).

Fish are the most common aquatic animal harmed by pollution. *Channa punctatus* is a freshwater edible fish that may survive even in a stressful environment owing to presence of an accessory respiratory organ and associated adaptations. In a polluted environment the fish are often get parasitized by ecto- and endo-parasites like ciliate *Trichodina* sp., the monogenean *Gyrodactylus* sp., and the larval stage (L4) of *Eustrongylides* sp. (Ogut and Palm, 2005; Guagliardo *et al.*, 2019; Gilbert and Avenant-Oldewage, 2021). Protozoan fish parasites act as causative agents of several diseases, sometimes enhance the chance of secondary infection and consequent mortality (Xu *et al.*, 2012).

Ciliophoran is one of the most important phyla of subkingdom Protozoa comprising ciliates are considered as the most harmful parasites of fish (Lom and Dykova, 1992). Most ciliates are ectoparasite of fish which heavily infest fish gills and reduce the breathing surface area for gaseous exchange. Consequently, fish faces respiratory distress and starts grasping for air (Durborow, 2003). Among ciliate protozoans, *Trichodina* sp. is a very common parasite of both freshwater and marine fishes. They are mainly ectoparasite of body surface, buccal cavity and gills. A few of them are endoparasites (Lom and Dykova, 1992). The parasitic load such as prevalence, mean intensity, and relative abundance of parasites can serve as potential indicators of seasonal water quality (Dias *et al.*, 2017; Vidal-Martínez *et al.*, 2019). The effect of water pollution on parasites may be positive or negative. Pollution can increase parasitic infestation by weakening the defence mechanism of the host or/and by increasing the population density of final and intermediate hosts. On the other hand, pollution can decrease parasitic infestation (Blanar *et al.*, 2009; Lacerda *et al.*, 2018). For example, eutrophication can increase parasitic infestation (Shah *et al.*, 2013), and heavy metal may decrease it (Lefcort *et al.*, 2002). The community structure of ectoparasite is related to a variety of physico-chemical properties of water such as temperature (Majumder *et al.*, 2015), pH (Macleoad and Poulin, 2012), salinity (Kua *et al.*, 2013), and effluents of factories and mills (Okereke *et al*., 2016). Low temperature and high EC directly affect parasite growth, development, and their lifecycle, whereas high concentrations of free CO_2, BOD, and low concentrations of DO affects host health and make them susceptible to parasitic infection (Zhang *et al.*, 2021; Koledoye *et al.*, 2022).

Very less research effort has been made to establish the synergetic effects of water quality parameters on parasitic load of both ecto- and endo-parasites of fish. There is very scarce information available in India on parasitic monitoring of environmental quality of the aquatic system in general, and riverine system in particular. The purpose of present study was to establish interrelationship between degraded water quality and parasitic infection, and to investigate the feasibility of developing fish parasite (ectoparasites such as protozoan *Trichodina* sp.) as response indicators for monitoring and assessing aquatic health.

MATERIALS AND METHODS

Study area

The river Saraswati is a distributary of the river Ganga (river Hooghly), which flows through Hooghly region of West Bengal, India. The sampling area (Fig. 1) is about 4 km away from the point of connection with the river Ganga and receives heavy organic loading from agricultural and surface run-off, and a variety of anthropogenic activities such as domestic wastewater discharge, seasonal jute retting, and effluents from a few small to moderate scale beer and brick kiln factories.

Collection of water samples and physicochemical analyses

The water samples were collected from the polluted river once a month from March 2017 to February 2020 for the analysis of physicochemical parameters. Water temperature, pH, TDS, turbidity, salinity, and EC were monitored periodically using a digital water and soil analysis kit (Electronics India, model -172). The concentrations of DO, free CO_2, BOD and COD were determined as per the standard methods (APHA, 2012).

Collection of host fish and parasites

The flow and level of water in the polluted river stay too low during the dry summer season (March, April and May) for any fish to survive. The host fish (*Channa punctatus* Bloch, 1792) was collected throughout a three-year period (March, 2017 to February, 2020), from June to February by local fishermen with the help of fishing gears (bamboo fish cage and fish net). River Saraswati is the distributary of the river Ganga. The host fish (*Channa punctatus*) entered the river Saraswati along with the tidal water of the river Ganga. During summer, the river Saraswati was almost dried up as well

Fig. 1: Sampling area and connection point of the polluted river Saraswati with river Ganga from Hooghly, West Bengal, India

as the water of the river Ganga did not reach up to the sampling point (The sampling area is about 4 km away from the connection point of two river). As a result, the host fish were not available during the summer; instead, they became available beginning with the monsoon season, along with water availability. Therefore, it was impossible to collect parasites throughout the summer. Host fish were studied for protozoan parasites. *Trichodina* sp. were collected from the body surface mucous and gill of fish. The skin, fins, and gill of the host fish were examined for ectoparasites. These were slaughtered by inserting needle into the fish's neurocranium, then the mucus from the body of the host fish was collected for observation of parasites (Fautama *et al.*, 2019). Collected parasites were immediately fixed in formaldehyde solution (10%) and counted under a light microscope (Zeiss Primo Star, Germany) at 10x magnification. Collected parasites were taxonomically identified by adapting the works of Basson and As (1989) for *Trichodina* sp. Prevalence, mean intensity and abundance for each sampling period were calculated as per Margolis *et al.* (1982) and Bush *et al.* (1997).

$$\text{Prevalence (\%)} = \frac{\text{Number of host infected}}{\text{Number of host examined}} \times 100 \qquad (1)$$

$$\text{Mean intensity} = \frac{\text{Total number of parasite}}{\text{Number of infected host}} \qquad (2)$$

$$\text{Abundance} = \frac{\text{Total number of parasite}}{\text{Number of host examined}} \qquad (3)$$

Statistical analyses

The data were subjected to appropriate statistical validation (Fekri-Soofi Abadi *et al.*, 2019). The data was analysed by one-way ANOVA using statistical software SPSS 20 to compare the physicochemical parameters of water. After parasite enumeration, the values were expressed in terms of prevalence, mean intensity and abundance. Also, similar monthly variation of parasitic load for *Trichodina* sp. were statistically compared. For understanding the effect of water parameters and pollution on the parasitic load, the data were compared by Spearman's correlation (Fekri-Soofi Abadi *et al.*, 2019). Further, to identify the patterns of correlated and non-correlated variables the data were subjected to statistical validation by principal components analysis (PCA) using Origin pro.

Hierarchical cluster analysis

Hierarchical cluster analysis is a technique for detecting patterns in data that may have gone unnoticed earlier. It is used to locate groupings of similar objects, where items in one cluster are more alike than those in other clusters. The out-put of this method is a 2D circular or tree-like dendrogram representing the series of nested clusters (Orsolic *et al.,* 2021; Hulme *et al.,* 2022).

RESULTS AND DISCUSSION

Physicochemical parameters of water

Ten physicochemical parameters i.e., temperature, EC, TDS, turbidity, salinity, pH, DO, free CO_2, BOD and COD were monitored during March 2017 to February 2020, from the river Saraswati. Significant variations were found in eight (temperature, EC, turbidity, DO, and BOD; $p < 0.001$ and TDS, free CO_2, and COD; $p < 0.05$) out of the ten water quality parameters using one-way ANOVA (Table 1). The mean water temperature ranged from 19.0 to 36.8°C throughout the study period. The maximum temperature (36.8°C) was recorded in July 2019 while the minimum temperature (19°C) was recorded in January 2018. The significant difference (ANOVA; $p < 0.001$) was observed among the monthly mean values of water temperature. The mean maximum EC value (793.33 ± 66.91 moh cm^{-1}) was recorded in March and differed significantly by one-way ANOVA ($p < 0.001$). In August, the lowest mean EC value (280 ± 30.55 moh cm^{-1}) was recorded, which progressively increased until December, then slightly dropped during the month of January and February EC values of water have a strong positive relationship with conductive ions derived from dissolved salts and inorganic elements (Zhu *et al.*, 2020). Water temperature, evaporation, rainfall, change in water level, tidal stage, and other factors influence seasonal fluctuations in EC of water. EC is positively correlated with temperature and evaporation rate (Sallam and Elsayed, 2018). As a result, the mean value of EC in the polluted river was substantially greater in the summer. Rainfall and inflow of water, on the other hand, were negatively correlated with EC because they dilute and wash out the concentration of dissolved minerals in the water. Because the monsoon and post-monsoon seasons were frequently accompanied by high rainfall frequency and intensity, as well as influx of water from the Ganga during tidal events, the EC value was lower than in winter (December, January, and February), despite the temperature difference (Ewaid *et al.,* 2018). The mean turbidity value of a polluted river followed the opposite pattern as the EC. The mean turbidity of water in the polluted river was high (9.33 ± 2.90 to 57.33 ± 1.76 NTU) and exhibited an increasing trend during April to August (9.33 ± 2.90 to 57.33 ± 1.76 NTU) followed by drastic decline during September (40.83 ± 2.48 NTU) to March (15.0 ± 0.57 NTU). Turbidity is caused by solid particles being suspended in a liquid. High turbidity measurements usually indicate lower water quality, so turbidity cannot be used as a sole indicator of pollution. It can be used in conjunction with other essential water parameters to assess water quality (Luis *et al.*, 2019). Turbidity in river water is primarily caused by suspended solids that are combined with water from riverbed material during heavy rainfall (Kamboj and Kamboj, 2019). Besides, the turbidity of water is also caused by industrial wastes, sewage plankton, and eutrophication (Mondal *et al.*, 2017). Discernible monthly variation in the mean turbidity ($p < 0.001$) was observed throughout the study period. The mean DO concentration in polluted river registered much lower, below the standard limit (5 mg L^{-1}), ranging from 0.2 to 4.5 mg L^{-1}. The study sites showed a distinct monthly variation in mean DO concentration ($p < 0.001$). A distinct pattern of temporal variations in DO concentrations for the study site was observed, i.e., remarkable rise from August (3.46 ± 0.65 mg L^{-1}) to October (3.78 ± 0.46 mg L^{-1}), followed by a drastic drop in DO levels from November (1.76 ± 0.27 mg L^{-1}) to June (0.98 ± 0.04 mg L^{-1}). In polluted river the mean concentration of free CO_2 was very high and ranged from 44.0 ± 1.0 to 64.66 ± 7.53 mg L^{-1}. It illustrated an opposing relationship with DO which was reflected in their spatial and temporal patterns. The lowest level of DO was caused by a combination of organic loading, reduced water volume, stagnation, seasonal eutrophication, and a lack of water influx from the Ganga. Seasonal variation of the mean concentration of free CO_2 was not significant in polluted river. It was recorded minimum in winter which was much higher than the

Table 1: The monthly variations in the physicochemical parameters of water from polluted river Saraswati (India) during March 2017 to February, 2020

Month		Temperature (ºC)	EC (µmoh cm^{-1})	TDS (ppt)	Salinity (ppt)	Turbidity (NTU)	pH range	DO (mg L^{-1})	Free CO_2 (mg L^{-1})	BOD (mg L^{-1})	COD (mg L^{-1})
January	Mean	20.60 ±	686.66 ±	426.66 ±	283.33 ±	21.33 ±	7.4 ±	1.3 ±	44.0 ±	5.41 ±	26.51 ±
	(SE)	0.85	3.33	12.01	16.66	0.66	0.17	0.05	1.0	0.33	0.52
	Min	19	680	410	250	20	7.1	1.2	42	4.75	25.5
	Max	21.9	690	450	300	22	7.4	1.4	45	6	27.24
February	Mean	23.50 ±	573.3 ±	376.6 ±	266.6	15.33 ±	7.43 ±	1.44 ±	49.66 ±	5.56 ±	32.45 ±
	(SE)	2.13	6.66	14.52	± 66.66	0.88	0.03	0.12	3.28	0.69	2.5
	Min	20.2	560	350	200	14	7.4	1.21	45	4.2	28.36
	Max	27.5	580	400	400	17	7.5	1.62	56	6.5	37
March	Mean	29.13 ±	793.33 ±	533.33 ±	266.66 ±	15.0 ±	7.27 ±	1.19 ±	58.1 ±	7.36 ±	35.57 ±
	(SE)	1.67	66.91	61.73	88.19	0.57	0.09	0.26	3.12	1.18	2.52
	Min	26.6	660	440	150	14	7.1	0.8	52	5	30.53
	Max	32.3	870	650	400	16	7.41	1.7	62.3	8.6	38.2
April	Mean	32.60 ±	653.33 ±	413.33 ±	266.66 ±	9.33 ±	7.34 ±	2.30 ±	64 ±	8.08 ±	29.82 ±
	(SE)	0.44	73.10	52.06	66.66	2.90	0.06	0.46	2.08	0.5	4.21
	Min	30	540	320	200	8	7.25	1.5	60	7.25	22
	Max	34	790	500	400	12	7.47	3.1	67	9	36.47
May	Mean	34.66 ±	750.0 ±	566.66 ±	400.0 ±	14.16 ±	7.32 ±	1.14 ±	61.67 ±	7.45 ±	34.77 ±
	(SE)	0.44	5.77	27.28	57.73	1.09	0.24	0.29	3.76	0.24	1.98
	Min	34	740	530	300	12	7.05	0.8	55	7	32.6
	Max	35.5	760	620	500	15	7.47	2.32	68.03	7.5	38.73
June	Mean	34.26 ±	323.33 ±	336.66 ±	83.33 ±	21.66 ±	7.22 ±	0.98 ±	65.07 ±	7.88 ±	34.37 ±
	(SE)	1.44	49.1	89.50	8.81	2.72	0.07	0.04	1.22	0.11	2.37
	Min	31.8	260	160	50	18	7.1	0.2	63	7.65	29.7
	Max	36.8	420	450	160	27	7.36	1.56	67.23	8	37.43
July	Mean	34.80 ±	280.0 ±	283.33 ±	150.0 ±	31.66 ±	7.35 ±	0.81 ±	63.33 ±	7.80 ±	28.14 ±
	(SE)	0.90	30.55	61.19	28.86	3.48	0.11	0.06	0.88	0.4	1.32
	Min	33	220	170	80	26	7.12	0.7	62	7	25.5
	Max	35.7	320	380	200	38	7.5	0.94	65	8.2	29.5
August	Mean	31.70 ±	290.0 ±	250 ±	216.66 ±	57.33 ±	7.22 ±	3.46 ±	57.66 ±	10.88 ±	21.63 ±
	(SE)	0.58	45.82	51.31	44.09	1.76	0.13	0.65	6.88	0.93	0.33
	Min	30.6	230	180	0	54	7.07	2.22	44	9.25	21.3
	Max	32.6	380	350	300	60	7.5	4.44	66	12.5	22.31
September	Mean	31.30 ±	363.33 ±	280±43.	183.33±	40.83±2.4	7.45±0	3.78±0.3	61.16±3.	11.3±0.6	21.38 ±
	(SE)	1.10	55.47	58	16.66	8	.1	4	456	6	2.89
	Min	29.5	260	200	0	37	7.35	3.1	55	10.2	16.2
	Max	33.3	450	350	200	45.5	7.65	4.24	67	12.5	26.2
October	Mean	30.00 ±	426.66 ±	323.33±	133.33±	37.0 ±	7.26 ±	3.78 ±	63.33 ±	10.25 ±	22.11 ±
	(SE)	0.52	17.63	29.62	33.33	1.0	0.11	0.46	1.66	0.9	4.43
	Min	29	400	280	100	35	7.15	2.9	60	8.5	15.4
	Max	30.8	460	380	200	38	7.5	4.5	65	11.5	30.5
November	Mean	26.81 ±	430.0 ±	326.66 ±	133.33 ±	25.83 ±	7.32 ±	1.76 ±	64.66 ±	9.71 ±	24.61 ±
	(SE)	1.03	25.16	32.82	33.33	4.86	0.07	0.27	7.53	1.31	3.81
	Min	25	400	280	100	10	7.18	1.21	50	7.25	19.3
	Max	28.6	480	390	200	25.5	7.45	2.07	75	11.75	32
December	Mean	22.83 ±	693.33 ±	400 .0 ±	200.0 ±	28.83 ±	7.67 ±	1.15 ±	50.5 ±	5.08 ±	24.88 ±
	(SE)	0.33	29.62	40.0	57.73	0.16	0.01	0.05	7.5	0.36	2.82
	Min	22.5	650	360	100	28.5	7.66	1.1	35.5	4.5	21.46
	Max	23.5	750	480	300	29	7.7	1.25	58	5.75	30.5
$CD_{0.05}$	$F_{11, 35}$	17.97	21.35	4.21	1.63	36.34	1.08	10.77	2.65	8.21	3.64
	p value	<0.001	<0.001	<0.05	>0.1	<0.001	>0.1	<0.001	<0.05	<0.001	<0.05

standard limit (10 mg L^{-1}). The lowest winter values of free CO_2 indicated organic load induced by intense heterotrophic activity and higher biotic respiration rates. The mean BOD value of water samples ranged from 5.08 ± 0.36 to 11.3 ± 0.66 mg L^{-1} and differed significantly. From August to

November, the BOD level was much higher and all the seasonal BOD level crossed the standard limit (5 mg L^{-1}). The mean COD value was relatively higher from April to June and monthly variation was not prominent ($p > 0.05$). In polluted river BOD level increased in post-monsoon by 88-99% as compared to winter. On the other hand, mean COD concentration was low in post-monsoon and increased by 43-47% in summer. BOD is the amount of oxygen consumed by the microorganisms for oxidation of biodegradable organic matter as food sources. COD is the amount of oxygen required for oxidization of oxidizable organic and inorganic substances present in water. The values of both BOD and COD are negatively correlated with the concentration of DO (Verma and Singh, 2013). In polluted river the mean concentration of DO was relatively high in post-monsoon along with low COD value reflecting negative correlation because oxidization of organic and inorganic substance utilized more DO. The DO level in polluted river was very low throughout the year except in post-monsoon, and BOD level was very high due to organic load and high microbial activity.

Parasitic load of Trichodina sp.

A total number of 404 fish (*Channa punctatus*) were collected from the polluted river during monsoon, post-monsoon and winter seasons from March 2017 to February 2020. During dry summer season, the flow and level of water in the polluted river were too low to contain any fish. The number of fish that are infected, the number of fish that are not infected, and the number of parasites all determine the parasitic load or infection indices (prevalence, mean intensity, and abundance). Evaluation therefore was not possible without host fish during the summer. Prevalence, mean intensity and abundance of *Trichodina* sp. were calculated for the quantification of parasite number in a host sample or population. The descriptive statistics associated with the parasitic load of host fish are given in Table 2. The lowest mean prevalence was observed in July (16.43 ± 4.46%), which progressively grew and reached a peak in November (49.84 ± 8.45%), before declining sharply in January. The mean intensity of *Trichodina* sp. was lowest in June (8.5 ± 0.76%) which steadily increased till it reached a peak in January (27.18 ± 1.85%). A similar pattern was seen in the mean abundance of *Trichodina* sp., where the mean abundance value steadily increased from August (2.82 ± 0.20) to September (6.12 ± 0.25) (Fig. 2). To test the hypothesis that monthly variation in water quality parameters affected parasite load, one-way ANOVA was performed. The analysis revealed significant monthly variation in prevalence ($F_{8, 26} = 6.376$, $p < 0.01$), mean intensity $F_{8, 26} = 31.824$, $p < 0.001$) and abundance ($F_{8, 26} = 24.545$, $p < 0.001$) of *Trichodina* sp. Further, the assumption of homogeneity of variances for prevalence, mean intensity and abundance of *Trichodina* sp. were satisfied based on Levene's test (prevalence, $F_{8, 26} = 2.297$, $p = 0.068$; mean intensity, $F_{8, 26} = 2.235$, $p = 3.577$, $p = 0.054$; abundance, $F_{8, 26 =}$ 2.023, $p = 0.102$). Thus, the null hypothesis of no difference between the mean was rejected. The value of mean prevalence, mean intensity and abundance of *Trichodina* sp. was low in monsoon which increased by 112, 80, and 276%, respectively, during post-monsoon and 85, 128, and 336%, respectively, during winter.

Table 2: The number of infected and uninfected host fish (*Channa punctatus*), mean (± SE), values of prevalence, mean intensity and abundance of *Trichodina* sp. collected from river Saraswati in each month, except summer

Month	Fish examined (No.)	Infected fish (No.)	Parasites collected from infected fish (No.)	Prevalence (%)	Mean intensity	Abundance
June	23	4	33	18.33 ± 4.41	8.5 ± 0.76	1.49 ± 0.26
July	25	4	50	16.43 ± 4.46	12.33 ± 0.33	2.05 ± 0.62
August	28	7	79	25.00 ± 2.89	11.39 ± 0.56	2.82 ± 0.20
September	35	12	213	34.70 ± 2.75	17.75 ± 0.66	6.12 ± 0.25
October	36	14	287	39.74 ± 5.59	20.47 ± 0.29	8.17 ± 1.26
November	41	20	390	49.84 ± 8.45	19.79 ± 1.48	9.62 ± 0.86
December	80	32	808	40.00 ± 2.31	25.26 ± 1.00	10.07 ± 0.37
January	82	25	670	31.11 ± 1.60	27.18 ± 1.85	9.55 ± 1.06

February	54	21	442	39.21 ± 2.53	21.05 ± 1.79	8.18 ± 0.49

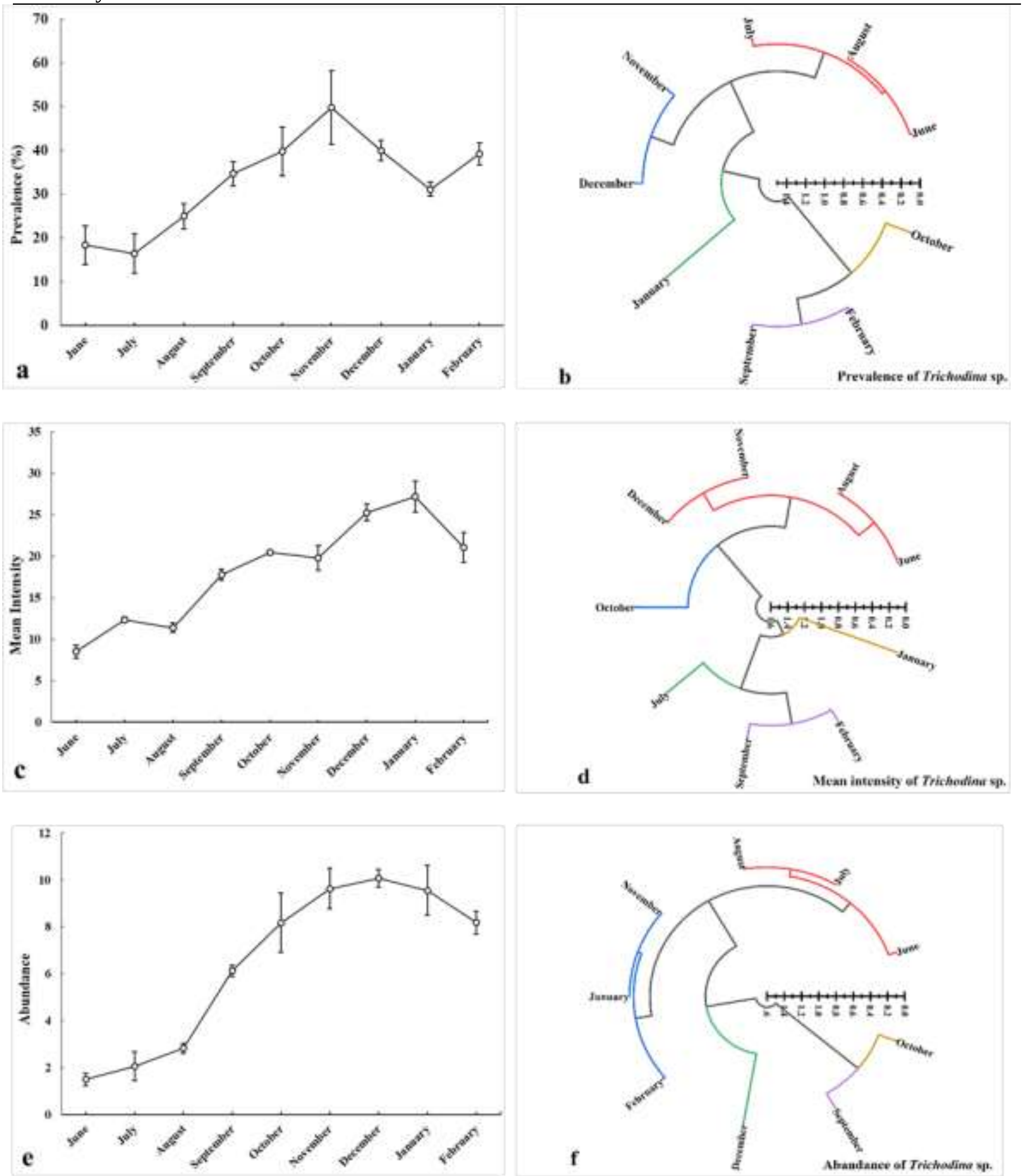

Fig. 2: Month-wise mean (±SE) of prevalence, mean intensity and abundance of *Trichodina* sp. with hierarchical clustering analysis; a) and b) prevalence, c) and d) mean intensity, e) and f) abundance

The months with the most similar parasitic load were selected using hierarchical clustering (Fig. 2). The mean prevalence of *Trichodina* sp. was organised into three groups: cluster 1 (June, July, and August), cluster 2 (November and December), and cluster 3 (September, February and October). The mean prevalence of January remained fully distinct from the rest of the cluster. The clustering pattern of mean intensity of *Trichodina* sp. differed from that of mean prevalence and was organised into three clusters; cluster 1 (June and August), cluster 2 (November and December), and cluster 3 (October and February). Mean intensity of the month of July, October, and January remained notably different from the others. The mean abundance of *Trichodina* sp. showed a similar pattern to that of prevalence and was categorized into three clusters: cluster 1 (June, July and August), cluster 2 (November, January and February) and cluster 3 (September and October) on the other hand the mean abundance value of December plotted separately in the circular dendrogram. The parasite load is dependent on seasonal fluctuations, according to a cluster analysis of monthly prevalence, mean intensity, and abundance of *Trichodina* sp. Because the water quality parameters of clustered months are more or less identical in nature therefore, parasitic load of *Trichodina* sp. influenced by seasonal variation and the synergetic effect of water parameters.

The correlation between parasitic load of *Trichodina* sp. and water quality was significant (Fig. 3). Prevalence of *Trichodina* sp. showed weak negative correlation with temperature ($R^2 = 0.2411$; $p < 0.01$) but weak to moderate positive correlation with EC ($R^2 = 0.2044$; $p < 0.05$). Mean intensity and abundance of *Trichodina* sp. were positively correlated with EC (mean intensity; $R^2 = 0.6931$, abundance; $R^2 = 0.5835$; $p < 0.001$), while negatively with temperature (mean intensity; $R^2 = 0.7147$, abundance; $R^2 = 0.6151$; $p < 0.001$). Mean intensity showed weak negative correlation with free CO_2 ($R^2 = 0.2035$; $p < 0.05$) and BOD ($R^2 = 0.1766$; $p < 0.05$) (Fig. 4). Overall, the parasitic load of the ectoparasite in terms of mean intensity and abundance were found to be mainly regulated by temperature and EC - the two physical factors of the ambient environment as key determinants (Zhang *et al.*, 2021; Koledoye *et al.*, 2022).

The PCA plot explained 75.9% (PC 1 = 54.1%, PC 2 = 21.8%) variation among water parameters and parasitic load. The comparison was based on Eigen value. All the indices of parasitic infestation are positively associated with both the PCs, while temperature was negatively associated with the variables. Free CO_2, BOD, DO, and turbidity were negatively associated with PC1 and positively with PC2; while EC was positively associated with PC 1 (Fig. 5). Overall, the PCA biplot indicated that the parasitic load was conditioned by a suite of environmental parameters facilitating parasitization. Fish health was implicated with the alteration of physicochemical properties of water which favours parasitic mode of adaptation to cope with the degraded water quality (Blanar *et al.*, 2009). The PCA output bears significant connotation for parasitic load on host fish conditioned by the physicochemical attributes of their habitat inclusive of their synergistic effects.

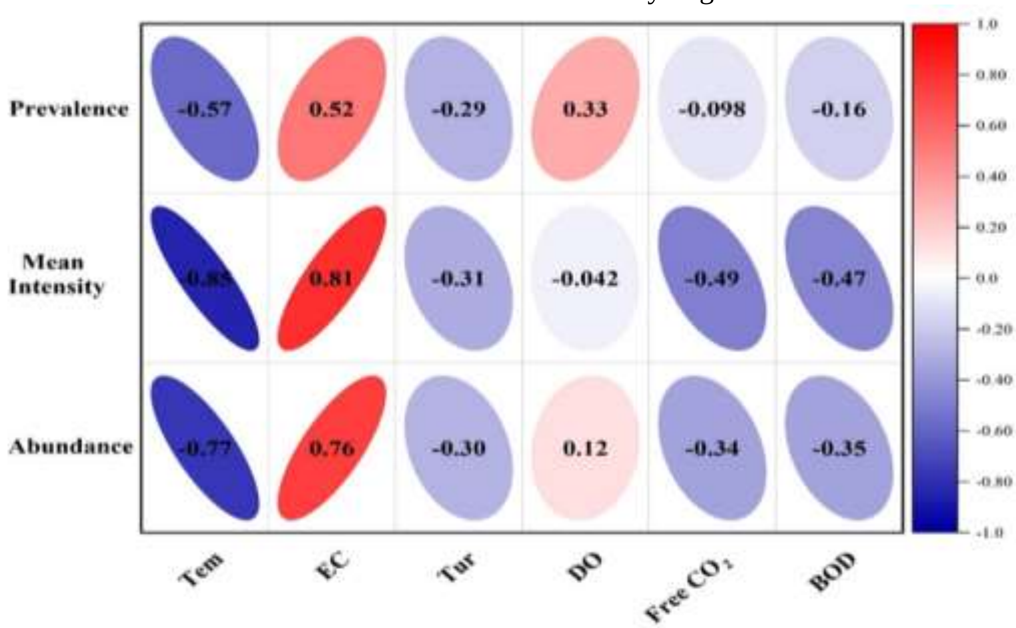

Fig. 3: Correlations among physicochemical parameters of water and parasitic load of *Trichodina* sp.

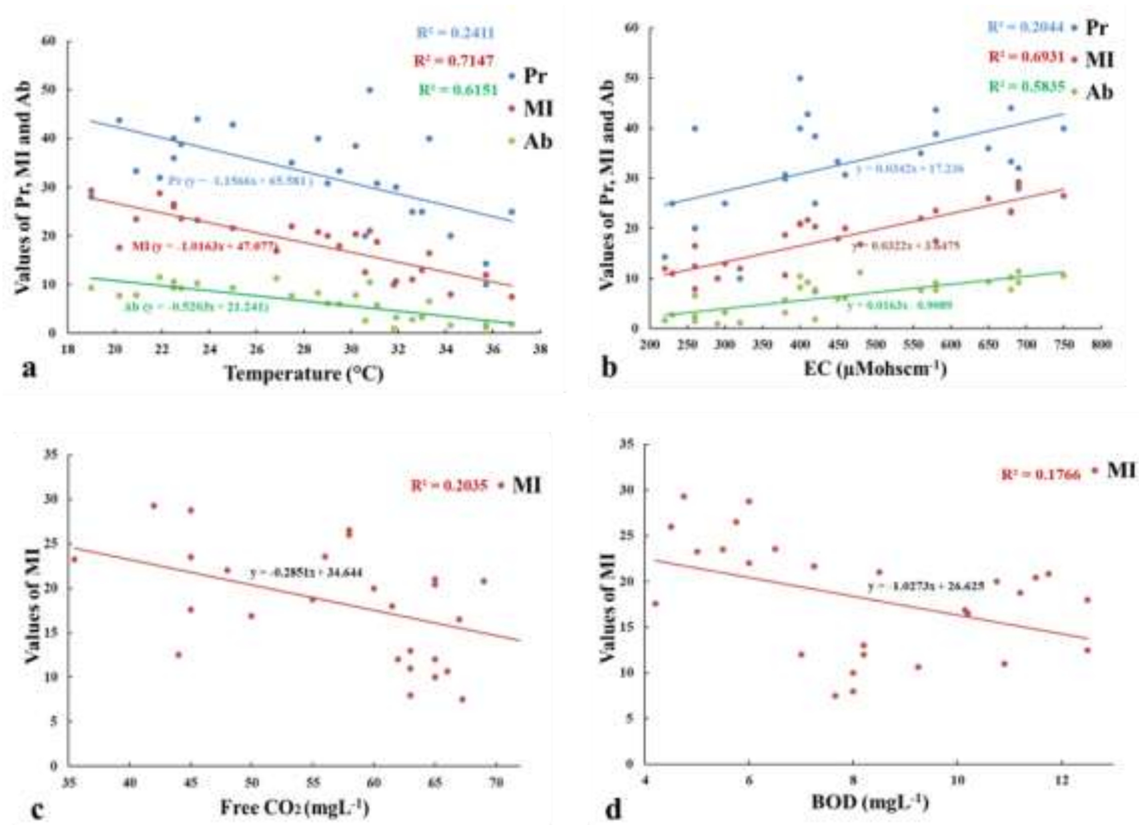

Fig. 4: Regression analysis (with equations and R^2 values) of water quality parameters and parasitic load [prevalence (Pr); mean intensity (MI); and abundance (Ab)] of *Trichodina* sp. a) Negative correlation with temperature; b) Positive correlation with EC; c) and d) MI correlated negatively with free CO_2 and BOD

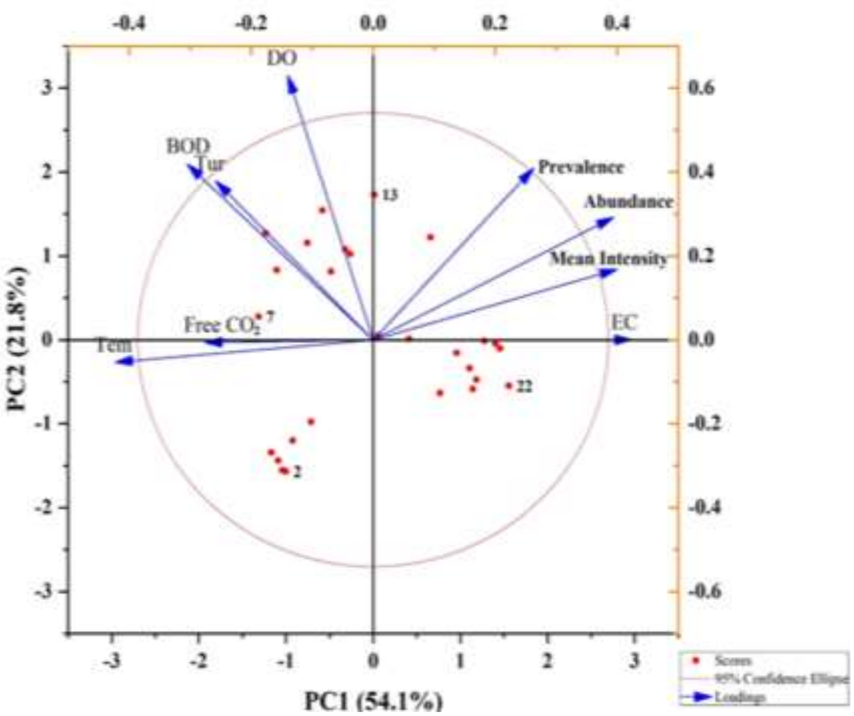

Fig. 5: Principal components analysis of water parameters, parasitic load of *Trichodina* sp. The two principal components (PC1 and PC2) explained 75.9% of the total variation between water parameters and parasitic load. Prevalence, mean intensity and abundance of *Trichodina* sp. are also indicated.

The ectoparasite Trichodinids requires a single host for completion of their life cycle, hence considered as monoxenous parasite. The mode of reproduction is mainly by binary fission (Martins *et al.,* 2015). *Trichodina* sp. can grow and reproduce optimally within a temperature range of 21 to 26°C; hence winter appears as the most suitable season for their reproduction. The process of binary fission is inhibited by high temperature (Fitriani *et al.,* 2019). Higher EC value indicates the nutrient enrichment induced pollution status of water, which can cause suppression of immunity in the host fish. Thus, parasitic load was found to be increased in the immuno-compromised fish host living in a polluted environment (Biswas and Pramanik, 2016; Maceda *et al.,* 2019). Growth, metabolism and swimming behaviour of host fish are directly affected by higher BOD level and lower DO concentration while availability of nutrients is indirectly associated with these two parameters (Onada *et al.,* 2015). On the other hand, hypoxic condition of water is responsible for respiratory trouble, therefore, host fish lose their mobility, and become susceptible to parasitic infection. Parasite load was not directly affected by high BOD and low DO concentration, because both the ecto- and endo-parasite adapt with hypoxic condition by adopting anaerobic respiration, which is an important parasitic mode of adaptation (Harada *et al.,* 2013). In polluted river the free CO_2 concentration was high throughout the season which restricted diffusion rate of CO_2 from fish blood during exhalation. The CO_2 level of fish blood rises that leads to acidosis and reduces oxygen carrying capacity of haemoglobin (Montgomery *et al.,* 2019). Gaseous exchange from the respiratory surface (gill) is restricted by low DO and high free CO_2 level. Most of the time the DO level remains below the threshold value (4 mg L^{-1}) (Osmond *et al.,* 1995) in polluted river. Under such adverse ambience the host fish (*Channa punctatus*) survives adopting the salvage strategy aided by the activities of the accessory respiratory organ and increase in the ventilation rate but it becomes restless, exhausted and weak as a fallout.

Conclusions: The study showed that the river Saraswati is under severe stress of environmental quality degradation due to diverse anthropogenic activities in general and organic load in particular. Degraded water quality causes deterioration of fish health, resulting in a debilitated immune system and more vulnerability to parasitic infection. The parasitic load of ciliate *Trichodina* sp. was directly influenced by the physicochemical stressors. Several other factors such as size, genetic attributes, sex, population density, of host fish need to be considered to earn a better insight into the pollution and related environmental stressor-induced alterations in parasitic load of *Trichodina* sp. on host fish.

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REFERENCES

APHA. 2012. *Standard Methods for the Examination of Water and Wastewater* (22nd edn.). American Public Health Association (APHA)/ American Water Works Association (AWWA)/Water Environment Federation (WEF), Washington, USA.

Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A. and Dar, S.A. 2020. Concerns and threats of contamination on aquatic ecosystems. pp. 1-26. **In**: *Bioremediation and Biotechnology*. Springer Nature, Switzerland. [*https://doi.org/10.1007/978-3-030-35691-0_1*].

Basson, L. and Van As, J.G. 1989. Differential diagnosis of the genera in the family Trichodinidae (Ciliophora: Peritrichida) with the description of a new genus ectoparasitic on freshwater fish from Southern Africa. *Systematic Parasitology,* **13**(2): 153-160.

Biswas, J. and Pramanik, S. 2016. Assessment of aquatic environmental quality using *Gyrodactylus* sp. as a living probe: Parasitic biomonitoring of ecosystem health. J*ournal of Advances in Environmental Health Research,* **4**(4): 219-226.

Blanar, C.A., Munkittrick, K.R., Houlahan, J., MacLatchy, D.L. and Marcogliese, D.J. 2009. Pollution and parasitism in aquatic animals: A meta-analysis of effect size. *Aquatic Toxicology*, **93***:* 18-28.

Bloch, M.E. 1792. Naturgeschichte der ausländischen Fische. Siebenter Theil. *J. Morino & Co. Berlin*, **12**: 85-90. [*https://doi.org/10.5962/bhl.title.63303*].

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *The Journal of Parasitology*, **83**(4): 575-583.

Dias, K.G., Alves, C.A., Silva, R.J., Abdallah, V.D. and Azevedo, R.K. 2017. Parasitic communities of *Hoplosternum littorale* (Hancock, 1828) as indicators of environmental impact. *Anais da Academia Brasileira de Ciências,* **89**: 2317-2325.

Durborow, R.M., Wise, D.J. and Terhune, J.S. 2003. *Saprolegniasis* (winter fungus) and branchiomycosis of commercially cultured channel catfish. *Southern Regional Aquaculture Center*.**4700**:1-4.

Ewaid, S.H., Abed, S.A. and Kadhum, S.A. 2018. Predicting the Tigris river water quality within Baghdad, Iraq by using water quality index and regression analysis. *Environmental Technology & Innovation,* **11**: 390-398.

Fautama, F.N., Zulfahmi, I., Muliari, M. and Anas, A.A. 2019. Prevalence and intensity of ectoparasites on *Clariasgariepinus* from aquaculture pond in Aceh Besar district, Indonesia. *Jurnal Biodjati*, **4**(1): 58-67.

Fekri-Soofi Abadi, M., Fekri, M., Moradabadi, A., Vahidi, R., Shamsi-Meymandi, S., Donya Dabiri, D. and Dabiri, S. 2019. Ability of real-time PCR for differential diagnosis of various forms of cutaneous leishmaniasis: A comparative study with histopathology. *BMC Research Notes*, **12**: 615. [*https://doi.org/10.1186/s13104-019-4666-5*].

Fitriani, E.N., Arief, M. and Suprapto, H. 2019. Prevalence and intensity of ectoparasites in Gabus fish (*Channa striata*) at Cangkringan Fishery Cultivation Technology Development Center, Sleman, Yogyakarta. pp. 1-9 (012095). **In**: *IOP Conference Series: Earth and Environmental Science*, *Volume 236).* IOP Publishing. [*https://doi.org/ 10.1088/1755-1315/236/1/012095*].

Gilbert, B.M. and Avenant-Oldewage, A. 2021. Monogeneans as bioindicators: A meta-analysis of effect size of contaminant exposure toward Monogenea (Platyhelminthes*). Ecological Indicators*, **130***:* 108062. [*https://doi.org/10.1016/j.ecolind.2021.108062*].

Guagliardo, S., Viozzi, G. and Brugni, N. 2019. Pathology associated with larval *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) infection in *Galaxias maculatus* (Actinopterygii: Galaxiidae) from Patagonia, Argentina. *International Journal for Parasitology: Parasites and Wildlife*, **10***:* 113-116.

Harada, S., Inaoka, D.K., Ohmori, J. and Kita, K. 2013. Diversity of parasite complex II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, **1827**(5)*:*658-667.

Heneash, A.M., Alprol, A.E., Abd El-Hamid, H.T., Khater, M. and El Damhogy, K.A. 2021. Assessment of water pollution induced by anthropogenic activities on zooplankton community in Mariout lake using statistical simulation. *Arabian Journal of Geosciences,* **14**(7): [*https://doi.org/10.1007/s12517-021-06977-9*].

Hulme, P.E. 2022. Hierarchical cluster analysis of herbicide modes of action reveals distinct classes of multiple resistance in weeds. *Pest Management Science*, **78**(3): 1265-1271.

Kamboj, N. and Kamboj, V. 2019. Water quality assessment using overall index of pollution in riverbed-mining area of Ganga-river Haridwar, India. *Water Science,* **33**(1)*:* 65-74.

Koledoye, T.Y., Akinsanya, B., Adekoya, K.O. and Isibor, P.O. 2022. Physicochemical parameters of the Lekki Lagoon in relation to abundance of *Wenyonia* sp. Woodland, 1923 (Cestoda: Caryophyllidae) in *Synodontis clarias* (Linnaeus, 1758). *Environmental Challenges,* **100453**. [*https://doi.org/10.1016/j.envc.2022.100453*].

Kua, B.C., Choong, F.C. and Leaw, Y.Y. 2014. Effect of salinity and temperature on marine leech, *Zeylanicobdella arugamensis* (De Silva) under laboratory conditions. *Journal of Fish Diseases*, **37**(3)*:* 201-207.

Lacerda, A., Roumbedakis, K., Bereta Junior, J., Nuñer, A., Petrucio, M.M. and Martins, M.L. 2018. Fish parasites as indicators of organic pollution in southern Brazil. *Journal of Helminthology,* **92**(3)*:* 322-331.

Lefcort, H., Aguon, M.Q., Bond, K.A., Chapman, K.R., Chaquette, R., Clark, J., Kornachuk, P., Lang, B.Z. and Martin, J.C. 2002. Indirect effects of heavy metals on parasites may cause shifts in snail species compositions. *Archives of Environmental Contamination and Toxicology*, **43**(1): 34-41.

Lom, J. and Dykova, I. 1992. Protozoan parasites of fishes. *Developments in Aquaculture and Fisheries Science*. *Volume 26.* Elsevier, Amsterdam, Netherland.

Luis, K.M.A., Rheuban, J.E., Kavanaugh, M.T., Glover, D.M., Wei, J., Lee, Z. and Doney, S.C. 2019. Capturing coastal water clarity variability with Landsat 8. *Marine Pollution Bulletin,* **145:** 96-104.

Maceda-Veiga, A., Mac Nally, R., Green, A.J., Poulin, R. and de Sostoa, A. 2019. Major determinants of the occurrence of a globally invasive parasite in riverine fish over large-scale environmental gradients. *International Journal for Parasitology,* **49**(8): 625-634.

MacLeod, C.D. and Poulin, R. 2012. Host–parasite interactions: A litmus test for ocean acidification? *Trends in Parasitology,* **28**(9): 365-369.

Majumder, S., Panda, S. and Bandyopadhyay, P.K. 2015. Effect of temperature on the prevalence of different parasites in *Cirrhinus mrigala* Hamilton of West Bengal. *Journal of Parasitic Diseases,* **39**(1): 110-112.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. and Schad, G. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *The Journal of Parasitology,* **68**(1): 131-133.

Martins, M.L., Cardoso, L., Marchiori, N. and Benites de Pádua, S. 2015. Protozoan infections in farmed fish from Brazil: diagnosis and pa. *Revista Brasileira de Parasitologia Veterinária,* **24**: 1-20.

Mondal, A., Das, S., Sah, R.K., Bhattacharyya, P. and Bhattacharya, S.S. 2017. Environmental footprints of brick kiln bottom ashes: Geostatistical approach for assessment of metal toxicity. *Science of the Total Environment,* **609***:* 215-224.

Montgomery, D.W., Simpson, S.D., Engelhard, G.H., Birchenough, S.N.R. and Wilson, R.W. 2019. Rising CO_2 enhances hypoxia tolerance in a marine fish. *Scientific Reports,* **9**(1). [*https://doi.org/10.1038/s41598-019-51572-4*].

Ogut, H. and Palm, H.W. 2005. Seasonal dynamics of *Trichodina* sp. on whiting (*Merlangius merlangus*) in relation to organic pollution on the eastern Black sea coast of Turkey. *Parasitology Research,* **96**(3)*:* 149-153.

Okereke, J.N., Ogidi, O.I. and Obasi, K.O. 2016. Environmental and health impact of industrial wastewater effluents in Nigeria - A review. *International Journal of Advanced Research in Biological Sciences,* **3**(6)*:*55-67.

Onada, O.A., Akinwole, A.O. and Ajani, E.K. 2015. Study of interrelationship among water quality parameters in earthen pond and concrete tank. *PeerJPrePrints*, **3**: p.e845v1. [*https://doi.org/10.7287/peerj.preprints.845v1*].

Orsolic, D., Pehar, V., Šmuc, T. andStepanić, V. 2021. Comprehensive machine learning based study of the chemical space of herbicides. *Scientific Reports,* **11**(1): [*https://doi.org /10.1038/s41598-021-90690-w*].

Osmond, D.L., Line, D.E., Gale, J.A., Gannon, R.W., Knott, C.B., Bartenhagen, K.A. and Lehning, D.W. 1995. Water, soil and hydro-environmental decision support system. [*www.water. ncsu. edu/watersheds/info/hmetals.html*].

Sallam, G.A. and Elsayed, E.A. 2018. Estimating relations between temperature, relative humidity as independent variables and selected water quality parameters in Lake Manzala, Egypt. *Ain Shams Engineering Journal,* **9**(1)*:*1-14.

Sarkar, R., Ghosh, A.R. and Mondal, N.K. 2020. Comparative study on physicochemical status and diversity of macrophytes and zooplanktons of two urban ponds of Chandannagar, WB, India. *Applied Water Science,* **10**(2): 1-8.

Shah, H.B., Yousuf, A.R., Chishti, M.Z. and Ahmad, F. 2013. Seasonal changes in infra-populations of *Diplozoon kashmirensis* Kaw, 1950 (Monogenea: Diplozoidae) along a eutrophic gradient. *Parasitology Research*, **112**(9)*:* 3347-3356.

Sures, B. 2004. Environmental parasitology: Relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology*, **20**(4): 170-177.

Verma, A.K. and Singh, T.N. 2013. Prediction of water quality from simple field parameters. *Environmental Earth Sciences*, **69**(3): 821-829.

Vidal-Martínez, V.M., Velázquez-Abunader, I., Centeno-Chalé, O.A., May-Tec, A.L., Soler-Jiménez, L.C., Pech, D. and Aguirre-Macedo, M.L. 2019. Metazoan parasite infra-communities of the dusky flounder (*Syacium papillosum*) as bioindicators of environmental conditions in the continental shelf of the Yucatan Peninsula. *Mexico. Parasites & Vectors,* **12**(1)*:* 1-18.

Xu, D.H., Pridgeon, J.W., Klesius, P.H. and Shoemaker, C.A. 2012. Parasitism by protozoan *Ichthyophthirius multifiliis* enhanced invasion of *Aeromonas hydrophila*in tissues of channel catfish. *Veterinary Parasitology,* **184**(2-4): 101-107.

Yurova, Y. and Shirokova, V. 2020. Geoecological assessment of Anthropogenic impacts on the Osetr river basin. *Geosciences,* **10**(4): 121. [*https://doi.org/10.3390/geosciences10040121*].

Zhang, X., Shang, B., Cheng, Y., Wang, G. and Li, W. 2021. Effects of different strategies of low temperature on egg hatching of *Dactylogyrus vastator* (Monogenea: Dactylogyridae). *Research Square,* 1-9. [*https://doi.org/10.21203/rs.3.rs-560786/v1*].

Zhu, G., Xiong, N., Wang, X., Hursthouse, A.S. and Marr, A. 2020. Correlation characteristics of electrical conductivity of surface waters with the fluorescence excitation-emission matrix spectroscopy-parallel factor components of dissolved organic matter. *Journal of Fluorescence*, **30**(6): 1383-1396.