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RESEARCH

Fish parasites as proxy bioindicators of degraded water quality of River Saraswati, India

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Abstract The nature and intensity of water pollution determine the effects on aquatic biota and aquatic ecosystem health. The present study aimed at assessing the impact of the degraded physicochemical regime of river Saraswati, a polluted river having a historical legacy, on the parasitic infection and the role of fish parasite as a bioindicator of water quality. Two Water Quality Indices (WQIs) were adopted as useful tools for assessing the overall water quality status of polluted river based on 10 physicochemical parameters. Total 394 fish (*Channa punctata*) were examined. Ectoparasite *Trichodina* sp., *Gyrodactylus* sp., and endoparasites *Eustrongylides* sp. were collected from the host fish. Prevalence, mean intensity and abundance for each sampling period were calculated for the determination of parasitic load. The parasitic load of *Trichodina* sp. and *Gyrodactylus* sp. was significantly ($p < 0.001$) higher in winter, whereas the parasitic load of *Eustrongylides* sp. showed no significant ($p > 0.05$) seasonal fluctuation. The parasitic load of ectoparasites was negatively correlated with temperature, free carbon dioxide, biochemical oxygen demand, and WAWQI but positively correlated with electrical conductivity and CCMEWQI. Fish health was found to be adversely affected by degrading water qualities and parasitic infection. A 'vicious cycle' develops as a result of the interplay among deteriorating water quality, withering fish immunological defence, and amplifying parasitic infection. Since parasitic load was strongly conditioned by the combined influence of a suite of water quality parameters the fish parasites can be used as a powerful indicator of deteriorating water quality.

Keywords Environmental pollution · Riverine health · Water Quality Index · Fish; Parasitic infection · Biomonitoring

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Introduction

The aquatic environment can be studied either directly by regular monitoring of physicochemical parameters of water or indirectly by using bioindicators. Both free-living and parasitic organisms are sensitive to pollutants and physiological and biochemical conditions are indicative of the environmental quality (Sures et al., 2017). Physicochemical changes can adversely affect living organisms and biological processes in the aquatic system, leading to biological degradation (Bhat et al., 2022). 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), biological oxygen demand (BOD), chemical oxygen demand (COD) etc. (Sarkar et al., 2020).

The water quality index (WQI) is a metric that is used for assessing the quality of water. The objective of formulating a WQI is to know the quality and usefulness of water by classifying it based on physicochemical and biological properties (Pramanik et al., 2023; Uddin et al., 2021). Thus, a wide range of variables are rationally assembled and aggregated into a single index value to portray the overall quality of water for a particular aquatic environment at a specific location and time (Siraj, et al., 2023).

Some organisms show different degrees of sensitivity upon exposure to pollutants of diverse nature and types which can be explored and exploited for monitoring aquatic pollution and restoration of aquatic health (Dar & Bhat, 2020). Till now numerous free-living organisms have been used as a bioindicator for aquatic ecosystems such as macrophytes (Singh & Singh, 2020), planktons (Hemraj et al., 2017), annelids (Abubakr et al., 2018), crustacean (de Almeida Rodrigues et al., 2022), molluscs (Hong et al., 2020) and fish (Jia & Chen, 2013; Marin et al., 2023; Tashla et al., 2018). A new approach is selected to monitor ecosystem health by the parasite as a bioindicator for water pollution. The aquatic environment can be studied indirectly by using fish parasites as bioindicators (Biswas & Pramanik, 2016). Environmental parasitologists acknowledge the role of fish parasites as a bioindicator for aquatic health (Suthar et al., 2022) but fish biologists and ecologists disagree with the opinion (Ridall & Ingels, 2021; Santoro et al., 2020; Timi & Poulin, 2020). The impact of water pollution may be positive or negative for parasites of aquatic animals. Accumulation of pollutants within parasites is responsible for negative effects on them, while organic pollution exerts a positive effect on the parasitic population and thereby acts as an effect indicator (Abdel-Gaber et al., 2017; Maceda-Veiga et al., 2019). Concentrations of various pollutants and environmental stressors have significant effects on the parasite. Ecosystem health can be assessed using ecto- and endoparasite of fish as bio-indicators (Sures et al., 2023). Parasites of freshwater fish are sensitive to water pollution and they can be considered as qualitative indicators of freshwater system. Fish parasites respond to both biotic and abiotic stressors and make them a promising tool for ecological indicators for detecting water quality (Sures & Nachev, 2022; Taglioretti et al., 2018). Physicochemical factors of water have distinct and/or combined effects on parasite infection.

Channa punctata (Bloch, 1793) is a common freshwater edible fish also known as Lata fish that may survive in a stressful environment owing to presence of an accessory respiratory organ and associated adaptations. In a polluted environment the fish are often found to be parasitized by ecto- and endoparasites such as the ciliate *Trichodina* sp., the monogenean *Gyrodactylus* sp., and the larval stage (L4) of *Eustrongylides* sp. (Gilbert & Avenant-Oldewage, 2021; Guagliardo et al., 2019; Zulfahmi et al., 2021). 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). Water temperature is one of the most important factors for seasonal patterns and transmission of monogenean parasite *Gyrodactylus* sp. There is a distinct relation among water temperature, and their microenvironment and the number of *Gyrodactylus* sp. (Rubio-Godoy et al., 2016). The load of ectoparasite, such as *Trichodina* sp. is found to be related with the concentration of environmental pollutants. Prolonged exposure to pollutants increases prevalence and abundance of parasite and coincides with pathological changes, hence can be used as a biomarker (Khan, 2004; Kochmann et al., 2023). Not only micro-parasites but macro-parasites such as nematode and arthropod can also be used as potent bioindicators for aquatic ecosystems (Morales-Serna et al., 2019). Parasite enhances the toxic effect of pollutants by interfering with host's defence mechanism and physiological homeostasis (Mehana et al., 2020). Combined effects of parasites and pollutants on the host are of interest in parasitological and eco-toxicological research (Sures et al., 2017). *Trichodina* sp. is a complete ectoparasite of fish, which is influenced by water quality parameters. *Gyrodactylus* sp. lives inside the gills and is somewhat impacted by environmental conditions on the other hand, *Eustrongylides* sp. which is an endoparasite may be unaffected by the external environment.

Little research effort has been made to establish a novel, non-stereotypical, unconventional bioindicator for environmental quality of the aquatic system beyond the oft-used traditional structural bioindicators (e.g. phytoplankton cell size and biomass; zooplankton body size and biomass; zooplankton to phytoplankton ratio; species diversity, etc.) and functional bio-indicators (e.g. algal carbon assimilation ratio, resource use efficiency, community production, gross production to respiration ratio, gross production to standing biomass ratio, etc.) or the conventional sensitive-tolerant bioindicator categories.

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. It is hypothesised that since degradation of water quality of a river adversely affect fish health making them more vulnerable to parasitic infection and higher parasitic load, fish parasites can serve as a proxy bioindicators of degraded water quality of that waterbody. The purpose of the present study is 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., monogenean *Gyrodactylus* sp. and endoparasites such as *Eustrongylides* sp.) as response indicators for monitoring and assessing aquatic health.

Materials and methods

Study area

The river Saraswati is a distributary of River Ganga (Hooghly River), which flows through the Hooghly region of West Bengal, India. The sampling area is about 4 km away from the point of connection with 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 sample and physicochemical analyses

The water samples were collected from the polluted river once a month from March 2017 to February 2020 during summer, monsoon, post-monsoon and winter season for analysis of different physicochemical parameters. The months from March to May represent the first quarter of the year (Q1), the summer season; June to August represent the monsoon season (Q2); September to November represent the post-monsoon (Q3); and December to February represent the winter season (Q4). Water temperature, pH, total dissolved solids (TDS), turbidity, salinity, and electrical conductivity (EC) were monitored periodically using a digital water and soil analysis kit {Electronics India (EI); Model – 172}. The concentrations of dissolved oxygen (DO), free carbon dioxide (CO_2), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined as per the standard methods (APHA, 2012).

Determination of Water Quality Index (WQI)

WQIs for the selected sites of the polluted river were determined based on 10 physicochemical parameters of water. The water quality was assessed using two alternative WQI formulations: Weighted Arithmetic Water Quality Index (WAWQI) and the Canadian Council of Ministers of the Environment Water Quality Index (CCMEWQI). When computing both types of WQIs, the standard values of water quality parameters suggested by WHO (2004), Bureau of Indian Standards (BIS) (1983), and ICMR (1975) were utilized as 'benchmarks' for assessing the quality of individual parameters.

Weighted Arithmetic Water Quality Index (WAWQI)

WAWQI was calculated as proposed by Horton (Horton, 1965) and developed by Brown et al. (1972) as stated below.

$$\mathrm{WAWQI} = \frac{\sum_{i=1}^{n} Wi \times Qi}{\sum_{i=1}^{n} Wi} \tag{1}$$

n = number of variables of parameters

W = Relative weight of the n^{th} parameters

Q = Water quality rating of the n^{th} parameters

Base on the WAWQI values water qualities are classified under five categories i.e. 0 to 25—excellent; 26 to 50—good; 51 to 75—poor; 76 to 100—very poor; > 100—unsuitable.

Canadian Council of Ministers of the Environmental Water Quality Index (CCMEWQI)

CCMEWQI is based on three measurements; factor 1 (F1) represents the scope; factor 2 (F2) represents the mean frequency; and factor 3 (F3) represents the amplitude. The values of F1, F2 and F3 are calculated using the following formulae (CCME, 2001)

$$F1 = \frac{\text{Number of failed variables}}{\text{Total number of variables}} \times 100 \tag{2}$$

$$F2 = \frac{\text{Number of failed tests}}{\text{Total number of tests}} \times 100 \tag{3}$$

$$F3 = \frac{nse}{0.01nse + 0.01} \tag{4}$$

where nse stands for normalised sum excursion which is derived as the ratio between sum of excursion and total number of tests.

The value of CCMEWQI is calculated by using the following formula

$$\text{CCMEWQI} = 100 - \frac{\sqrt{F_1^2 + F_2^2 + F_3^2}}{1.732} \tag{5}$$

The vector length can reach $\sqrt{100^2 + 100^2 + 100^2}$ = $\sqrt{3000}$ = 173.2, so division by the factor 1.732 is only to adjust CCMEWQI into 0 to 100 scale (Kachroud et al., 2019).

From CCMEWQI values water are classified into five categories i.e., ≤ 44—poor, 45 to 64—bad, 65 to 79—marginal, 80 to 94—good, and 95 to 100—excellent.

Collection of host fish and parasites

A total number of 394 host fish *Channa punctata* (Bloch, 1793) (Table 3) were collected from the polluted river during monsoon, post-monsoon and winter seasons from March 2017 to February 2020, by local fishermen with the help of fishing gears (bamboo fish cage and fish net). Host fish were investigated for protozoan and metazoan parasites. *Trichodina* sp. and *Gyrodactylus* sp. representing protozoan and monogenean ectoparasites respectively were collected from the body surface mucous and gill of fish. *Eustrongylides* sp. (nematode) is endoparasite collected from dissected host fish. These three parasites were strategically selected for this study in order to assess differential impact of water quality on prevalence, mean intensity and abundance of three different types of parasites. *Trichodina* sp. is a complete ectoparasite of fish, and it is strongly influenced by ambient water quality parameters. On the other hand, *Eustrongylides* sp., is an endoparasite, which may remain unaffected by the external environmental physicochemical factors. *Gyrodactylus* sp. lives inside the gills, and is moderately impacted by the extrinsic environmental factors. Collected parasites were immediately fixed by formaldehyde solution (10%) and counted under the light microscope or naked eye in case of macroscopic parasite *Eustrongylides* sp. Collected parasites were taxonomically identified by adapting the works of Basson and As (1989) for *Trichodina* sp., Bakke et al. (2002) for *Gyrodactylus* sp., and Moravec et al. (2009) for *Eustrongylides* sp. Prevalence, mean intensity and abundance for each sampling period were calculated according to Bush et al. (1997).

$$\text{Prevalence (\%)} = \frac{\text{Number of host infected}}{\text{Number of host examined}} \times 100 \tag{6}$$

$$\text{Mean intensity} = \frac{\text{Total number of parasite}}{\text{Number of infected host}} \tag{7}$$

$$\text{Abundance} = \frac{\text{Total number of parasite}}{\text{Number of host examined}} \tag{8}$$

Statistical analyses

All the data obtained were subjected to appropriate statistical validation. The values were analysed by one-way ANOVA followed by Fisher LSD using the statistical software SPSS 20 in order to compare the physicochemical parameters and WQI of water seasonally. Similar type of statistical analysis was applied to compare the seasonal variation of parasitic load for three different parasites. For understanding the effect of water parameters and pollution on parasitic load, the data were compared by Spearman's correlation. Further, to identify the patterns of correlated and non-correlated variables the data were subjected to statistical validation by principal components analysis (PCA) using OriginPro.

Results

The seasonal data of all the physicochemical parameters of water sample from the polluted river (Saraswati) are presented in Table 1.

The mean water temperature ranged from 22.31 ± 0.78 °C to 33.59 ± 0.45 °C throughout the period of study. The maximum temperature (36 °C) was recorded in July 2019 while the minimum temperature (19 °C) was recorded in January 2018. The significant difference (LSD test; $p < 0.01$) in temperature was observed among the seasonal mean values in the following order of variation: monsoon (33.59 ± 0.45 °C), summer (32.13 ± 0.91 °C) > post-monsoon (29.37 ± 0.76 °C) > winter (22.31 ± 0.78).

The mean EC value of the water samples ranged from 297.78 ± 37.7 mS cm^{-1} to 732.22 ± 34.9 mS cm^{-1} during the period of investigation. The maximum EC value (870 mS cm^{-1}) was recorded in March 2018. The mean EC value increased gradually in winter (651.11 ± 6.75 mS cm^{-1}) compared to monsoon and post-monsoon to reach the maximum in summer (732.22 ± 34.9 mS cm^{-1}). The mean EC values of summer and winter differed significantly (one-way ANOVA, $F_{3, 8} = 44.84$, $p < 0.001$; LSD test, $p < 0.001$) from that of monsoon and post-monsoon season.

The mean turbidity of water in the polluted river was much high ranging from 12.83 ± 1.22 NTU to 44.22 ± 1.12 NTU. The maximum turbidity value (56 NTU) was recorded in August 2017 from the polluted. The mean turbidity value was lower in summer (12.83 ± 1.22 NTU) and exhibited an increasing trend during monsoon (35.77 ± 1.94 NTU) and post-monsoon (31.22 ± 2.56) followed by drastic decline in winter (21.83 ± 0.53). Discernible seasonal variation in the mean turbidity ($F_{3, 8} = 94.50$, $p < 0.001$) was

Table 1 Seasonal variations of the physicochemical parameters (mean ± SE) of water from the polluted river (Saraswati). Instead of mean, pH represents range of values monitored during different seasons

Locality	Seasons	Tem (°C) Mean ±SE	EC (mS cm^{-1}) Mean ±SE	TDS (ppm) Mean ±SE	Salinity (ppm) Mean ±SE	Tur (NTU) Mean ±SE	pH Range	DO (mg L^{-1}) Mean ±SE	Free CO_2 (mg L^{-1}) Mean ±SE	BOD_5 (mg L^{-1}) Mean ±SE	COD (mg L^{-1}) Mean ±SE
River Saraswati	Summer	32.13 ±0.91	732.22 ±34.9	504.44 ±8.01	288.88 ±55.55	12.83 ±1.22	7.15 – 7.53	1.55 ±0.09	61.26 ±2.07	7.63 ±0.31	33.39 ±1.28
	Monsoon	33.59 ±0.45	297.78 ±37.7	290 ± 65.0	66.66 ±15.91	35.77 ±1.94	7.18 – 7.35	1.75 ±0.35	62.02 ±2.56	8.85 ±0.36	28.05 ±1.29
	Post-monsoon	29.37 ±0.76	406.66 ±31.7	309.99 ±31.79	133.33 ±38.48	31.22 ±2.56	7.23 – 7.53	3.11 ±0.22	63.05 ±2.99	10.42 ±0.36	22.70 ±3.68
	Winter	22.31 ±0.78	651.11 ±6.75	401.11 ±13.51	255.55 ±29.39	21.83 ±0.53	7.40 – 7.59	1.29 ±0.05	48.05 ±3.69	5.35 ±0.35	27.95 ±0.44
	Overall	**29.35 ± 0.83**	**521.94 ±32.25**	**376.38 ±19.57**	**186.11 ±22.93**	**25.41 ±2.23**	**7.15 – 7.59**	**1.92 ±0.19**	**58.59 ±1.51**	**8.06 ±0.38**	**28.02 ±1.08**

Parameters are represented here with respective abbreviations: temperature (Tem), electrical conductivity (EC), total dissolved solids (TDS), turbidity (Tur), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD)

The values in bold indicate the overall seasonal values of the physicochemical parameters of the water observed in summer and winter compared to monsoon and post-monsoon (LSD test; $p < 0.001$).

The mean DO concentration in the polluted river registered much lower values ranging from 1.29 ± 0.05 mg L^{-1} to 3.11 ± 0.22 mg L^{-1}. The minimum DO concentration (0.5 mg L^{-1}) was recorded during June 2017. The study sites showed a distinct seasonal variation in mean DO concentration ($F_{3, 8} = 13.652$, $p < 0.005$), and the mean DO concentration of post-monsoon (3.11 ± 0.22 mg L^{-1}) differed significantly (LSD test; $p < 0.005$) from summer (1.55 ± 0.09 mg L^{-1}), monsoon (1.75 ± 0.35 mg L^{-1}) and winter (1.29 ± 0.05 mg L^{-1}). A distinct pattern of temporal variations in DO concentrations was observed, registering a remarkable rise from monsoon to post-monsoon season, followed by a drastic drop in DO levels in winter.

In polluted river the mean concentration of free CO_2 ranged from 48.05 ± 1.25 mg L^{-1} to 63.05 ± 2.99 mg L^{-1}. It illustrated an opposing relationship with DO which was reflected in their spatial and temporal patterns. The maximum free CO_2 concentration was recorded in November 2018. No seasonal variation in mean free CO_2 concentration was observed ($F_{3, 8} = 0.182$, $p = 0.906$). The free CO_2 concentration decreased markedly in winter resulting in seasonal variation between winter (48.05 ± 3.69 mg L^{-1}) and other three seasons (summer, 61.26 ± 2.07 mg L^{-1}; monsoon, 62.02 ± 2.56 mg L^{-1}; post-monsoon, 63.05 ± 2.99 mg L^{-1}) by LSD test; $p < 0.001$.

The mean BOD value of the water samples ranged from 5.35 ± 0.35 mg L^{-1} to 10.42 ± 0.36 mg L^{-1}, The minimum BOD value (5.12 mg L^{-1}) was recorded in September 2017 and the maximum BOD value (12.5 mg L^{-1}) was recorded in August 2018. The mean concentration of BOD was lower in winter (5.3 ± 0.35 mg L^{-1}) and differed significantly from other seasons (summer = 7.63 ± 0.31 mg L^{-1}; monsoon = 8.85 ± 0.36 mg L^{-1} and post-monsoon = 10.42 ± 0.36 mg L^{-1}) by one-way ANOVA ($F_{3, 8} = 37.295$, $p < 0.001$) followed by LSD test, $p < 0.001$.

The maximum concentration of COD (38.33 mg L^{-1}) was recorded in May 2019 in PR-2 and the minimum concentration of COD (20 mg L^{-1}) was recorded in October 2018. The mean COD concentration of summer (33.39 ± 1.28 mg L^{-1}) differed significantly (LSD test; $p < 0.05$) from post-monsoon (22.70 ± 1.08 mg L^{-1}) therefore, seasonal variation was not prominent. In polluted river the COD level was minimum on post-monsoon and exhibited an increasing trend during the following seasons.

Water quality index (WQI)

Two WQI methods were adopted for quality classification of water. The mean values and usefulness of CCMEWQI and WAWQI are presented in Table 2.

Table 2 Season-wise mean (±SE), minimum and maximum values of both the WQIs (CCMEWQI and WAWQI) and quality classification

Sources of water	Season (2017 to 2020)		CCMEWQI		WAWQI	
			Value	Class of quality	Value	Class of quality
Polluted River (River Saraswati)	Summer	Mean ± (SE)	31.75 ± 1.49	Poor	182.12 ± 5.12	Unsuitable
		Min	30.21		169.92	
		Max	34.73		186.97	
	Monsoon	Mean ± (SE)	37.00 ± 0.48	Poor	225.10 ± 14.57	Unsuitable
		Min	36.07		190.39	
		Max	37.72		240.53	
	Post-Monsoon	Mean ± (SE)	42.25 ± 2.76	Poor	212.15 ± 9.83	Unsuitable
		Min	37.05		210.29	
		Max	46.46		241.10	
	Winter	Mean ± (SE)	45.35 ± 0.16	Bad	167.36 ± 7.64	Unsuitable
		Min	45.08		157.30	
		Max	45.65		182.74	
	Overall	Mean ± (SE)	**39.09 ± 1.70**	**Poor**	**196.68 ± 8.16**	**Unsuitable**
		Min	**30.21**		**157.30**	
		Max	**46.46**		**241.10**	

The values in bold indicate the overall WQI values and corresponding quality classes

The mean index value of CCMEWQI differed significantly and exhibited seasonal variation by one-way ANOVA ($F_{3, 8} = 14.171$, $p < 0.001$). No significant difference was observed between the average CCMEWQI value of winter (45.35 ± 0.16) and post-monsoon (42.25 ± 2.76) (LSD test; $p > 0.05$). The water quality of summer, monsoon and post-monsoon indicated poor water quality whereas winter indicated bad water quality according to water quality rating of CCMEWQI. The average index value of WAWQI during monsoon (225.10 ± 14.57) and post-monsoon (212.15 ± 9.83) differed significantly from summer (182.12 ± 5.12) and winter (167.36 ± 7.64) therefore, exhibited seasonal variation (ANOVA; $F_{3, 8} = 17.096$, $p < 0.001$ LSD test; $p < 0.05$). According to quality rating, water quality of the polluted river was unsuitable for drinking throughout the year.

Enumeration of parasites and effect of seasonality on parasitic load

During the dry summer season, the flow and level of water in the polluted river were too low to contain any fish. The descriptive statistics associated with the parasitic load of host fish are represented in Table 3.

The lowest mean prevalence ($20.08 \pm 3.92\%$) of *Trichodina* sp. was observed in the monsoon while the highest mean prevalence ($41.2 \pm 1.25\%$) was recorded in post-monsoon season. On the other hand, both the lowest mean intensity (10.89 ± 0.42) and abundance (2.15 ± 0.35) of *Trichodina* sp. were recorded in monsoon while the highest mean intensity (24.58 ± 0.66) and abundance (9.01 ± 0.51) were observed in winter. To test the hypothesis that seasonal variation had an effect on parasite load, one-way ANOVA was performed. The analysis exhibited significance seasonal variation in prevalence ($F_{2, 24} = 16.651$, $p < 0.001$), mean intensity $F_{2, 24} = 63.915$, $p < 0.001$) and abundance ($F_{2, 24} = 58.774$, $p < 0.001$) of *Trichodina* sp. Furthermore, the assumption of homogeneity of variances for prevalence, mean intensity and abundance of *Trichodina* sp. were tested and satisfied based on Levene's test (prevalence, $F_{2, 24} = 1.037$, $p = 0.370$; mean intensity, $F_{2, 24} = 2.235$, $p = 0.129$; abundance, $F_{2, 24} = 3.577$, $p = 0.054$). Thus, the null hypothesis of no difference between the mean was rejected. To evaluate the nature of the difference between the three means of *Trichodina* sp. further, the statistically significant ANOVA was followed by Fisher's LSD post-hoc tests (Hayter, 1984). The difference between the mean prevalence and abundance of *Trichodina* sp. of monsoon was statistically significant from both the post-monsoon and winter ($p < 0.001$) but no significant difference was observed for the mean prevalence and abundance between post-monsoon and winter seasons ($p = 0.246$ for prevalence; $p > 0.05$ for abundance). Mean intensity of *Trichodina* sp. of monsoon, post- monsoon and winter was statistically significant by LSD test; $p < 0.001$ (Fig. 1a, b, c). Therefore, parasitic load of *Trichodina* sp. was highly impacted by seasonal variation.

Similar types of statistical analysis were performed to determine the relationship between seasonality and parasitic load of *Gyrodactylus* sp. and *Eustrongylides* sp. One-way ANOVA exhibited significant seasonal variations for the mean value of prevalence ($F_{2, 24} = 4.339$, $p < 0.05$) mean intensity $F_{2, 24} = 10.702$, $p < 0.001$) and abundance ($F_{2, 24} = 6.931$, $p = 0.05$) of *Gyrodactylus* sp. in the order of winter > post-monsoon > monsoon (Table 3). Levene's test showed the equality of variance for all the parameters (prevalence, $F_{2, 24} = 1.172$, $p = 0.327$; mean intensity, $F_{2, 24} = 3.280$, $p > 0.05$; abundance, $F_{2, 24} = 1.286$, $p = 0.295$). Thus, like *Trichodina* sp., the null hypothesis of no difference between the mean was also rejected for *Gyrodactylus* sp. The mean values of the three parameters tested i.e. prevalence, mean intensity and abundance in monsoon season differed significantly from those of post-monsoon and winter (LSD test, $p < 0.05$) but the parametric differences were not significant between post- monsoon and winter (LSD test, prevalence, $p = 0.761$; mean intensity, $p = 0.130$, abundance, $p = 0.264$) (Fig. 1d, e, f). Thus, unlike *Trichodina* sp. the seasonal effect on parasitic load of *Gyrodactylus* sp. was not so prominent.

Seasonality and parasitic load exhibited different pattern in *Eustrongylides* sp. The highest mean values of three parameters were recorded in post-monsoon period followed by winter and monsoon (Table 3). The assumption of homogeneity of variance was met in mean prevalence (Levene's test: $F_{2, 24} = 1.78$, $p = 0.189$) and mean abundance (Levene's test, $F_{2, 24} = 2.938$, $p = 0.07$). On the other hand, homogeneity of variances was violated in mean intensity (Levene's test: $F_{2, 24} = 5.617$, $p = 0.01$) of *Eustrongylides* sp. The mean prevalence of *Eustrongylides* sp. did not differ significantly

Table 3 Number of the infected and non-infected host fish (*Channa punctata*), mean (±SE), of prevalence, mean intensity and abundance of three parasites (*Trichodina* sp., *Gyrodactylus* sp. and *Eustrongylides* sp.) collected from river Saraswati during monsoon, post-monsoon and winter seasons (2017 to 2020)

Parasite	Sampling Period	Year	No. of fish examined	Number of infected fish	Number of parasites	Prevalence	Mean intensity	Abundance
Trichodina sp.	Monsoon	2017–18	20	4	42	20	10.5	2.1
		2018–19	30	4	47	13.33	11.75	1.56
		2019–20	26	7	73	26.92	10.42	2.8
	Mean ± SE					**20.08 ± 3.92**	**10.89 ± 0.42**	**2.15 ± 0.35**
	Post-monsoon	2017–18	35	15	296	42.85	19.73	8.45
		2018–19	40	15	307	37.5	20.46	7.67
		2019–20	37	16	287	43.24	17.93	7.75
	Mean ± SE					**41.2 ± 1.85**	**19.37 ± 0.75**	**7.96 ± 0.24**
	Winter	2017–18	63	23	535	36.5	24.43	8.92
		2018–19	80	27	680	36.25	24.37	8.83
		2019–20	63	25	633	41.26	25.03	10.33
	Mean ± SE					**36.64 ± 1.71**	**24.58 ± 0.66**	**9.01 ± 0.51**
Gyrodactylus sp.	Monsoon	2017–18	20	1	6	5	6	0.3
		2018–19	30	3	16	10	5.33	0.5
		2019–20	26	3	17	11.53	5.66	0.65
	Mean ± SE					**8.84 ± 1.97**	**5.66 ± 0.19**	**0.49 ± 0.1**
	Post-monsoon	2017–18	35	6	36	17.14	6	1.02
		2018–19	40	6	43	15	7.16	1.07
		2019–20	37	6	47	16.21	7.83	1.27
	Mean ± SE					**16.11 ± 0.62**	**7 ± 0.53**	**1.12 ± 0.07**
	Winter	2017–18	63	10	72	15.87	7.2	1.14
		2018–19	80	14	162	17.5	11.57	2.02
		2019–20	63	9	71	14.28	7.88	1.12
	Mean ± SE					**15.88 ± 0.92**	**8.88 ± 1.35**	**1.43 ± 0.29**
Eustrongylides sp.	Monsoon	2017–18	20	3	13	15	4.33	0.65
		2018–19	30	5	20	16.66	4	0.66
		2019–20	26	1	5	3.84	5	0.19
	Mean ± SE					**11.83 ± 4.02**	**4.44 ± 0.29**	**0.5 ± 0.15**
	Post-monsoon	2017–18	35	7	37	20	5.28	1.05
		2018–19	40	12	54	30	4.5	1.35
		2019–20	37	8	46	21.62	5.75	1.24
	Mean ± SE					**23.87 ± 3.09**	**5.17 ± 0.36**	**1.21 ± 0.08**
	Winter	2017–18	63	11	46	17.46	4.18	0.73
		2018–19	80	22	58	27.5	2.63	0.72
		2019–20	63	14	58	22.22	4.14	0.92
	Mean ± SE					**22.39 ± 2.8**	**3.65 ± 0.5**	**0.79 ± 0.06**

The values in bold indicate the overall seasonal mean of parasitic prevalence, intensity and abundance

(ANOVA: $F_{2,\ 24} = 3.289$, $p > 0.05$) among three seasons. Significant but marginal (ANOVA $F_{2,\ 24} = 3.44$, $p = 0.048$) seasonality was exhibited by mean abundance of *Eustrongylides* sp. Only the mean abundance of monsoon (0.5 ± 0.15) differed significantly from post-monsoon (1.21 ± 0.08) and winter (0.79 ± 0.06) by LSD test, $p < 0.05$ (Fig. 1g, h, i). The overall statistical analysis indicated that the null hypothesis of no effect of season on parasitic load was accepted for *Eustrongylides* sp.

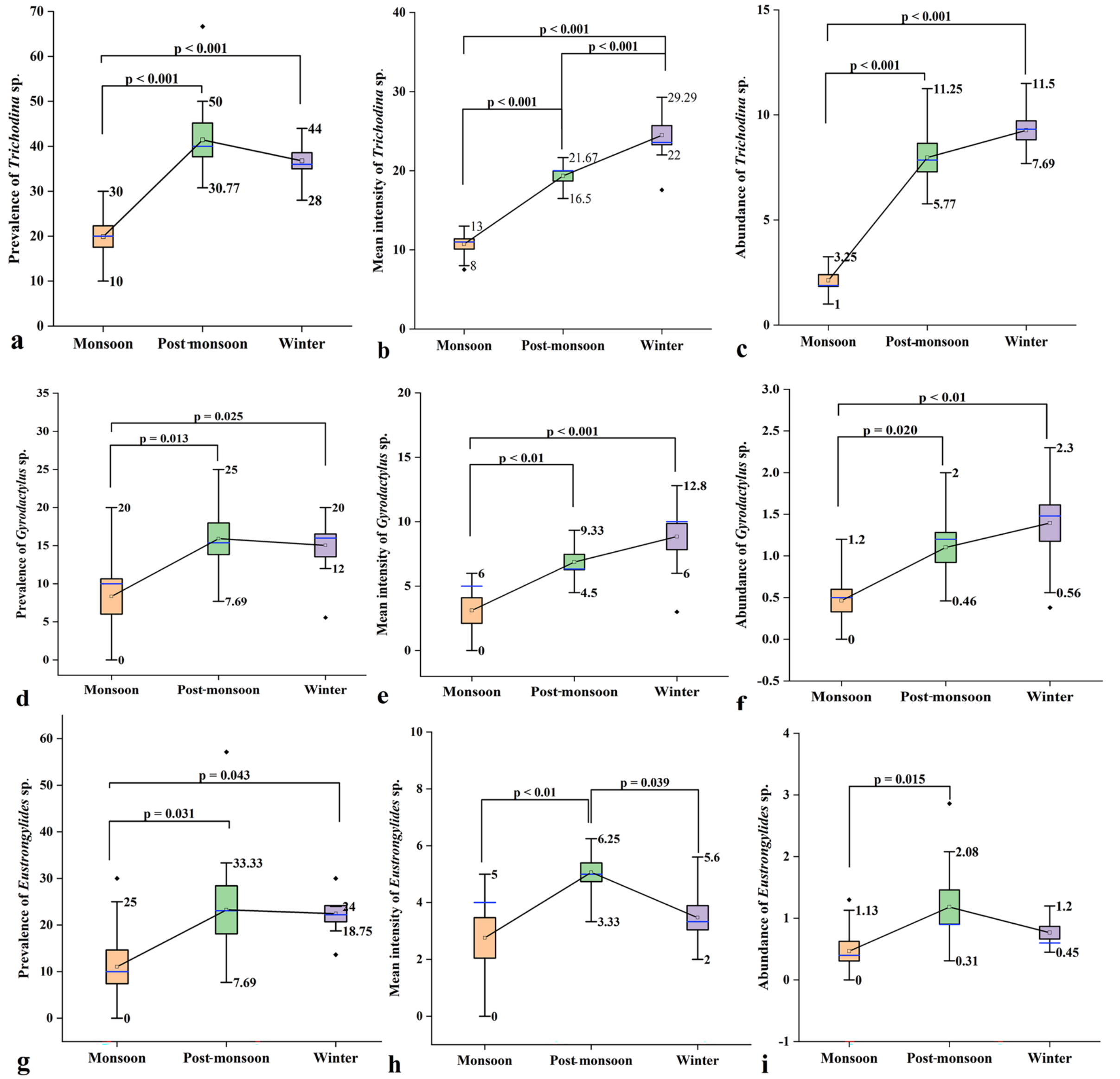


Fig. 1 Seasonal effect on parasitic load of three parasites (*Trichodina* sp., *Gyrodactylus* sp. and *Eustrongylides* sp. collected from host fish (*Channa punctata*) of river Saraswati as revealed by post-hoc analysis (Fisher LSD) of prevalence, mean intensity and abundance of three parasites associated with seasons (monsoon, post-monsoon and winter) and some descriptive statistics such as mean, median, standard error upper and lower boundary, etc. (**a**, **b** and **c**) prevalence, mean intensity and abundance of *Trichodina* sp. (**d**, **e** and **f**) prevalence, mean intensity and abundance of *Gyrodactylus* sp. (**g**, **h** and **i**) prevalence, mean intensity and abundance of *Eustrongylides* sp

Discussion

Physicochemical profile of the river water

In polluted river, seasonal variation of water quality parameters was more prominent in the mean value of temperature, EC, TDS, and, turbidity while the variation was less prominent in the mean concentration of DO, free CO_2, BOD, and COD. The seasonal variation was absent in the mean value of pH and salinity suggesting their very minor role in shaping the overall water quality status of the study sites. The mean EC value of summer was higher and crossed the standard limit (500 mS cm^{-1}) compared to both monsoon and post-monsoon. In summer EC increased by 80.34% and 146.55% respectively than post-monsoon and monsoon while in winter it was increased by 60.37% from post-monsoon and 119.22% from the monsoon. EC values of water display a significant positive correlation with conductive ions coming from dissolved salts and inorganic materials (Zhu et al., 2020), and TDS value indicates the number of dissolved particles in the water sample and is correlated with EC (Alvareda et al., 2020). Seasonal variations of both EC of water depend on several factors such as temperature, evaporation, rainfall, change of water level, tidal stage, etc. Temperature and evaporation rate are positively correlated with EC (Sallam & Elsayed, 2018). Therefore, the polluted river, the mean value of EC was much higher in summer. On the other hand, EC was negatively correlated with rainfall and the inflow of water because they dilute and wash out the concentration of dissolved minerals from the water. So as monsoon and post-monsoon often were accompanied by high rainfall frequency and intensity, as well as influx of water from the Ganges during tidal events, EC value was lowered compared to that in winter despite the difference in temperature (Ewaid et al., 2018). Therefore, the mean EC value recorded was found to be higher in winter than that during monsoon and post-monsoon. Mean turbidity value of polluted river exhibited an opposite pattern compared to EC. Mean turbidity values of monsoon and post- monsoon were increased by 63.85% and 43.01% respectively from winter and more than one and half-fold than summer. Turbidity is caused by solid particles being suspended in a liquid. High turbidity values typically imply lower water quality so, turbidity cannot be used as the sole indicator of water pollution. It may be used as an associate indicator to assess water quality along with other important water quality parameters (Luis et al., 2019). In river water, high turbidity is mainly due to suspended solids that are mixed with water from riverbed material by heavy rainfall (Kamboj & Kamboj, 2019). Besides, industrial wastes, sewage plankton, and eutrophication are also responsible for the turbid condition of water (Mosley et al., 2023). The mean concentration of DO of polluted river was recorded as maximum in post-monsoon but below the standard limit (5 mg L^{-1}), which exhibited seasonal variation. The mean DO value in post-monsoon (3.11 ± 0.22 mg L^{-1}) was recorded as maximum and exhibited decreasing seasonal pattern in the following order: winter (58.52%) > summer (50.10%) > monsoon (43.72) (LSD test; $p < 0.05$). The lowest concentration of dissolved oxygen was the combined effect of organic loading, lowered water volume, stagnancy, seasonal eutrophication and almost absence of water influx from the river 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 standard limit (10 mg L^{-1}). In the other three seasons, the mean concentration of free CO_2 was increased by ~ 30% than in winter. The lowest winter values of free CO_2 indicated organic loading induced intense heterotrophic microbial activity and higher biotic respiration rates. The mean concentration of BOD of polluted river was recorded minimum in winter (5.35 ± 0.35 mg L^{-1}) and all the seasonal BOD level crossed the standard limit (5 mg L^{-1}). In the polluted river it increased in post-monsoon by 88–99% compared to winter. On the other hand, mean COD concentration was lower in post-monsoon and it was increased by 43–47% in summer. The mean COD value crossed the standard limit (20 mg L^{-1}) in all the seasons. 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 & Singh, 2013). In polluted river the mean concentration of DO was relatively higher in post-monsoon along with lower COD value reflecting negative correlation because oxidization of organic and inorganic substance utilized more DO. The DO level was very low during winter (1.20 mg L^{-1} to 1.37 mg L^{-1}) and summer (1.37 mg L^{-1} to 1.71 mg L^{-1}), and BOD level was very high due to organic loading and high microbial activity.

Relationship between water quality and seasonal parasitic load

Host infectivity and parasitic load

In summer the host fish were almost absent in polluted river due to low water volume, and a higher level of water pollution which was approved by both the WQI (CCMEWQI = 31.75 ± 1.49 and WAWQI = 182.12 ± 5.12) methods. Host fish were available from monsoon season onwards because at that time volume of the water increased due to rainfall and influx of water from the river Ganga. The fish came in the polluted river along with the tidal water of the river Ganga.

Impact of seasonal variation in water quality on parasitic load

The parasitic load of host fish was analysed by three statistical methods: prevalence, mean intensity, and abundance. All the parameters of parasitic load depended on the sampling period. The value of mean prevalence (20.08 ± 3.92%), mean intensity (10.89 ± 0.42) and abundance (2.15 ± 0.35) of *Trichodina* sp. was low in monsoon which increased 112%, 80%, and 276%, respectively during post-monsoon and 85%, 128%, 336%, respectively during winter. A similar pattern of seasonal variation was noticed in *Gyrodactylus* sp. The mean prevalence of post-monsoon and winter was increased by 90.99% and 80.55% compared to monsoon. A similar increasing trend was also noticed for the mean intensity which was increased by 120.9% and 184.24% respectively during post-monsoon and winter compared to monsoon. The abundance was also increased by 139.13% during post-monsoon and 202.17% during winter compared to monsoon. These two ectoparasite species showed a distinct seasonal pattern of prevalence, mean intensity, and abundance. The values of those three parasitic traits were highest during winter followed by post-monsoon and monsoon. In case of endoparasite *Eustrongylides* sp., only the mean prevalence was significantly higher (LSD test; $p < 0.05$) in post-monsoon and winter compared to monsoon. Thus, it was evident that mean intensity and abundance of the parasite was found not to be conditioned by seasonal variation. Overall, the statistical analysis exhibited that the null hypothesis of no effect of seasonal change on parasitic load was accepted for *Eustrongylides* sp. indicating its season-neutral nature from parasitic infectivity standpoint. On the other hand, it was rejected for *Trichodina* sp. and *Gyrodactylus* sp. establishing their infectivity being conditioned by season.

Impact of water quality change on parasitic load

The related water quality and parasitic parametric data were compared by Spearman's correlation to identify the degree of relationship and to understand the effect of water quality parameters and pollution on parasitic load. Further, the data were subjected to statistical validation by principal components analysis (PCA) to identify the patterns of correlated and non-correlated variable highlighting their emerging similarity and difference.

Relationship between parasitic load with water quality parameters and WQIs

Correlation between parasitic load of *Trichodina* sp. and water quality was significant (Fig. 4). 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 ($R^2 = 0.2044$; $p < 0.05$) and CCMEWQI ($R^2 = 0.3457$; $p < 0.01$). Mean intensity and abundance of *Trichodina* sp. were positively correlated with CCMEWQI (mean intensity – $R^2 = 0.5436$, abundance – $R^2 = 0.5744$; $p < 0.001$), EC (mean intensity – $R^2 = 0.6931$, abundance – $R^2 = 0.5835$; $p < 0.001$), salinity (mean intensity – $R^2 = 0.4196$, abundance – $R^2 = 0.3223$; $p < 0.001$, 0.01), and TDS (mean intensity – $R^2 = 0.2356$, abundance – $R^2 = 0.2016$; $p < 0.05$) while negatively with WAWQI (mean intensity – $R^2 = 0.4333$, abundance – $R^2 = 0.336$; $p < 0.001$, 0.01) and 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. 2). 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 (Koledoye et al., 2022).

The correlation analyses performed among parasitic load of *Gyrodactylus* sp. and different physicochemical parameters of water showed mixed findings comprising the majority of correlations being insignificant (Fig. 4). The positive correlation was exhibited between parasitic load (except prevalence) and CCMEWQI (mean intensity – $R^2 = 0.2976$, abundance – $R^2 = 0.2281$; $p < 0.05$), EC (mean intensity – $R^2 = 0.3893$, abundance – $R^2 = 0.634$; $p < 0.05$), TDS (mean intensity – $R^2 = 0.1196$, abundance – $R^2 = 0.1223$; $p < 0.05$), salinity (mean intensity – $R^2 = 0.3196$, abundance – $R^2 = 0.2223$; $p < 0.05$), and pH (mean intensity – $R^2 = 0.2951$, abundance – $R^2 = 0.2186$; $p < 0.05$). On the other hand, temperature was negatively correlated (mean intensity – $R^2 = 0.4112$, abundance – $R^2 = 0.3545$; $p < 0.05$) with parasite load of *Gyrodactylus* sp. (Fig. 3). The correlation tests revealed that the parasitic load of the gill parasite in terms of mean intensity and abundance were found to be partially regulated by two external physical factors, temperature and EC. Because of transitional nature of gill parasites between the ectoparasites and endoparasites, they are not

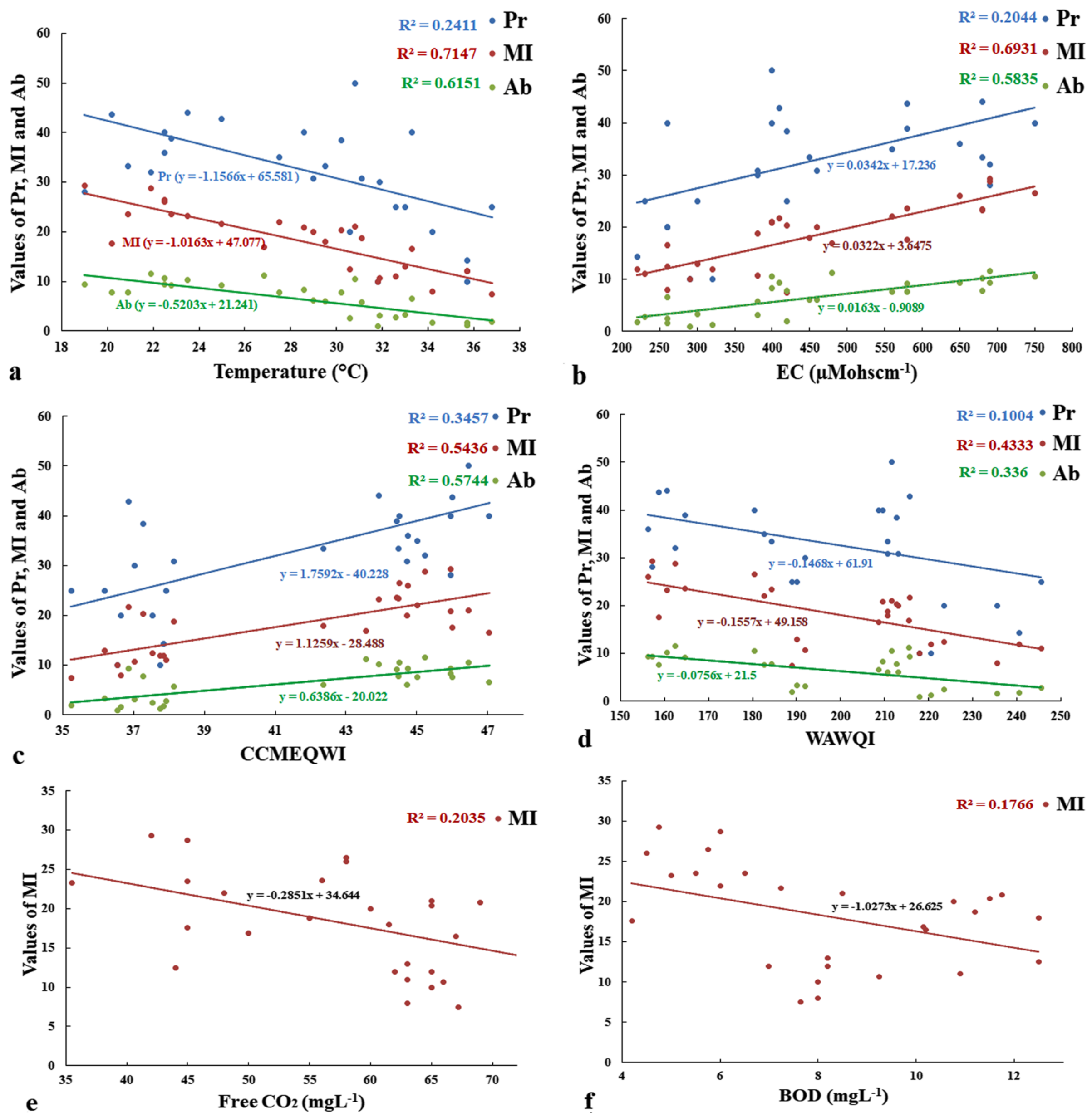


Fig. 2 Regression analysis (with equations and R^2 values) of water quality parameters and parasitic load [prevalence (Pr); mean intensity (MI); and abundance (Ab)] of *Trichodina* sp. (**a**) Negative correlation with temperature; **b** Positive correlation with EC; **c** Positive correlation with CCMEWQI;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

totally immune to extrinsic physical factors but their mean intensity and abundance were partially conditioned by the ambient physical factors (Vidal-Martínez et al., 2019).

In parasitic load of *Eustrongylides* sp. the result was different. No significant correlation was established between parasitic load of *Eustrongylides* sp. and water quality parameters except DO and COD. The mean intensity was positively correlated with DO ($R^2 = 0.2527$; $p < 0.01$) and negatively with COD ($R^2 = 0.3736$; $p < 0.001$) (Fig. 4). This implies that the endoparasite harbours the internal environment which remains almost un-impacted or far less influenced

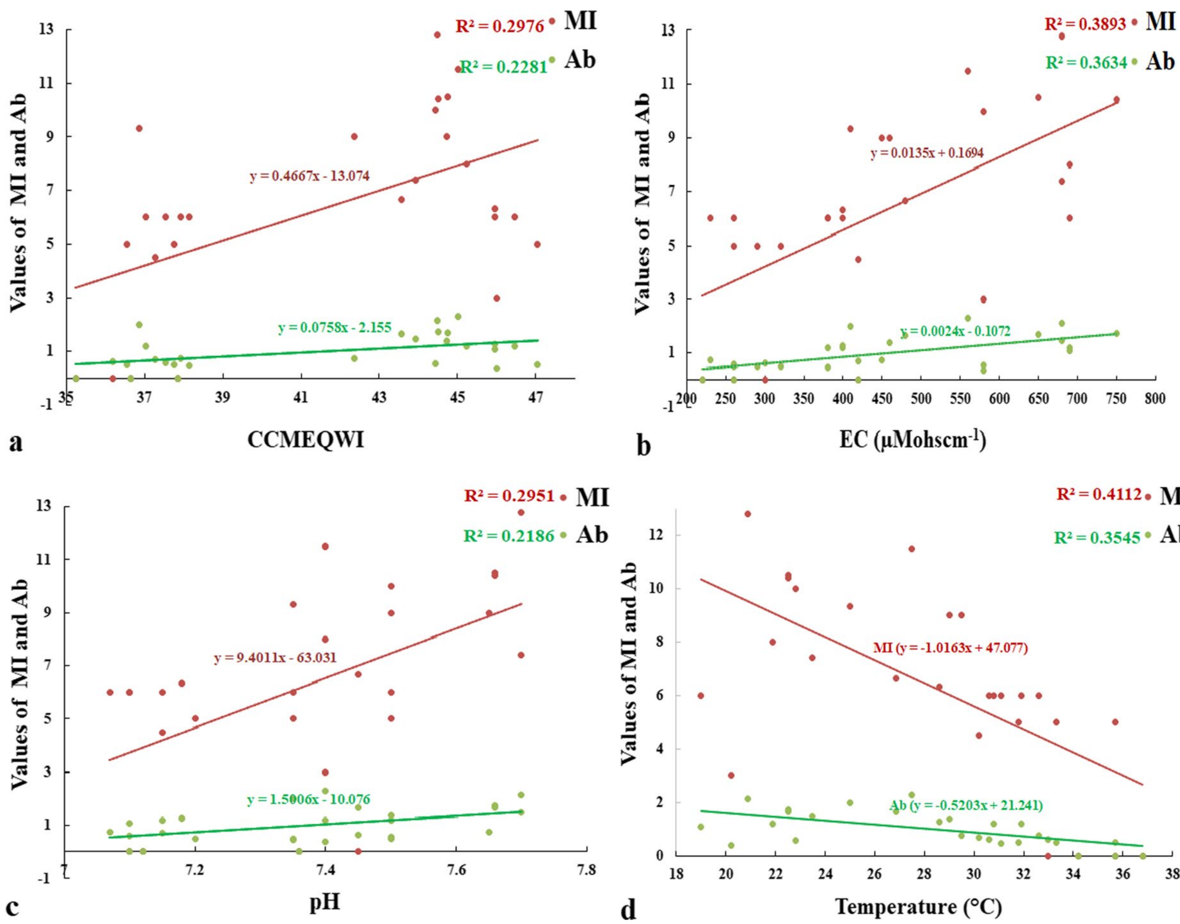


Fig. 3 Regression analysis (with equations and R^2 values) of water parameters and parasitic load [mean intensity (MI); and abundance (Ab)] of *Gyrodactylus* sp. (**a**) Positive correlation with CCMEWQI; **b** Positive correlation with EC; **c** Positive correlation with pH; **d** Negative correlation with temperature

(even indirectly) by majority of the external physical factors. Since DO and COD have significant impact on the health and physiological performance of the host fish the parasitic infectivity was shown to be correlated with them (Al-Hasawi, 2019).

PCA analysis of parasitic infestation, water parameters and WQIs

The PCA plot explained 63.4% (PC 1 = 42.8%, PC 2 = 20.6%) of the variation among water parameters, water quality indices and parasitic load. The comparison was based on Eigen value. The CCMEWQI, and all the indices of parasitic infestation are positively associated with both the PCs (PC 1 and PC 2), while COD is negatively associated with the variables. Temperature, free CO_2, BOD, DO, turbidity and WAWQI were negatively associated with PC 1 and positively with PC 2 while, EC, TDS, salinity, and pH were positively associated with PC 1 and negatively with PC 2 (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 of the ambient water (Blanar et al., 2009; Öktener & Bănăduc, 2023). 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.

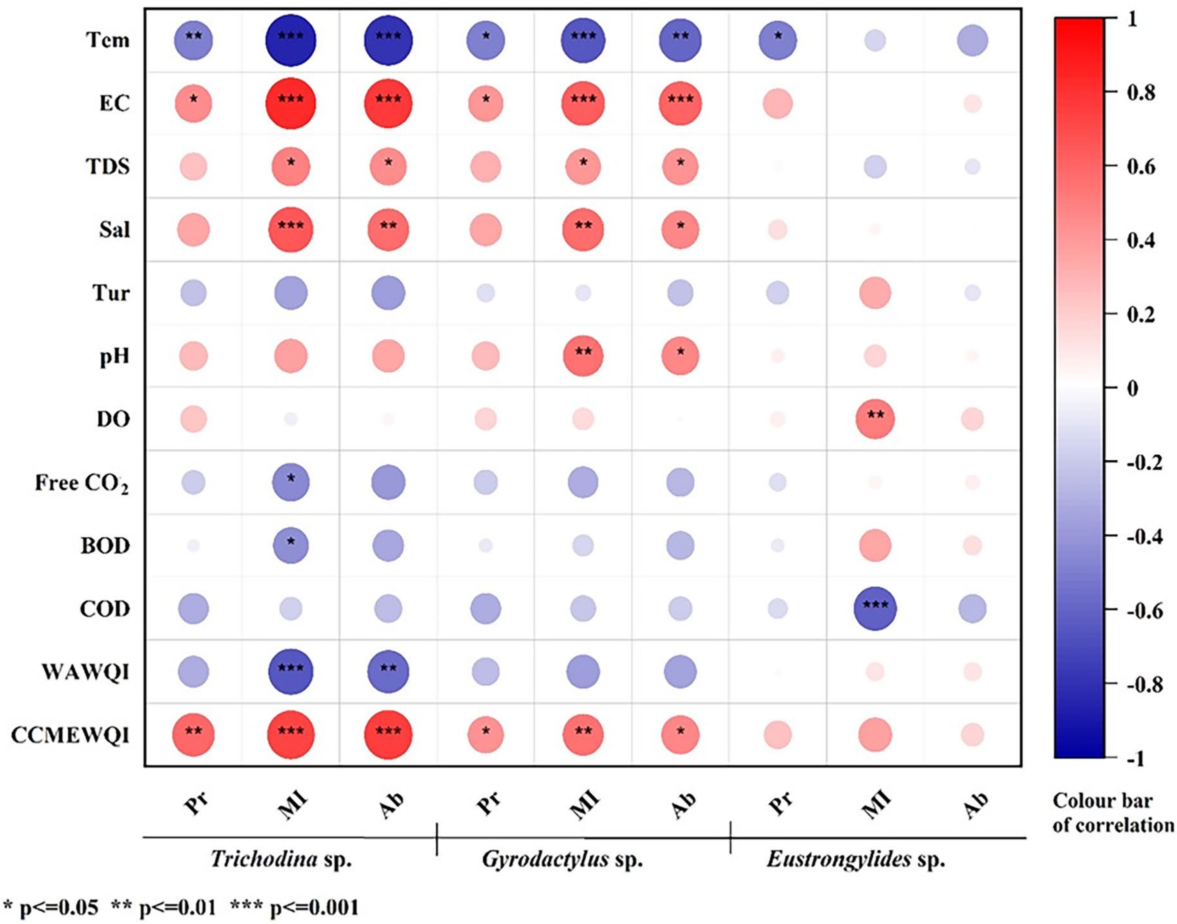


Fig. 4 Correlations among physicochemical parameters of water and parasitic load of three parasites (*Trichodina* sp., *Gyrodactylus* sp. and *Eustrongylides* sp.): Prevalence (Pr), mean intensity (MI) and abundance (Ab)

Potential of fish parasites in biomonitoring of water quality

The seasonal variation of ectoparasitic load depends on the physicochemical attributes of water. The ectoparasite trichodinids require a single host for completion of their life cycle, hence called 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 °C to 26 °C; hence winter appears as the most suitable season for their reproduction. Growth and development of parasite is inhibited by high temperature (Ghosh et al., 2021). The prevalence, mean intensity and abundance of *Trichodina* sp. were found to be the lowest in monsoon. The values increased in post-monsoon and reached the maxima in winter. Water temperature is one of the most essential factors for the life cycle, transmission, and microenvironment of monogenean parasite *Gyrodactylus* sp. (Rubio-Godoy et al., 2016; Urdes & Hangan, 2023). In monogenean ectoparasite, the time and duration of egg hatching are negatively associated with temperature, with an intermediate range (20 to 25 °C) of temperature offering optimum condition for egg hatching (Al-Hasawi, 2019; Zhang et al., 2021).

The EC of water is directly proportional to the concentration of dissolved minerals such as calcium, magnesium, etc., and the overall ionic composition

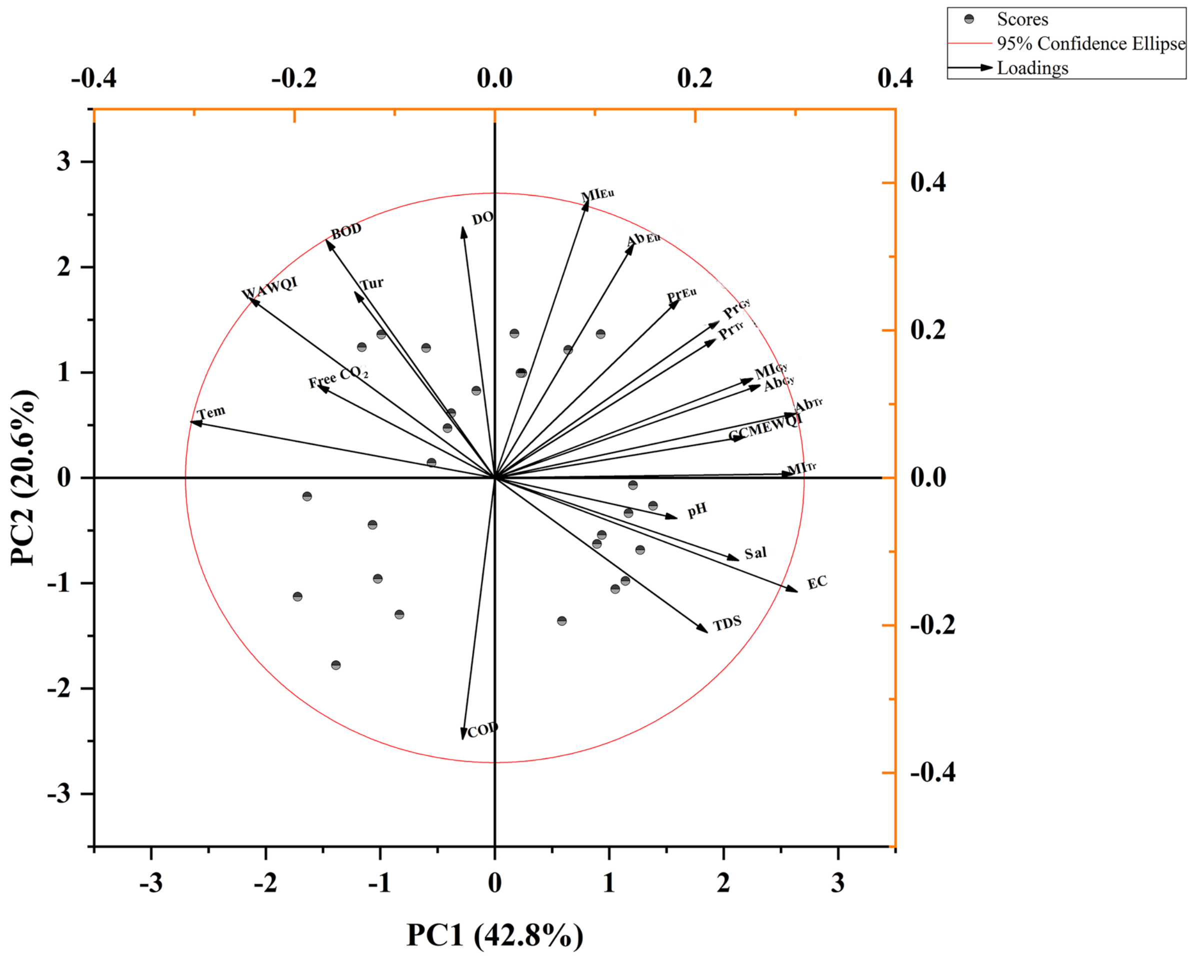
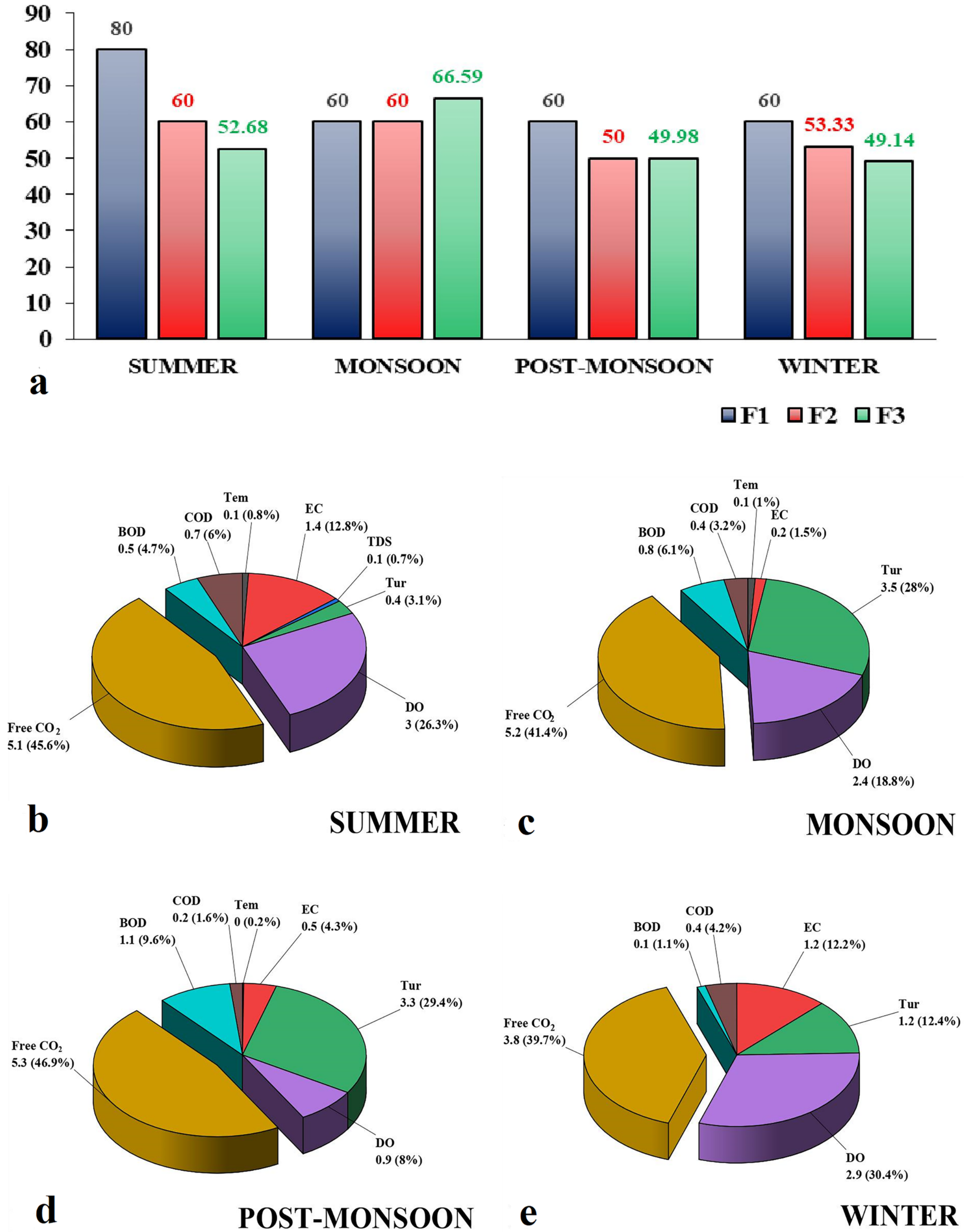


Fig. 5 Principal Components Analysis (PCA) of water parameters, pollution indices and parasitic load of three parasites (*Trichodina* sp. *Gyrodactylus* sp. and *Eustrongylides* sp.) of fish. Two principal components (PC 1 and PC 2) explained 63.3% of the total variation between water parameters, pollution indices and parasitic load. Prevalence, mean intensity and abundance of *Trichodina* sp. (Pr_{Tr}, MI_{Tr} and Ab_{Tr}); prevalence, mean intensity and abundance of *Gyrodactylus* sp. (Pr_{Gy}, MI_{Gy} and Ab_{Gy}); prevalence, mean intensity and abundance of *Eustrongylides* sp. (Pr_{Eu}, MI_{Eu} and Ab_{Eu})

of water is often associated with sewage discharges (Colin et al., 2016). The mean EC values showed distinct seasonal differences with the following order of variation: winter > post-monsoon > monsoon. All the parameters of parasitic load of both the ectoparasites (*Trichodina* sp. and *Gyrodactylus* sp.) were observed to be positively correlated with EC (Fig. 4). 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 (Maceda-Veiga 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 (Deswati et al., 2023; Ficke et al., 2007). 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 endoparasite adapt with hypoxic condition by adopting anaerobic respiration, which is one of the most important parasitic mode of adaptation (Harada et al., 2013; Martínez-González


90
80
70
60
50
40
30
20
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0
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60
52.68
60
60
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50
49.98
60
53.33
49.14
SUMMER
MONSOON
POST-MONSOON
WINTER
a
F1 F2 F3
Tem 0.1 (0.8%)
COD 0.7 (6%)
BOD 0.5 (4.7%)
EC 1.4 (12.8%)
TDS 0.1 (0.7%)
Tur 0.4 (3.1%)
DO 3 (26.3%)
Free CO_2 5.1 (45.6%)
b
SUMMER
Tem 0.1 (1%)
COD 0.4 (3.2%)
EC 0.2 (1.5%)
BOD 0.8 (6.1%)
Tur 3.5 (28%)
Free CO_2 5.2 (41.4%)
DO 2.4 (18.8%)
c
MONSOON
COD 0.2 (1.6%)
Tem 0 (0.2%)
EC 0.5 (4.3%)
BOD 1.1 (9.6%)
Tur 3.3 (29.4%)
Free CO_2 5.3 (46.9%)
DO 0.9 (8%)
d
POST-MONSOON
COD 0.4 (4.2%)
BOD 0.1 (1.1%)
EC 1.2 (12.2%)
Tur 1.2 (12.4%)
Free CO_2 3.8 (39.7%)
DO 2.9 (30.4%)
e
WINTER

◂**Fig. 6** Relative contribution of individual water quality parameters: **a** The determinants of CCMEWQI i.e. F1, F2 and F3 for all the seasons of polluted river. Seasonal comparison for the contribution of individual water quality parameters related to sum excursion of F3. **b** Summer; **c** Monsoon; **d** Post-monsoon; **e** Winter

et al., 2022). In polluted river the free CO_2 concentration was much higher 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 mgL^{-1}) (Coffin et al., 2018) in the polluted river. Under such adverse ambience the host fish (*Channa punctata*) 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. In the polluted river the value of CCMEWQI was lower during summer, which indicated higher pollution load and exhibited increasing trend in monsoon, post-monsoon followed by winter. CCMEWQI is positively correlated with the parasitic load of both *Trichodina* sp. and *Gyrodcatylus* sp. (Fig. 2c, 3a). A similar type of relation between parasitic infestation and WQI was revealed by PCA. Based on the calculated WQI, the polluted river reflected *prima facie* the best seasonal water quality during winter. This was contested and proved wrong by the relative seasonal contributions of the main physicochemical parameters calculated using the values of F1, F2, F3, and the sum excursion of F3. The relative contribution of individual water quality parameters for CCMEQWI was different in each season. The underlying determinants of WQI i.e. F1 and F2 show the highest values in summer and F3 in monsoon (Fig. 6a).

The relative contribution of free CO_2 is the maximum (40 to 47%) for excursion of CCMEWQI in all seasons. In summer the relative contributions for other parameters are as follows: DO (26.3%) > EC (12.8%) > COD (6%) > BOD (4.7%) > turbidity (3.1%) (Fig. 6b). The comparison between summer and other three seasons shows that the contribution of turbidity is much higher (28 to 30%) in monsoon and post-monsoon while DO level contributes for 8 to 18% only. On the other hand, the contribution pattern of individual water quality parameters in winter (DO = 30.4% > turbidity 12.4% > EC = 12.2% > COD = 4.2%) is nearly similar to that of summer (Fig. 6c, d).

The overall outcome of this analysis indicates that the WQI value of monsoon and post-monsoon is mainly associated with free CO_2 and turbidity while in winter DO, EC and COD also contribute besides these two parameters. Turbidity cannot be used as the primary indicator of water pollution. Although it may be used as an associate indicator to assess water quality along with other important water quality parameters (Kamboj & Kamboj, 2019). Therefore, the water quality of monsoon and post-monsoon was considered much better than winter throughout the study.

During summer host fish was not available hence, parasitic load remained indeterminate. During monsoon and post-monsoon, the quantity of water increased and level of water pollution decreased as a result of rainfall and influx of water from the river Ganga. The load of both the ectoparasites (*Trichodina* sp. and *Gyrodcatylus* sp.) was lower in monsoon followed by a remarkable rise in post-monsoon and winter. *Eustrongylides* sp. is a heteroxenic endoparasite of fish and wading bird (*Casmerodius albus*, *Egretta thula*, and *Ardea herodias*) (Honcharov et al., 2022). Prevalence, mean intensity, and abundance of *Eustrongylides* sp. were not affected by seasonal change and not correlated with most of the physicochemical parameters of water. Both prevalence and efficiency of intermediate hosts play an important role in the transmission of endoparasite (Hanzelova & Gerdeaux, 2003). The prevalence, mean intensity and abundance of endoparasite depend on its life cycle, physicochemical parameters and quality of water (Adamba et al., 2020). It also depends on intermediate host where life cycle of parasite needs more than one host. Life cycle of *Eustrongylides* sp. requires three hosts. Fish acts as the second intermediate host, where encapsulated 3rd stage larva (L3) is transformed into 4th stage larva (L4) (Gupta, 2019).

Parasitism is a common phenomenon, and most of the living organisms on earth survive with the parasite. It is a hetero-specific relationship in which one (parasite) gets benefits at the cost of the other (host). To ensure long-term benefits, parasites harm the host but do not kill them. It may be considered as an ecological rule. Host-parasite interaction is one kind of reciprocal adaptation that allows survival and transmission of parasites for maintenance of suitable parasitic population. Many researchers attempted to understand the role of parasite in the ecosystem (Buck, 2019; Vannatta & Minchella, 2018). There are two different schools of thought concerning the importance of parasites in fish ecology. Fish biologists or ecologists ignore parasitism or parasite may be negligible in their studies (Lagrue & Poulin, 2015; Timi et al., 2020). On the other hand, environmental parasitologists think that parasitism is a potential relationship that delivers valuable ecological information hence, parasites must be considered as a potent bioindicator for environmental health (Guagliardo et al., 2019; Ridall & Ingels, 2021). In fish parasites, the effect of water pollution may be positive or negative (Sures, 2008). In most circumstances, negative effects happen when parasites accumulate pollutants. Sometime parasites accumulate more pollutants than host fish unknowingly in the form of accumulation indicators (Abdel-Gaber et al., 2017; Morales-Serna et al., 2019). The population size of the parasite is reduced by the negative effect of pollution. On the other hand, when water becomes polluted fish health deteriorates. Metabolism, respiration, excretion, immunity and behaviour of fish are affected by the synergetic effects of physicochemical attributes of water (Zeitoun & Mehana, 2014). Therefore, host fish becomes more vulnerable to be affected by the parasites losing their ability to escape from parasitism. The population size of the parasite is increased by the positive effect of pollution (Maceda-Veiga et al., 2019). In this study the severely degraded water quality exerts positive effect on the population of *Trichodina* sp. and *Gyrodactylus* sp. offering them an adaptive advantage for increasing the population size. The parasitic load of both the parasites was much higher in post-monsoon and winter because all the physicochemical properties of water were optimum for their growth, reproduction, transmission and life cycle. It is consistent with the findings of Suliman and Al-Harbi (2016) which showed that poor water quality characterized by inadequate water exchange, low dissolved oxygen level, high concentrations of ammonium-N, nitrite-N, and conductivity was responsible for the intensified parasitic infection. Ojwala et al (2018) also demonstrated strong influence of physicochemical regime comprising water temperature, conductivity, turbidity, pH, DO, and ammonia on fish health and body's defense against parasites and pathogens as reflected in the parasitic load and assemblage encountered in the host fish. Prevalence, mean intensity and abundance of *Trichodina* sp. were much higher compared to *Gyrodactylus* sp. and *Eustrongylides* sp. throughout the period of study (Table 3). Due to synergetic effects of water quality parameters significant seasonal variation was observed in both the ecoparasites (*Trichodina* sp. and *Gyrodactylus* sp.) while the population of endoparasite (*Eustrongylides* sp.) was not much affected by water quality and seasonal changes. Both ecto- and endoparasites regularly produce certain molecular signals in response to changes in their environmental milieu (Asif et al., 2018; Sures et al., 2023). Ectoparasites are important tools for providing wealth of information on physicochemical quality, environmental stressors, trophic interactions, population structure, biodiversity, etc. Environmental degradation influences the occurrence and intensity of fish parasites; thus, they may serve as sensitive living probes to monitor environmental factors and ecological status of the water body (Deflem et al., 2022). Parasitic load of endoparasites depends not only on the external environment but also on their life cycle stages, intermediate host and the definitive host; all the hosts serve as a microenvironment for endoparasites (Al-Hasawi, 2019). Therefore, endoparasites are considered as an accumulator and effect indicator for different levels (cellular to ecological) of organisation (Sures et al., 2017). For understanding the effects of fish parasite on their microenvironment (host body) haematological, serum biochemical, and histopathological studies are much necessary.

Conclusions

The results show that the river Saraswati in under severe stress of environmental quality degradation due to diverse anthropogenic activities in general and organic loading in particular. From the WQI scores, it was evident that the river is much polluted, with eight out of ten parameters monitored i.e. DO, BOD, COD, free CO_2, turbidity, EC, TDS, and temperature exceeding respective standard values. The parasitic load was found to be a function of a suite of related physicochemical factors comprising temperature, EC, free CO_2, BOD, COD, and DO. The parasitic load was increased under degraded water quality and low temperature regime. Parasite growth, development, and life cycle are aided by optimal temperature and high EC. Persistent high concentrations of free CO_2, BOD, COD, and low concentrations of DO cause deterioration of fish health, resulting in a debilitated immune system and more vulnerability to parasitic infection. Both protozoan and metazoan parasites are used as bioindicators for aquatic health, but they react differently depending on their needs and the hosts they use during their life cycle. The parasitic load of the ciliate *Trichodina* sp. and the monogenean *Gyrodactylus* sp. was directly influenced by the physico-chemical stressors operating in the ambient system. Contrarily, the population of endoparasite *Eustrongylides* sp. remained unaffected by ambient water quality for two reasons: (i) they are comparatively less exposed to environmental stressors; (ii) their life cycle depends on other alternative hosts. The present study establishes parasitic monitoring as a novel bio-monitoring tool for environmental quality management of the aquatic system, using parasitic load indicators (e.g. prevalence, mean intensity, abundance). This is indeed a step forward to establish such a non-conventional biological indicator beyond the oft-used traditional structural bioindicators (e.g. phytoplanktons cell size and biomass; zooplanktons body size and biomass; zooplanktons to phytoplanktons ratio; species diversity, etc.) and functional bio-indicators (e.g. algal carbon assimilation ratio, resource use efficiency, community production, gross production to respiration ratio, gross production to standing biomass ratio, etc.) or the conventional sensitive-tolerant bioindicator categories. Fish parasites, when combined with other indicator measures, will allow for a more accurate assessment of the causes and consequences of environmental changes in the aquatic habitats. Several other factors such as seasonal variations, size, genetic attributes, sex, population density, lack of food supply need to be considered to earn a better insight into the pollution and related environmental stressor-induced alterations in fish as an indicator of fish health.

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SP: Formal investigation; methodology; data collection, curation and statistical analysis; manuscript writing—original draft preparation; resources.

MK: Manuscript writing—original draft preparation; manuscript writing—review and editing.

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Data availability Available upon reasonable request.

Declarations

Ethical approval All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors as found in the Instructions for Authors and are aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.

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