

Coupling Water-Quality Gradients with Cestode Epidemiology in *Mastacembelus armatus*: A Two-Year Spatiotemporal Assessment of the Western Yamuna Canal, India

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Highlights

- Across 480 fish examined over 24 months, cestode prevalence was 36.9% (177/480) and the pooled mean intensity was 1.65 worms per infected fish.
- After adjustment for season and year, females had 2.24-fold higher odds of infection than males, while summer fish had 3.57-fold higher odds than monsoon fish.
- A two-harmonic cosinor model identified a reproducible bimodal annual cycle, with a primary April peak and a secondary October–November peak.
- Three rotated water-quality factors explained 69.7% of variance; the dominant factor represented organic loading/oxygen demand and was highest at Site II, particularly in summer.
- Lower dissolved oxygen in the preceding month was associated with higher mean parasite intensity (Spearman $\rho = -0.59$, FDR $q = 0.031$), whereas the broader water-quality PC1 did not remain independent of season.

Abstract

Freshwater parasite dynamics may reflect simultaneous changes in host biology, season and environmental quality, yet these domains are often analysed separately. This study integrated two consecutive annual cycles (July 2024–June 2026) of physicochemical monitoring of the Western Yamuna Canal, Haryana, India, with monthly cestode records from *Mastacembelus armatus*. Water was sampled twice monthly at three canal sites (144 site-date observations; $n = 143$ for nitrate because of one missing value), and 480 fish were examined over 24 months. Ten physicochemical variables were evaluated using factorial linear models, non-parametric confirmation, correlation analysis, weighted arithmetic water-quality indexing, hierarchical clustering and principal component analysis (PCA) with Kaiser–Meyer–Olkin and Bartlett diagnostics. Parasite prevalence, mean intensity and mean abundance were evaluated by chi-square procedures, binomial and Poisson generalized linear models, host-size models and harmonic (cosinor) regression. Water–parasite associations were assessed with concurrent and one-month-lagged Spearman correlations with Benjamini–Hochberg false-discovery-rate control and with quasi-binomial models based on monthly water-quality principal components. Overall cestode prevalence was 36.9% (177/480), with 292 cestodes recovered and a mean intensity of 1.65. Females had greater adjusted odds of infection than males (OR = 2.24, 95% CI 1.51–3.33), and summer odds were higher than monsoon odds (OR = 3.57, 95% CI 2.20–5.80). Summer mean intensity was 1.52 times the monsoon value ($p = 0.009$). A two-harmonic model strongly improved fit and identified a primary April peak and a secondary October–November peak; monthly prevalence profiles were highly reproducible between years ($r = 0.97$). Season significantly affected all ten water variables, while site affected nine of ten. Three rotated components explained 69.7% of water-quality variance: an organic-loading/oxygen-demand factor, a mineral/ionic factor and a climatic/thermal factor. Weighted arithmetic WQI values were predominantly in the “Poor” category and were highest in summer. Same-month single-parameter correlations with prevalence did not survive FDR correction. However, dissolved oxygen in the preceding month was inversely associated with mean parasite intensity ($\rho = -0.59$, $q = 0.031$). A monthly organic-loading/low-oxygen PC showed only borderline association with prevalence after overdispersion correction (OR = 1.16, $p = 0.067$) and no independent contribution after season was included ($p = 0.888$). The findings therefore support a reproducible host–season pattern and a temporally coupled host–environment signal, but not a demonstrated causal effect of water-quality deterioration on infection.

Keywords: *Mastacembelus armatus*; cestode; Western Yamuna Canal; dissolved oxygen; seasonality; principal component analysis; water quality index; environmental parasitology.

1. Introduction

Freshwater ecosystems integrate hydrological variation with human pressures such as urban wastewater, agricultural runoff, industrial discharge and land-use change. These pressures alter dissolved oxygen, dissolved solids, ionic composition and nutrient availability, with consequences for ecosystem functioning and fish health (Baron et al., 2002; Priya et al., 2023; Edegbene et al., 2024; Zhang et al., 2025). In regulated systems such as canals, the signal can be particularly complex because flow management and seasonal dilution operate together with local inputs. Studies from Indian rivers and canals have repeatedly shown strong seasonal and spatial heterogeneity in physicochemical conditions (Alkhadher et al., 2025; Kumari & Awasthi, 2025; Saha et al., 2022; Sruthi et al., 2023).

The Western Yamuna Canal (WYC) is important for irrigation, domestic supply and regional economic activity in north India. Earlier work in and around Yamuna Nagar has reported spatial differences in dissolved oxygen, dissolved solids, conductivity and other water-quality variables in reaches affected by urban and industrial activity (Bhatnagar et al., 2009; Hassan et al., 2017; Jaiswal et al., 2019; Sharma et al., 2024). Industrial effluents, agricultural runoff and domestic inputs can also alter fish physiology and habitat quality (Kaur & Bbraich, 2022; Mishra & Kumar, 2021; Suthar et al., 2009). Recent multivariate hydrochemical studies further show that correlated variables are often better interpreted as environmental gradients than as isolated measurements (Benchamin et al., 2024; Songara et al., 2025).

Fish parasites are components of aquatic food webs as well as potential indicators of ecological change. Their occurrence can respond to contaminant exposure, water quality, temperature, hydrology, host immunity and the availability of intermediate hosts, although responses are strongly parasite- and host-specific (Brázová et al., 2021; Gilbert & Avenant-Oldewage, 2021; Jerônimo et al., 2022; Sures et al., 2023). Field studies have reported associations between parasite assemblages or infestation and physicochemical gradients in freshwater fishes (Adamba et al., 2020; Ageng'o et al., 2024; Dumbo et al., 2020; El-Khayat et al., 2024; Latief et al., 2024). These associations do not necessarily imply that deteriorating water quality increases infection: pollution may suppress parasite stages or intermediate hosts in some systems and facilitate infection in others.

Seasonality is therefore a central confounder and biological driver. A meta-analysis showed that aquatic parasite infections commonly exhibit seasonal structure, but the direction and timing of peaks vary among taxa and environments (Poulin, 2020). Temperature, flow, host feeding, immunity and intermediate-host dynamics may all contribute (Löhmus & Björklund, 2015; Lymbery et al., 2020; Scharsack & Franke, 2022). In *M. armatus*, previous work has also reported season-, sex- and size-related variation in cestode infection (Vankara & Vijayalakshmi, 2015). Consequently, an apparent water–parasite association can arise simply because both water chemistry and parasite prevalence change in the same months.

The present study was designed to integrate two complete annual cycles of water-quality and fish-parasite observations from the WYC. The objectives were to (i) quantify seasonal, annual and spatial variation in canal physicochemistry; (ii) identify multivariate water-quality processes and WQI patterns; (iii) characterize annual, seasonal, sex- and host-size patterns of cestode infection in *M. armatus*; (iv) model periodicity in monthly prevalence; and (v) determine whether concurrent or preceding water conditions were associated with parasite prevalence or burden after accounting for multiple testing and seasonality. The analytical emphasis was on effect sizes, reproducibility and multivariate structure rather than on isolated p-values. Because the design is observational, environmental associations were interpreted as associations rather than evidence of causation.

2. Materials and methods

2.1 Study area and sampling design

The study covered two consecutive annual cycles, July 2024–June 2025 and July 2025–June 2026, along the Western Yamuna Canal in Yamuna Nagar district, Haryana, India. Three representative sites were monitored: Site I at Old Saharanpur Road Bridge, Site II at the Railway Bridge and Site III near Old Hamida Barrage. Water was sampled twice monthly (fortnightly) at each site, giving 3 sites × 2 sampling occasions × 24 months = 144 site-date observations. One nitrate measurement was missing for Site III in June 2026, so nitrate analyses

used $n = 143$ available observations. Seasons were defined a priori as monsoon (July–October), winter (November–February) and summer (March–June). Fish records were monthly rather than site-resolved; therefore, site-specific parasite prevalence could not be tested.

2.2 Physicochemical analysis and water-quality indexing

Water temperature, pH, total dissolved solids (TDS) and electrical conductivity (EC) were measured in the field using standard meters. Dissolved oxygen (DO) was fixed immediately and determined by the modified Winkler iodometric method. Total alkalinity, total hardness, chloride, free carbon dioxide (free CO_2) and nitrate were determined by standard titrimetric or volumetric procedures following standard water-analysis practice (American Public Health Association, 2017). The same set of ten variables was retained across both annual cycles.

For contextual screening, observations were compared with the acceptable ranges or limits used in the analysis workbook, including BIS IS 10500:2012 for drinking-water parameters and CPCB designated-best-use thresholds for aquatic life where relevant (Bureau of Indian Standards, 2012). A weighted arithmetic water quality index (WQI) was calculated from pH, TDS, EC, DO, alkalinity, hardness, chloride and nitrate. The index was used as a comparative multivariable screening summary rather than as a substitute for parameter-specific regulatory interpretation.

2.3 Fish examination and parasitological indices

Twenty *M. armatus* were examined each month (240 fish per annual cycle; 480 fish overall). Monthly numbers examined, numbers infected and total cestodes recovered were recorded, with male and female counts available separately. Prevalence was calculated as the proportion of examined fish infected; mean intensity was the number of parasites divided by the number of infected fish; and mean abundance was the number of parasites divided by the number of fish examined (Bush et al., 1997; Rózsa et al., 2000). Wilson 95% confidence intervals were calculated for prevalence. Body-length and body-weight class summaries from the source records were analysed as grouped host-trait data. The 2025–26 body-weight table contained 93 infected fish across classes whereas the monthly records contained 94; therefore, the 2025–26 weight-class inferential result was treated as provisional and was not used as a primary conclusion.

2.4 Data audit and statistical analysis

Physicochemical variables were summarized by year, season and site as mean $\pm$ standard deviation, standard error, range and coefficient of variation. Spatial and temporal effects were tested using Type II linear models containing year, season, site and the season $\times$ site interaction. Partial eta-squared (η^2) was used as an effect-size measure. Residual normality and variance homogeneity were assessed with Shapiro–Wilk and Levene tests; Kruskal–Wallis tests were used as non-parametric confirmation where model assumptions were imperfect. Tukey HSD was used for planned pairwise comparisons. Pearson correlations described relationships among water variables.

PCA was applied to z-standardized physicochemical observations after checking sampling adequacy with the Kaiser–Meyer–Olkin statistic and Bartlett’s test of sphericity. Components with eigenvalues >1 were retained and subjected to varimax rotation for interpretation (Jolliffe & Cadima, 2016). Agglomerative hierarchical clustering used Ward linkage and Euclidean distances on standardized monthly means; dendrogram fidelity was summarized by the cophenetic correlation coefficient.

Associations of infection with year, sex, season, month and host size/weight class were tested with chi-square procedures and Cramér’s V. The sex effect across years was summarized by a Mantel–Haenszel odds ratio and a Breslow–Day homogeneity test. Multivariable infection risk was estimated with a binomial logistic regression using month $\times$ sex cells and predictors sex, season and year; adjusted odds ratios (OR) with 95% confidence intervals were reported. Parasite counts among infected hosts were analysed with a Poisson generalized linear model using $\log(\text{number infected})$ as an offset, so exponentiated coefficients represent mean-intensity ratios.

Seasonal periodicity in prevalence was modelled with binomial harmonic (cosinor) regression containing annual and semi-annual sine–cosine terms. Nested models were compared by likelihood-ratio tests and Akaike information criterion (AIC), and peak timing was derived from the fitted curve (Cornelissen, 2014). Reproducibility between annual cycles was assessed from paired monthly profiles.

Water–parasite links were explored with Spearman rank correlations between site-averaged monthly water variables and prevalence, mean intensity and mean abundance, both concurrently (lag 0) and using the preceding month’s water values (lag 1). Benjamini–Hochberg false-discovery-rate (FDR) correction was applied within each outcome × lag family of ten tests (Benjamini & Hochberg, 1995). To reduce multicollinearity, PCA was also re-computed on the 24 site-averaged monthly water profiles. Monthly prevalence was then modelled with binomial GLMs using water principal components and year. Because the ordinary binomial model showed overdispersion (Pearson dispersion = 2.34), the primary environmental model used quasi-binomial standard-error scaling. A sensitivity model excluded months containing unverified exact duplicate blocks identified during source-data audit, and a final model added season to test whether water PC1 provided information beyond the shared seasonal pattern. All tests were two-sided with $\alpha = 0.05$. Analyses were generated in Python 3.12 using SciPy and statsmodels, with supporting calculations and data checks in Microsoft Excel.

Table 1. Study design and analytical framework.

Element	Value	Detail
Study period	July 2024–June 2026	Two complete annual cycles
Water sampling	144 site-date observations	3 sites × 2 fortnightly samples × 24 months; nitrate n = 143
Fish examined	480	20 fish month ⁻¹ ; 240 per annual cycle
Water variables	10	Temperature, pH, TDS, EC, DO, alkalinity, hardness, chloride, free CO ₂ , nitrate
Parasite outcomes	3	Prevalence, mean intensity and mean abundance
Multivariate methods	PCA, clustering, WQI	Sample-level structure and monthly environmental gradients
Multiplicity control	Benjamini–Hochberg FDR	Applied to water–parasite correlation families

3. Results

3.1 Seasonal and spatial structure of water quality

Water chemistry showed strong and repeatable seasonal structure across the two annual cycles (Figure 1). In the factorial models, season significantly affected all ten measured variables. Seasonal effect sizes were large for temperature ($\eta p^2 = 0.642$), nitrate (0.562), TDS (0.395), DO (0.337), EC (0.314), free CO₂ (0.280) and alkalinity (0.273), and were also substantial for hardness, pH and chloride. Site affected nine of ten variables; temperature was the only parameter without a site effect. The season × site interaction was non-significant for every variable, indicating that the spatial ranking was broadly persistent across seasons. Year effects were limited primarily to alkalinity, free CO₂ and nitrate.

Site II consistently showed the pattern expected for the most impacted reach: lower DO together with higher TDS, EC, alkalinity, hardness, free CO₂ and nitrate. For example, pooled DO means were approximately 6.36, 5.57 and 6.17 mg/L at Sites I, II and III, respectively, whereas pooled EC means were about 283, 326 and 298 μ S/cm. In summer, mean DO fell to approximately 4.57 mg/L at Site II. The distributional plots confirmed strong seasonal shifts and persistent site differences for most chemical variables (Figure 2). Non-parametric tests reproduced the principal season and site conclusions, reducing concern that the findings were solely consequences of imperfect normality or unequal variances.

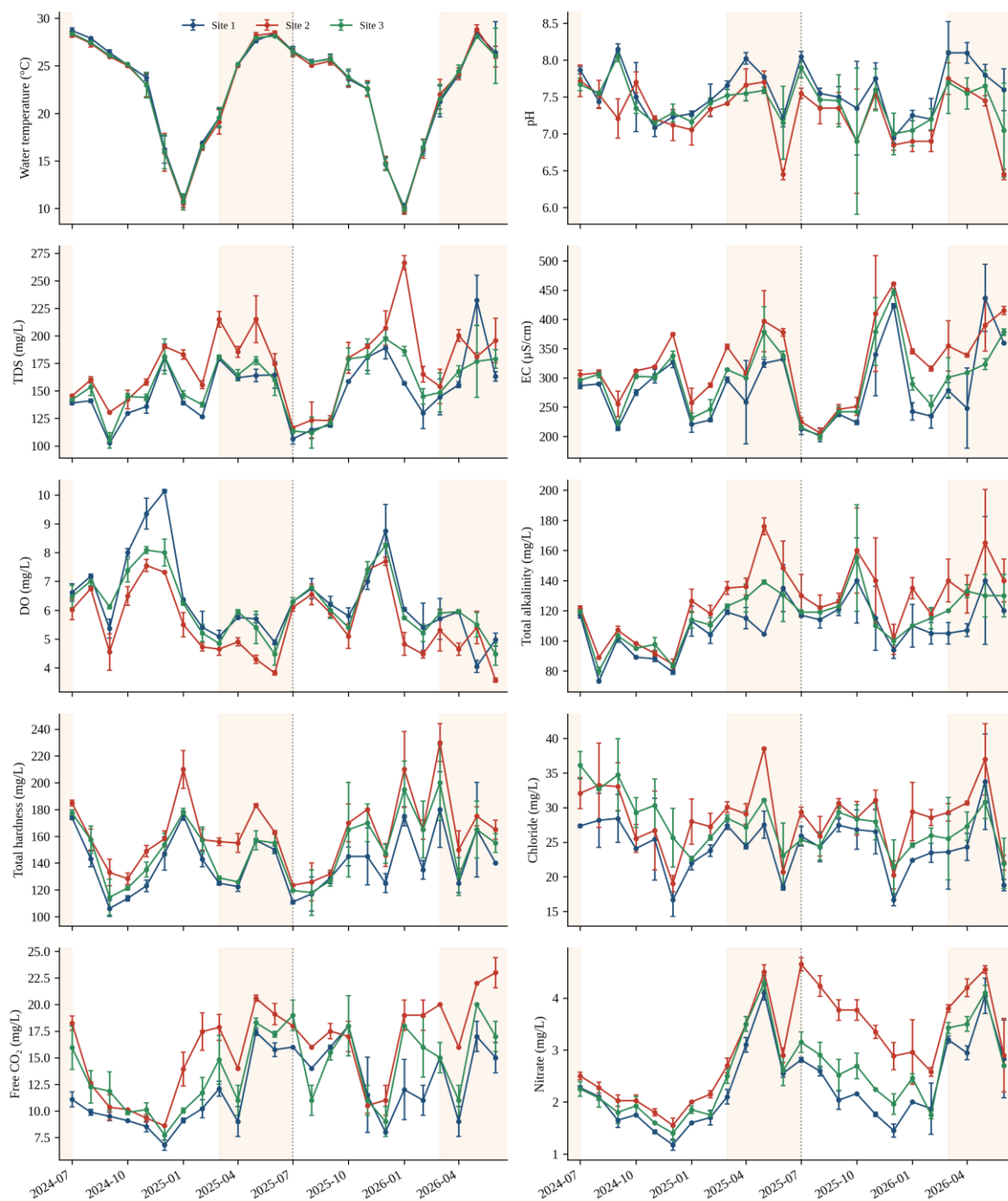


Figure 1. Two-year monthly trajectories of the ten physicochemical water-quality variables at Sites I–III of the Western Yamuna Canal. Vertical separation and recurrent peaks/troughs illustrate strong seasonal structure and persistent local differences.

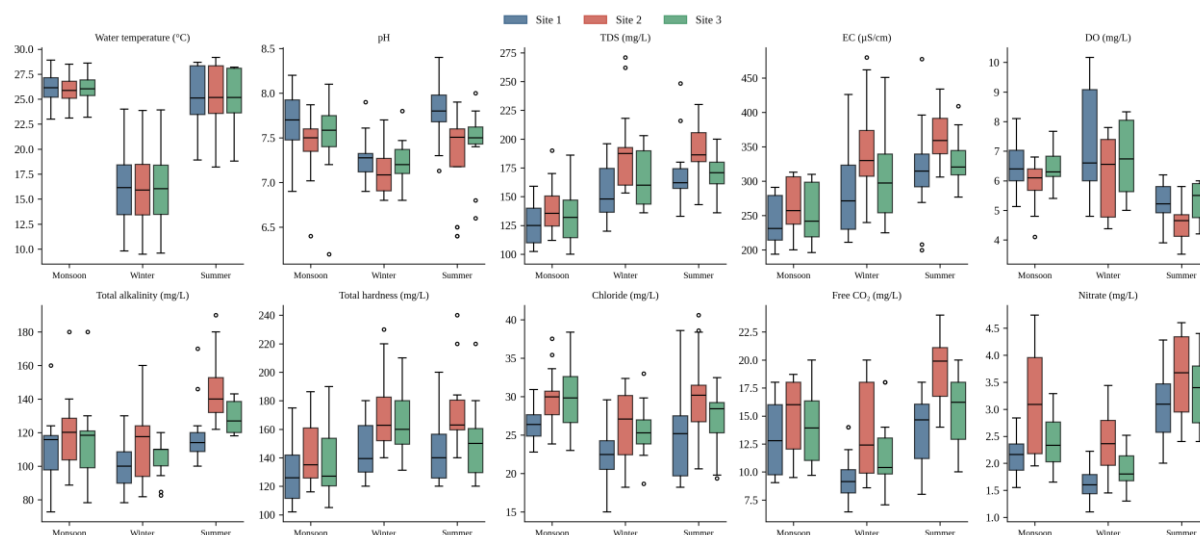


Figure 2. Distributions of physicochemical parameters by season and sampling site. Site II generally exhibits lower dissolved oxygen and higher ionic/organic-loading indicators than Sites I and III.

Table 2. Type II factorial models for water-quality variables. p-values are shown for year, season and site; η^2 values summarize the magnitude of season and site effects.

Variable	Year p	Season p	Site p	Season η^2	Site η^2	Season×site sig.
Temperature	0.165	<0.001	0.983	0.642	<0.001	No
pH	0.369	<0.001	<0.001	0.167	0.112	No
TDS	0.164	<0.001	<0.001	0.395	0.145	No
EC	0.555	<0.001	<0.001	0.314	0.099	No
DO	0.162	<0.001	<0.001	0.337	0.102	No
Alkalinity	<0.001	<0.001	<0.001	0.273	0.152	No
Hardness	0.321	<0.001	<0.001	0.159	0.134	No
Chloride	0.192	<0.001	<0.001	0.142	0.145	No
Free CO ₂	<0.001	<0.001	<0.001	0.280	0.215	No
Nitrate	<0.001	<0.001	<0.001	0.562	0.284	No

3.2 Correlation structure, multivariate gradients, clustering and WQI

The water variables were strongly intercorrelated (Figure 3). The strongest positive relationships included TDS–EC ($r = 0.73$), free CO₂–alkalinity ($r = 0.69$), free CO₂–nitrate ($r = 0.62$) and alkalinity–nitrate ($r = 0.62$), whereas DO was inversely related to free CO₂ ($r = -0.65$), alkalinity ($r = -0.62$) and nitrate ($r = -0.45$). This correlation structure justified multivariate dimension reduction rather than fitting many collinear environmental predictors separately.

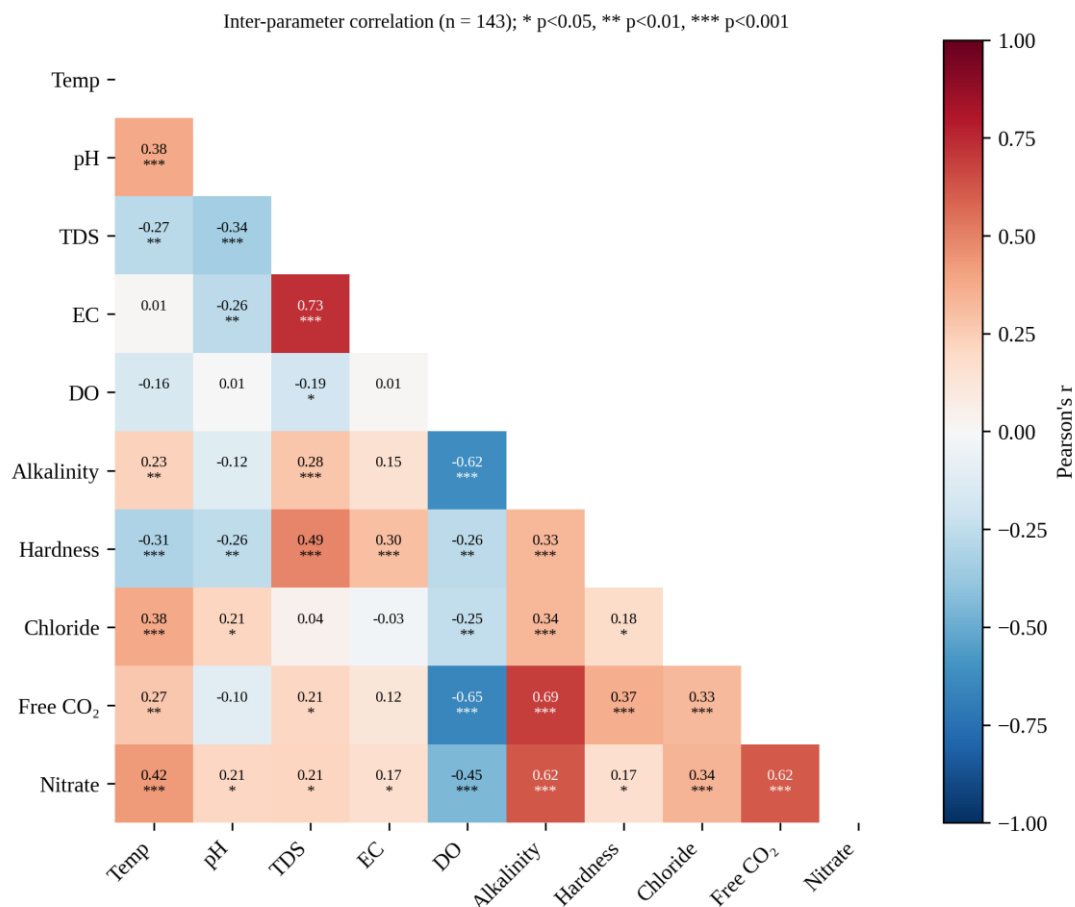


Figure 3. Pearson correlation matrix among physicochemical water-quality variables (n = 143 complete observations). The DO–free CO₂–alkalinity–nitrate block indicates a coordinated oxygen-demand/organic-loading gradient.

PCA diagnostics supported factor extraction (KMO = 0.679; Bartlett $\chi^2 = 645.93$, df = 45, $p < 0.001$). Three components had eigenvalues >1 and together explained 69.7% of total standardized variance. After varimax rotation, VF1 explained 30.9% and represented an organic-loading/oxygen-demand axis, with strong positive loadings for free CO₂ and alkalinity, a positive nitrate loading and a strong negative DO loading. VF2 explained 20.0% and represented a dissolved-mineral/ionic axis dominated by TDS and EC. VF3 explained 18.8% and represented a climatic/thermal axis dominated by temperature and, to a lesser degree, pH. Site II had the highest mean PC1 score in every season and the highest overall summer score, identifying it as the strongest organic-loading/low-oxygen hotspot within the monitored reach (Figure 4).

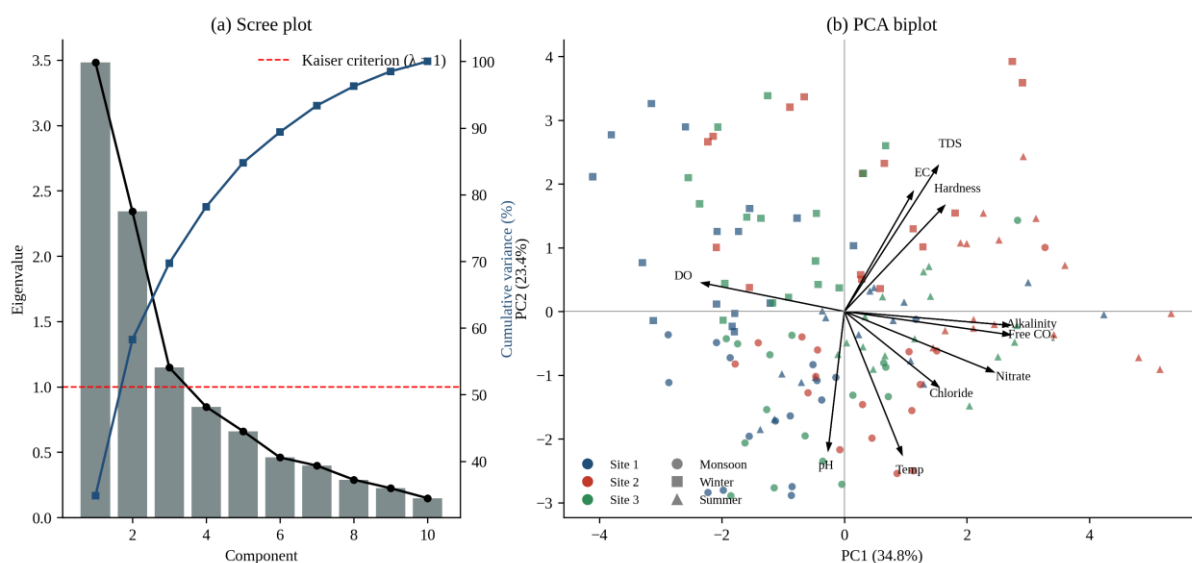


Figure 4. Principal component analysis of standardized water-quality observations. (a) Scree plot and cumulative explained variance; three components exceeded the Kaiser eigenvalue >1 criterion. (b) PCA biplot showing separation by site and season.

Table 3. Varimax-rotated loadings of the three retained water-quality factors. Values with |loading| ≥ 0.65 are the dominant contributors.

Variable	VF1: organic/oxygen demand	VF2: mineral/ionic	VF3: climatic/thermal
Temperature	0.194	-0.072	0.847
pH	-0.105	-0.279	0.691
TDS	0.203	0.872	-0.227
EC	-0.021	0.944	0.048
DO	-0.833	0.065	0.021
Alkalinity	0.850	0.159	0.078
Hardness	0.462	0.424	-0.409
Chloride	0.421	0.051	0.468
Free CO ₂	0.875	0.100	0.082
Nitrate	0.656	0.221	0.476
Variance explained	30.9%	20.0%	18.8%

Hierarchical clustering of the 24 monthly site-averaged profiles produced three broad hydrochemical groups (cophenetic correlation = 0.626; Figure 5). Cluster membership did not map perfectly onto calendar seasons, indicating that flow regulation and hydrological transitions also structured water quality. The warm, higher-alkalinity/higher-nitrate and lower-oxygen months were concentrated mainly in one cluster, whereas cooler, better-oxygenated months formed a contrasting group.

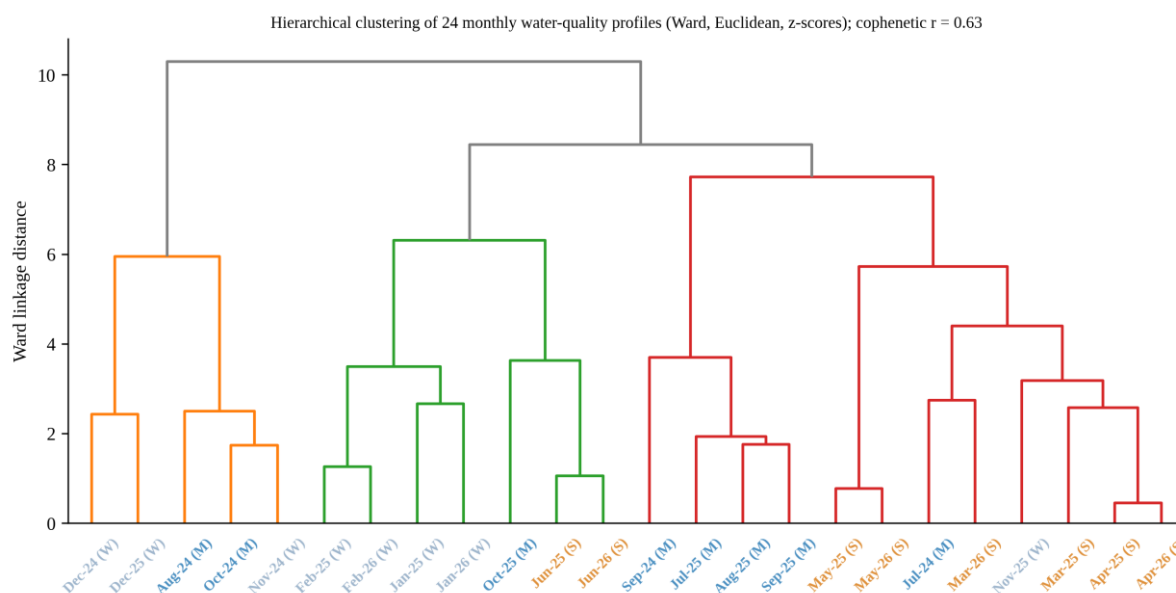


Figure 5. Hierarchical clustering of 24 monthly site-averaged water-quality profiles using Ward linkage and Euclidean distance after z-standardization. The three-cluster solution separates broad hydrochemical regimes rather than simply reproducing the calendar seasons.

The 72 site-month WQI values were predominantly classified as Poor: 57/72 (79.2%) were Poor, 9/72 (12.5%) Good and 6/72 (8.3%) Very poor. Mean WQI was 70.61 in summer, 63.19 in monsoon and 53.45 in winter. Season strongly influenced WQI ($p < 0.001$), whereas site ($p = 0.661$) and year ($p = 0.811$) did not. The index should be interpreted together with the component variables because its pattern was strongly influenced by DO. Parameter-specific screening showed that approximately 21.5% of all DO observations were below 5 mg/L, rising to nearly half of summer observations, whereas TDS, alkalinity, chloride and nitrate did not exceed the BIS acceptable limits used in the workbook (Figure 6).

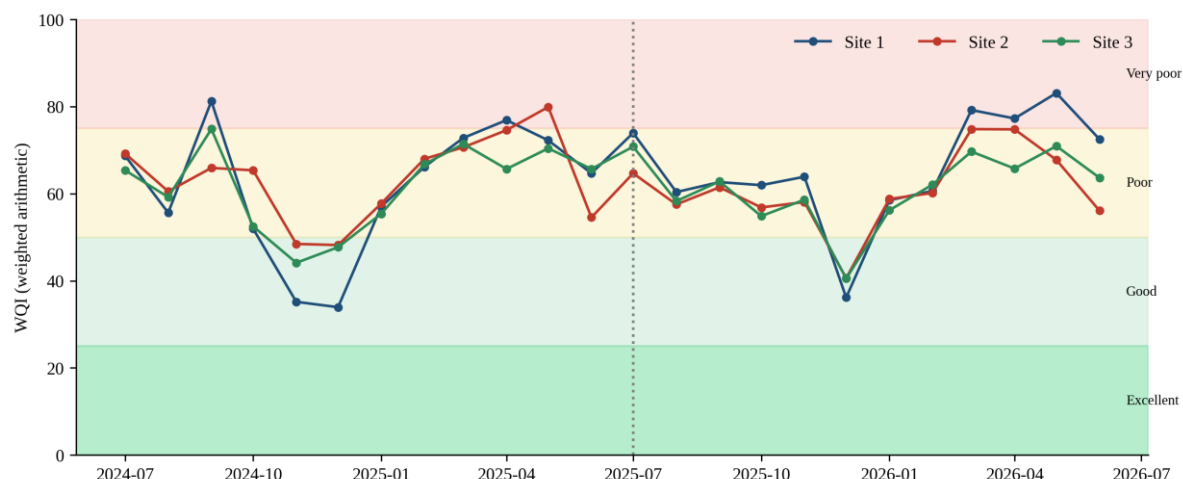


Figure 6. Monthly weighted arithmetic Water Quality Index (WQI) at the three sampling sites. Background bands show the index categories used in the statistical workbook; the site-month series is predominantly in the Poor range and peaks mainly during summer.

3.3 Cestode prevalence, intensity and host-associated effects

Across the 24-month study, 177 of 480 *M. armatus* were infected, giving an overall prevalence of 36.9% (95% Wilson CI approximately 32.7–41.3%). A total of 292 cestodes were recovered, corresponding to a pooled mean intensity of 1.65 and mean abundance of 0.61 parasites per examined fish. Prevalence was 34.6% (83/240) in 2024–25 and 39.2% (94/240) in 2025–26; the year difference was not significant ($\chi^2 = 1.08$, $p = 0.298$), and year remained non-significant after multivariable adjustment.

Sex differences were consistent and substantial. Males had a pooled prevalence of 28.2% (67/238), whereas females had a prevalence of 45.5% (110/242). The Mantel–Haenszel female-to-male OR was 2.13 (95% CI 1.46–3.11; $p < 0.001$), with no evidence of heterogeneity between years (Breslow–Day $p = 0.371$). In the mutually adjusted logistic model, females had 2.24-fold higher odds of infection than males (95% CI 1.51–3.33; $p < 0.001$).

Season had the largest host-level effect. Pooled prevalence was 25.0% in monsoon, 32.5% in winter and 53.1% in summer ($\chi^2 = 29.16$, $p < 0.001$). After adjustment for sex and year, summer fish had 3.57-fold higher odds of infection than monsoon fish (95% CI 2.20–5.80; $p < 0.001$), whereas winter did not differ significantly from monsoon (OR = 1.47, $p = 0.127$). The logistic model fitted adequately (deviance goodness-of-fit $p = 0.704$; Pearson dispersion = 0.854) and showed no sex $\times$ season interaction ($p = 0.864$). Mean intensity also increased in summer: the Poisson model estimated a 1.52-fold summer-to-monsoon intensity ratio (95% CI 1.11–2.08; $p = 0.009$), while the female intensity ratio was only marginal ($p \approx 0.065$). Figures 7–10 summarize these host and seasonal patterns.

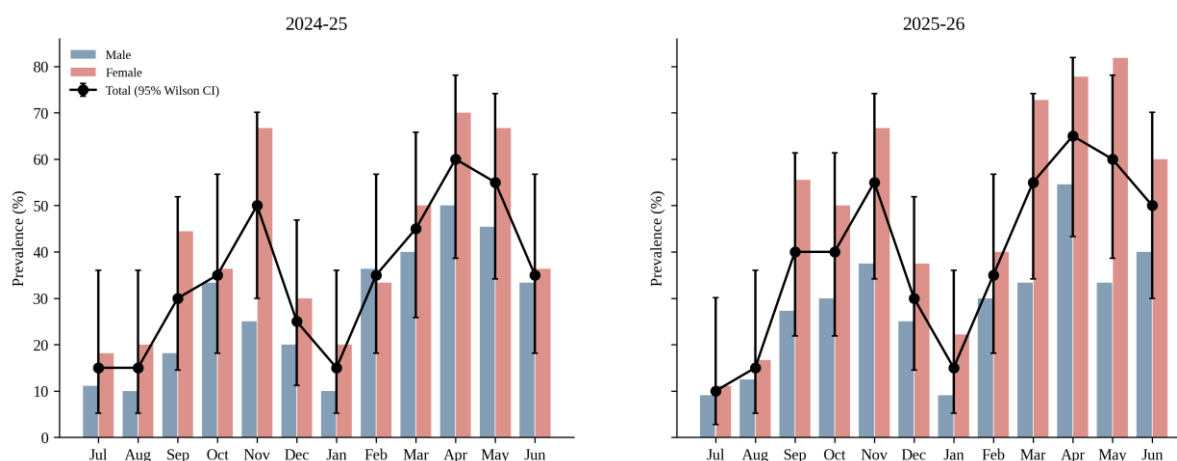


Figure 7. Monthly cestode prevalence in *M. armatus* by sex and annual cycle. Total prevalence is shown with 95% Wilson confidence intervals; females generally show higher prevalence and both years display similar seasonal peaks.

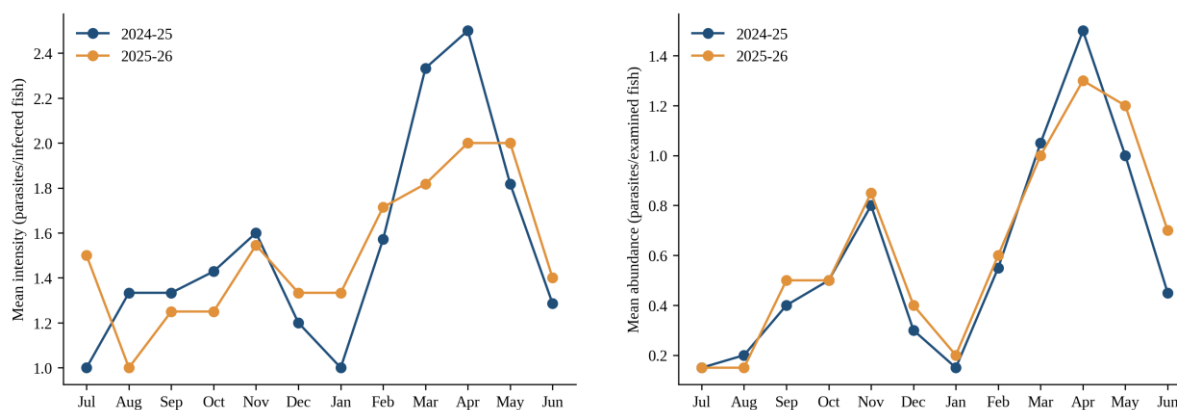


Figure 8. Monthly mean intensity and mean abundance of cestode infection in the two annual cycles. Burden rises during the main spring–summer infection window but is more variable than prevalence.

Host body length was significantly associated with infection in both years (2024–25: $\chi^2 = 10.33$, $p = 0.0159$; 2025–26: $\chi^2 = 23.77$, $p < 0.001$). Prevalence peaked in the 40.1–50.0 cm class (45.1% and 54.1% in the two annual cycles) and fell in the largest class. A pooled quadratic logistic term was significant ($p < 0.001$), supporting an inverted-U pattern rather than a simple monotonic increase. Mean intensity, in contrast, tended to increase with size, consistent with cumulative parasite burden among infected hosts. Body-weight classes showed a similar but weaker nonlinear pattern; because the 2025–26 weight table was short by one infected fish, its inferential result is considered provisional (Figure 9).

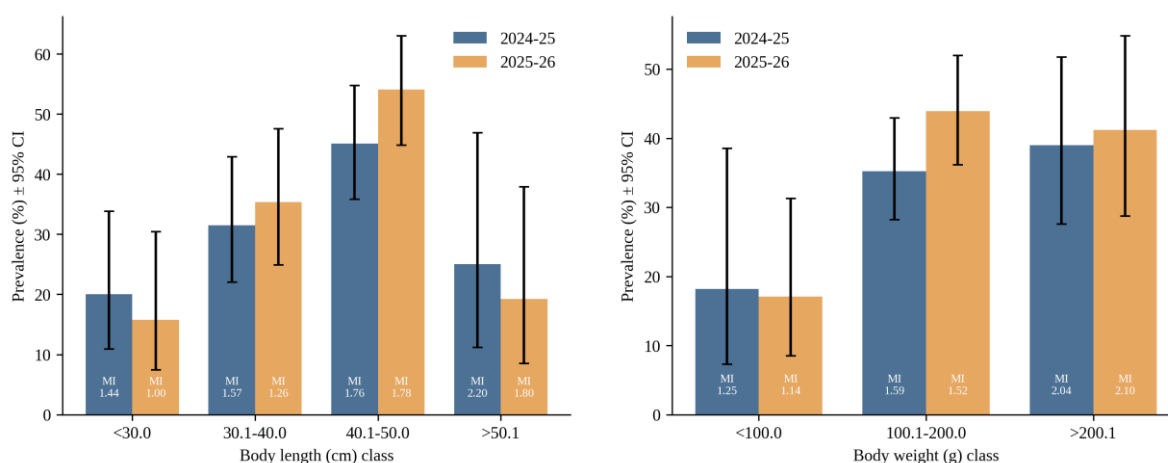


Figure 9. Cestode prevalence (bars with 95% Wilson confidence intervals) and mean intensity by host body-length and body-weight classes. The robust length pattern peaks at the intermediate 40.1–50.0 cm class.

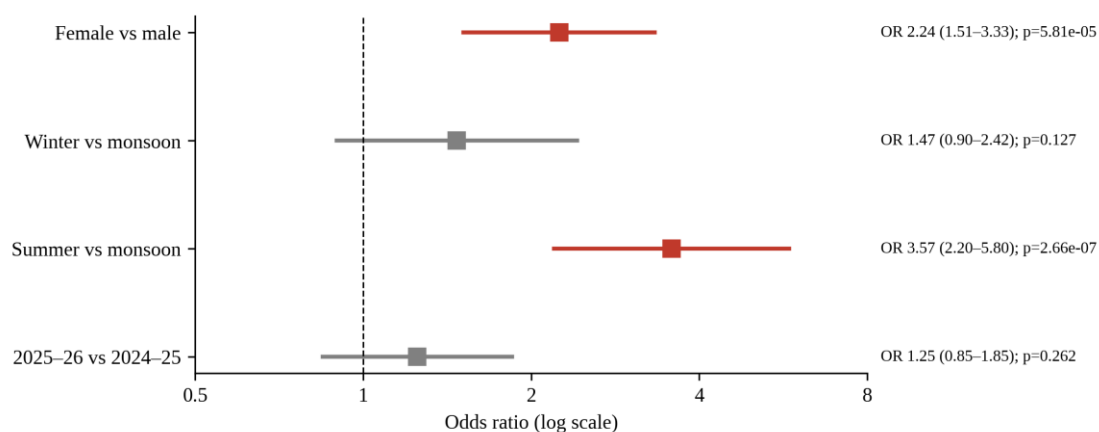


Figure 10. Adjusted odds ratios from the binomial logistic model of infection on sex, season and year. Error bars are 95% confidence intervals; the vertical reference line denotes OR = 1.

Table 4. Principal parasitological outcomes and effect estimates.

Outcome	Observed pattern	Effect/test	Significance
Overall prevalence	177/480	36.9%	—
Annual prevalence	34.6% vs 39.2%	$\chi^2 = 1.08$	$p = 0.298$
Female vs male	45.5% vs 28.2%	Adjusted OR = 2.24 (1.51–3.33)	$p < 0.001$
Summer vs monsoon	53.1% vs 25.0%	Adjusted OR = 3.57 (2.20–5.80)	$p < 0.001$
Winter vs monsoon	32.5% vs 25.0%	Adjusted OR = 1.47 (0.90–2.42)	$p = 0.127$
Mean intensity: summer vs monsoon	—	Intensity ratio = 1.52 (1.11–2.08)	$p = 0.009$
Body length, 2024–25	Peak at 40.1–50.0 cm	$\chi^2 = 10.33$	$p = 0.0159$
Body length, 2025–26	Peak at 40.1–50.0 cm	$\chi^2 = 23.77$	$p < 0.001$
Pooled length shape	Inverted-U	Quadratic term	$p < 0.001$

3.4 Harmonic seasonality and inter-annual reproducibility

The monthly pattern was not adequately represented by a single annual wave. Adding the annual harmonic to a year-only binomial model improved fit (LR $\chi^2 = 10.74$, $p = 0.0046$), but addition of the semi-annual harmonic produced a much larger improvement (LR $\chi^2 = 40.17$, $p < 0.001$) and reduced AIC from 134.4 to 98.2. The fitted two-harmonic model identified a primary peak around April (fitted prevalence approximately 64% in the 2024–25 baseline) and a secondary peak around October–November (approximately 45%), with troughs around January and July–August. Monthly prevalence profiles were highly concordant between the two annual cycles (Pearson $r = 0.966$, $p < 0.001$; Spearman $\rho = 0.966$, $p < 0.001$), while monthly mean-intensity profiles were also positively correlated ($r = 0.751$, $p = 0.0049$). Thus, the central epidemiological feature was a reproducible bimodal seasonal pattern rather than a progressive year-to-year increase (Figure 11).

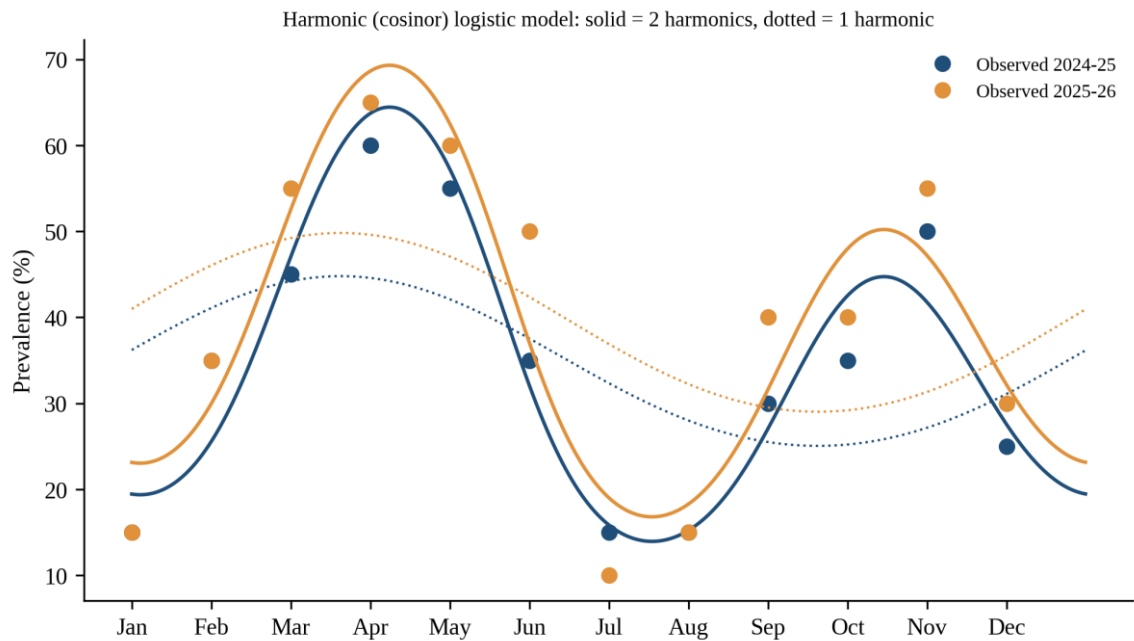


Figure 11. Observed monthly prevalence and fitted harmonic binomial curves. The preferred two-harmonic model identifies a primary spring peak and a secondary post-monsoon peak, while a single annual harmonic fails to capture the bimodal pattern.

3.5 Water–parasite associations

At lag 0, prevalence showed nominal positive Spearman correlations with alkalinity ($\rho = 0.533$, $p = 0.0073$), EC ($\rho = 0.476$, $p = 0.0188$) and nitrate ($\rho = 0.445$, $p = 0.0292$). However, none remained significant after FDR correction ($q = 0.073$, 0.094 and 0.097 , respectively). Same-month DO was negatively related to prevalence ($\rho = -0.340$) but was not significant. No concurrent water variable was FDR-significant for mean intensity. These results argue against selecting a single water parameter post hoc as the primary driver (Figure 12).

The lagged analysis produced one association that survived multiplicity correction: dissolved oxygen in the preceding month was inversely associated with mean parasite intensity in the following month (Spearman $\rho = -0.589$, raw $p = 0.0031$, FDR $q = 0.031$). The corresponding Pearson correlation was also negative ($r = -0.559$, $p = 0.0056$). Lagged DO was nominally associated with prevalence and mean abundance, but these outcomes

did not survive FDR correction. The lagged intensity signal is therefore the strongest parameter-specific environmental finding in the dataset, although it remains observational and cannot establish a mechanism.

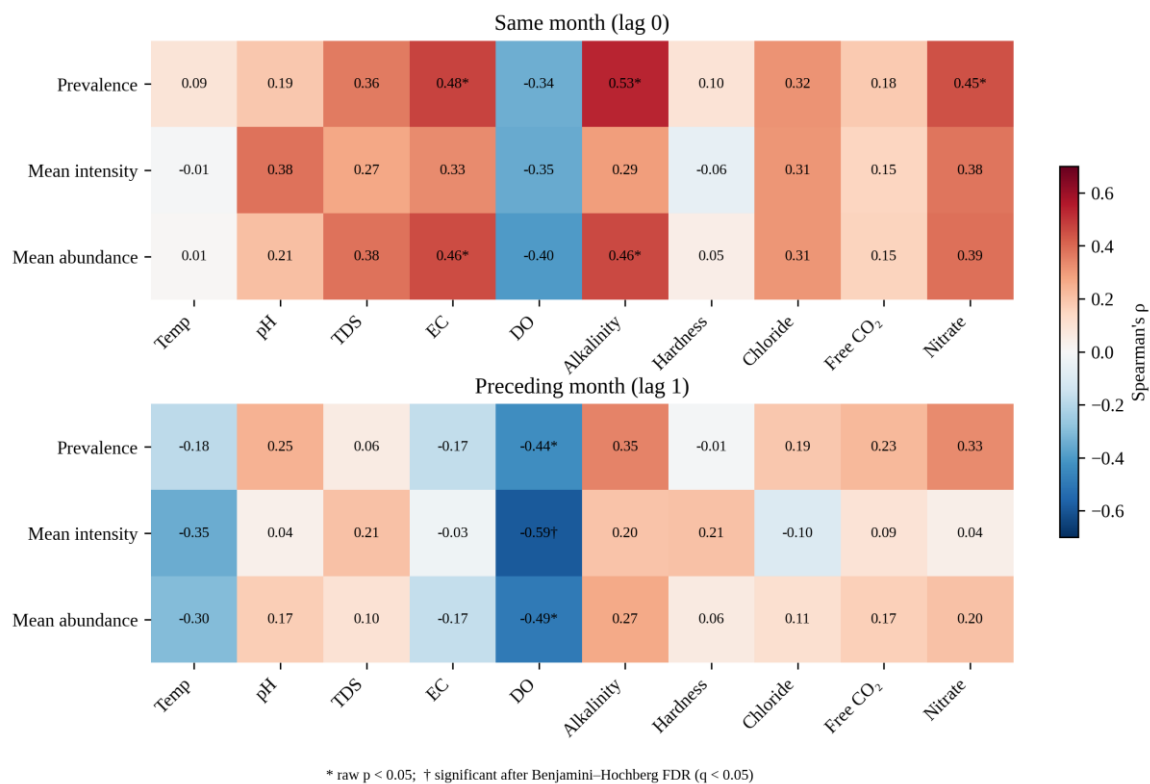


Figure 12. Spearman correlations between site-averaged monthly water-quality variables and parasite indices. The upper panel shows concurrent associations (lag 0) and the lower panel preceding-month associations (lag 1); significance should be interpreted using FDR-adjusted q-values.

Monthly water PCA gave a first component characterized by higher free CO₂, alkalinity and nitrate and lower DO. In an ordinary binomial model, PC1 appeared significant (OR = 1.16 per unit, p = 0.005), but the model was overdispersed (dispersion = 2.34). After quasi-binomial correction, the association was only borderline (OR = 1.16, 95% CI 0.99–1.37; p = 0.067). Its direction remained positive when months with unverified duplicate water-value blocks were excluded (OR = 1.18, p = 0.128) and when preceding-month PC1 was used (OR = 1.15, p = 0.089). Crucially, when season and year were included, PC1 contributed no additional information (OR = 1.02, 95% CI 0.82–1.25; p = 0.888), while summer remained strongly associated with prevalence (OR = 3.34, p < 0.001). Thus, the broader water-quality deterioration and parasite prevalence share the same seasonal timing, and the present dataset cannot separate a direct environmental effect from other seasonal processes (Figure 13).

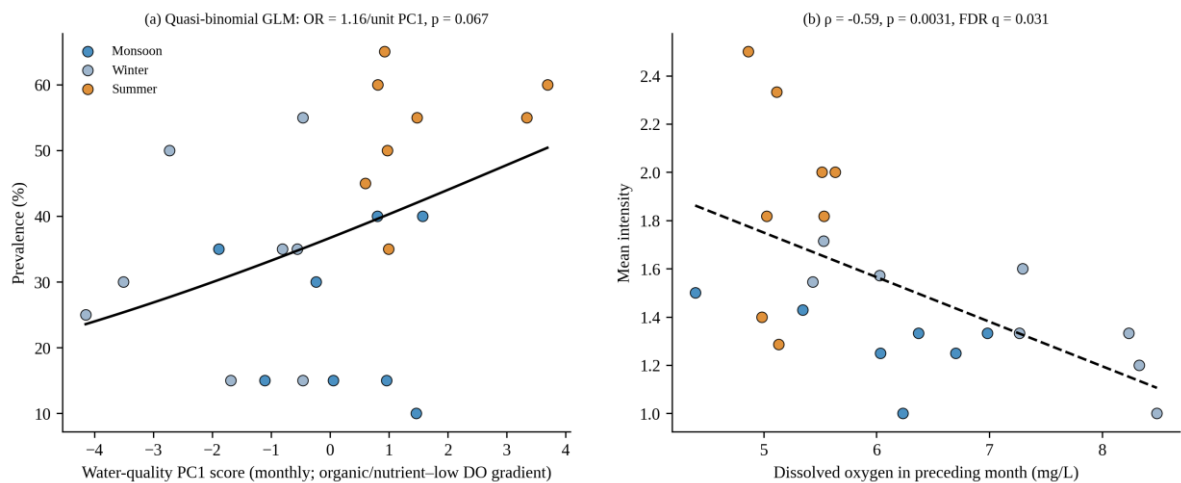


Figure 13. Integrated host–environment results. (a) Monthly prevalence plotted against the organic-loading/low-DO water PC1 with the quasi-binomial fitted trend; the association is borderline after overdispersion correction and is not independent of season. (b) Mean parasite intensity decreases with dissolved oxygen measured in the preceding month; this lagged association survives FDR correction.

Table 5. Key water–parasite association results after multiplicity and overdispersion control.

Analysis	Effect	Adjusted evidence	Interpretation
Prevalence vs alkalinity, same month	$\rho = 0.533$	$p = 0.0073$; $q = 0.073$	Exploratory only
Prevalence vs EC, same month	$\rho = 0.476$	$p = 0.0188$; $q = 0.094$	Exploratory only
Prevalence vs nitrate, same month	$\rho = 0.445$	$p = 0.0292$; $q = 0.097$	Exploratory only
Mean intensity vs DO, preceding month	$\rho = -0.589$	$p = 0.0031$; $q = 0.031$	FDR-significant
Prevalence vs monthly PC1	OR = 1.16/unit	Quasi-binomial $p = 0.067$	Borderline
Prevalence vs PC1 + season + year	OR = 1.02/unit	$p = 0.888$	No independent PC1 effect

4. Discussion

The integrated analysis resolves the dataset into three complementary levels: strong seasonal and spatial structuring of canal water quality, reproducible host- and season-associated cestode epidemiology, and a more cautious environmental association that is largely entangled with season. This distinction is important. The clearest biological results are the replicated host and seasonal effects, whereas the water–parasite findings provide mechanistic leads rather than proof that water-quality deterioration caused infection.

The water-quality results agree with the broader literature showing that regulated rivers and canals respond strongly to seasonal dilution, evaporation, flow and localized anthropogenic inputs (Alkhadher et al., 2025; Rahman et al., 2021; Saha et al., 2022). The large seasonal effects for temperature, nitrate, TDS, DO and EC and the absence of season $\times$ site interactions indicate that the hydrological cycle was the dominant temporal organizer, while local conditions shifted the baseline of most chemical variables. The persistent Site II pattern is consistent with earlier observations of spatially heterogeneous conditions in the Western Yamuna Canal and Yamuna system (Bhatnagar et al., 2009; Hassan et al., 2017; Jaiswal et al., 2019; Sharma et al., 2024). However, the present observations identify a water-quality hotspot; they do not directly apportion pollution sources.

The correlation, PCA and clustering results strengthen this interpretation by showing that the water variables do not behave independently. TDS and EC formed a strong mineral/ionic axis, whereas low DO occurred together with higher free CO₂, alkalinity and nitrate. The latter pattern is ecologically consistent with greater respiratory/organic-loading pressure, although the measurements do not include BOD, COD or direct organic-carbon characterization. Comparable multivariate approaches have proved useful for distinguishing hydrochemical regimes and contaminant signatures that are not obvious from univariate comparisons (Benchamin et al., 2024; Songara et al., 2025). The WQI analysis similarly summarized the seasonal deterioration but was strongly influenced by the weight given to DO. It should therefore be viewed as a screening index, not as a stand-alone declaration of potability or ecological status.

The host-level results are more direct. Female *M. armatus* were approximately twice as likely to be infected as males, and this effect persisted after adjustment for season and year and was homogeneous across annual cycles. Sex-biased parasitism can arise through differences in diet, habitat use, energy allocation, reproductive state, endocrine physiology or immune investment, and the direction of the bias varies among host–parasite systems. The present data do not contain gonadal stage, age or individual condition indices, so no single mechanism can be assigned. Nevertheless, the repeatability of the female excess and its agreement with previous observations in *M. armatus* (Vankara & Vijayalakshmi, 2015) make host sex an important covariate for future work.

Host length showed an equally informative nonlinear pattern. Prevalence rose from small fish to the 40.1–50.0 cm class and then fell in the largest class, whereas mean intensity generally continued to rise with body size. A purely cumulative-exposure model would predict monotonic prevalence; the observed inverted-U pattern instead suggests that ontogenetic shifts in feeding or habitat, selective mortality, or acquired resistance may alter the probability of infection in older/larger fish. These possibilities require individual-level diet, age, condition and parasite-count data. The body-weight pattern should be interpreted more cautiously because one infected fish could not be reconciled in the 2025–26 weight-class source table.

Seasonality was the strongest epidemiological result. Both annual cycles independently showed higher prevalence in summer, and the adjusted odds of infection were about 3.6 times those in monsoon. Mean intensity was also significantly higher in summer. More importantly, harmonic regression showed that the cycle was bimodal rather than a simple warm-season rise, with a major April peak and a secondary October–November peak. Seasonal parasite peaks can reflect parasite development rates, intermediate-host abundance, host feeding and movement, or hydrological effects, but no universal pattern applies across taxa (Poulin, 2020). Temperature can influence teleost immunity and pathogen interactions (Okon et al., 2024; Scharsack & Franke, 2022), yet temperature alone was not correlated with infection in this dataset. This supports the interpretation that season is a composite ecological driver rather than a proxy for temperature alone.

The water–parasite analysis provides an important caution against overinterpretation. Concurrent alkalinity, EC and nitrate were individually correlated with prevalence at nominal $p < 0.05$, but none survived FDR correction. Likewise, an ordinary binomial PC1 model produced a seemingly persuasive $p = 0.005$, but this result weakened to $p = 0.067$ after correcting the standard errors for overdispersion. When season was added, PC1 became completely non-significant. In other words, months with poorer organic-loading/low-oxygen conditions also tended to be months with higher infection, but the two rhythms are sufficiently synchronized that the present 24-month series cannot statistically identify which seasonal mechanism is responsible. This is consistent with the broader environmental-parasitology literature, where contamination can influence parasites through hosts, free-living stages and intermediate hosts, but direction and magnitude depend on the system (Gilbert & Avenant-Oldewage, 2021; Jerônimo et al., 2022; Sures et al., 2023).

The preceding-month DO association with mean intensity is more specific and therefore deserves attention. Lower DO one month earlier was associated with a heavier subsequent worm burden, and this was the only parameter-specific relationship that survived FDR correction. Hypoxia can affect fish physiology and stress responses, while oxygen conditions may also covary with organic loading and food-web processes that influence parasite transmission (Saari et al., 2018; Upadhyay et al., 2024). However, the lagged relationship still does not prove that oxygen depletion caused greater parasite establishment. It may reflect a shared underlying seasonal process or changes in feeding and intermediate-host availability. The result is best treated as a testable hypothesis for prospective sampling in which DO, fish physiology, intermediate hosts and individual parasite burdens are measured together.

The study therefore supports an integrated biomonitoring framework rather than use of the cestode as an isolated pollution indicator. Parasites can be sensitive sentinels because they integrate conditions experienced by the host and food web over time, but some stressors can increase infection while others reduce parasite transmission by suppressing free-living or intermediate stages (Brázová et al., 2021; Gilbert & Avenant-Oldewage, 2021; Pramanik & Biswas, 2024). In practical terms, the reproducible April and October–November infection windows provide useful periods for intensified monitoring, while water surveillance should emphasize summer oxygen depletion and the Site II chemical profile.

The main strengths of the study are two complete annual cycles, repeated water sampling at three sites, replicated seasonal parasite patterns, effect-size reporting, multiple-testing control and sensitivity analyses that explicitly account for overdispersion and source-data concerns. Limitations include the ecological alignment of monthly fish outcomes with site-averaged water quality, lack of site-specific fish data, absence of individual parasite counts for modelling aggregation, lack of intermediate-host, diet, age, reproductive and immune data, and the grouped nature of host size/weight records. Several exact duplicate blocks in the supplied water workbooks were flagged during data audit and should be checked against original field/laboratory registers before final submission; importantly, exclusion of the affected months did not change the direction of the integrated water–parasite model. Species-level parasite identification and complete fish collection/necropsy procedures should also be verified from the original laboratory records. These limitations restrict mechanistic inference but do not alter the replicated host and seasonal findings.

5. Conclusion

Across two annual cycles of the Western Yamuna Canal, cestode infection in *M. armatus* showed a reproducible and statistically strong host–season structure. Overall prevalence was 36.9%; females had about twice the infection odds of males, summer was the high-prevalence and high-intensity season, and prevalence

peaked nonlinearly in intermediate-sized hosts. Harmonic modelling further revealed two recurrent epidemiological windows, with a major April peak and a secondary October–November peak. Canal water quality was simultaneously organized by strong seasonal hydrology and persistent spatial differences, with Site II showing the strongest organic-loading/low-oxygen signature. Three rotated water-quality factors explained 69.7% of total variance, and the WQI was predominantly in the Poor category, particularly during summer. The strongest specific water–parasite association was an inverse relationship between preceding-month dissolved oxygen and subsequent mean parasite intensity ($\rho = -0.59$, FDR $q = 0.031$). In contrast, the apparent multivariate association between water PC1 and prevalence did not remain independent of season. The defensible conclusion is therefore not that pollution caused cestode infection, but that host susceptibility, parasite epidemiology and water-quality deterioration are seasonally coupled within the canal system. Future work should collect fish at identified sites, retain individual fish-level worm counts and host traits, quantify intermediate-host availability and fish physiological biomarkers, and verify parasite identity morphologically and/or molecularly to resolve the mechanisms behind the observed seasonal coupling.

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