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Helminth parasite communities in *Channa punctatus* (Bloch, 1793) from Deoria and Sultanpur districts of Uttar Pradesh, India

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ABSTRACT

Helminth parasites of freshwater fish represent important ecological and epidemiological components of aquatic ecosystems. This study examined abundance, intensity and prevalence of helminth parasites in the freshwater fish Channa punctatus (Bloch, 1793) from riverine localities of Deoria and Sultanpur districts of Uttar Pradesh, India. Based upon monthly host examination records and parasite counts per taxon. Standard indices were calculated following established definitions and nonparametric tests were applied to evaluate spatial variation. Pooled prevalence was comparable across sites and statistical analysis indicated no significant differences in overall infection levels. Community composition was similar with the same taxon rank order recorded in both localities. Abundance and mean intensity comparisons across matched months did not reveal site specific differences while variance to mean ratios supported aggregation of parasites in hosts. Findings suggested that helminth transmission dynamics were broadly consistent across both sites. The study emphasized the need for paired temporal sampling and robust statistical approaches in comparative parasitology and provided a baseline for fisheries health management in northern India.

Keywords: *Channa punctatus*, Helminths; Mean intensity, Parasite community, Prevalence

Fish are widely consumed and remain a comparatively affordable animal-source food, supporting diets and health across diverse populations (Golden *et al.* 2021, Byrd *et al.* 2022). they provide high-quality protein along with concentrated micronutrients such as vitamin B_{12}, retinol, iron, zinc and long-chain omega-3 fatty acids, making them valuable for sustaining nutrient adequacy in many dietary contexts (Shastak and Pelletier 2024, Singh *et al*. 2025, Shahzad 2024, Zamborain-Mason *et al.* 2025, Viana *et al.* 2023). In low and middle-income regions, greater access to fish has the potential to improve diet quality and reduce micronutrient deficiencies, especially when aquaculture emphasizes species with nutrient-dense profiles (Noreen *et al.* 2025, Shepon *et al.* 2021). The spotted snakehead, *Channa punctatus (C. punctatus)*, is a widely consumed species in South Asia and recent studies have confirmed its nutrient-rich proximate composition (Jahan *et al.* 2019, Sumi *et al.* 2023). Beyond its dietary role, snakehead species are traditionally valued during recovery and are increasingly studied for their bioactive compounds, including wound-healing properties demonstrated through preclinical and clinical investigations (Lee *et al.* 2022, Riviati *et al.* 2024). As a benthic, carnivorous and largely resident fish, *C. punctatus* often comes into contact with intermediate hosts and substrates that favour helminth colonization, a pattern which is well supported by molecular and field-based studies across South Asia (Williams *et al.* 2022, Farzana *et al.* 2019). Although helminths are natural elements of freshwater ecosystems, heavy infections affect host physiology, growth and reproduction through complex host-parasite-environment interactions (Buchmann 2022, Grabner *et al.* 2023). These infections also reduce aquaculture productivity and product quality while raising management costs, highlighting the importance of effective fish health practices (Madsen and Stauffer 2024, Maezono *et al.* 2025). Consequently, regular surveillance and seasonal monitoring are necessary, since recent investigations in India and neighbouring regions reveal substantial helminth burdens in *C. punctatus* and clear seasonal trends that indicate periods of elevated risk and management priority (Patil *et al.* 2025, Rana and Kaur 2021). The present study examined seasonal variation in helminth infections in *C. punctatus* from Deoria and Sultanpur districts of Uttar Pradesh (UP) while evaluating helminth biodiversity in this freshwater fish through pooled prevalence and paired

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comparisons of abundance mean intensity and dispersion patterns across the annual cycle.

MATERIALS AND METHODS

Study area and sample collection: The study was conducted at the Department of Zoology, Baba Raghav Das Post Graduate College, Deoria, between June 2024 and May 2025. A total of 400 specimens were obtained, 200 from each district. Sampling was conducted at four sites along Ghaghra and Chhoti Gandak rivers in Deoria district and at three sites along the Gomti river in Sultanpur (Fig. 1).

Isolation of parasites: Fish were sacrificed with approval from the Institutional Ethical Committee, DDU Gorakhpur University, Gorakhpur and all procedures complied with institutional animal care protocols. The gastrointestinal tract, liver and gall bladder were excised and placed in petri dishes containing 0.9% normal saline for immediate examination (Justine *et al.* 2012, Sepulveda and Kinsella 2013). The alimentary canal was dissected to recover helminths. Specimens were relaxed in saline to ease detachment from host tissues (Justine *et al.* 2012). Live worms recovered from the stomach and intestine were rinsed in saline and fixed in alcohol–formalin–acetic acid (AFA) solution prepared as 100 ml of 50% ethanol with 6 ml of formalin and 2.5 ml of acetic acid. Fixed specimens were stained with Semichon's acetocarmine, dehydrated through a graded ethanol series, cleared in xylene and mounted in dibutylphthalate polystyrene xylene (DPX) medium for light microscopy (Sepulveda and Kinsella 2013, Rittmeier and Holzinger 2023). Selected specimens for scanning electron microscopy were fixed in 2.5% glutaraldehyde, washed in buffer, dehydrated in an ascending ethanol series, dried in a critical point dryer and sputter coated prior to imaging under high vacuum (Gluenz *et al.* 2015).

Statistical Analyses: Pooled prevalence across sites was compared using the Pearson chi-square (χ^2) test applied to a 2×2 contingency table of infected and uninfected hosts by site, a standard approach for testing equality of proportions in categorical data samples. Monthly prevalence comparisons between sites were carried out with Fisher's exact tests because exact methods are recommended when expected cell counts are small (Agresti 2002). Monthly abundance and monthly mean intensity values for each parasite taxon were compared across sites using paired Wilcoxon signed rank tests, which provide a nonparametric method for matched pair comparisons without assuming normality of the data distribution (Wilcoxon 1945). Within a site, month effects on mean intensity were examined with Kruskal Wallis tests, applied separately for each parasite and site to evaluate rank-based differences among months (Kruskal and Wallis 1952). Aggregation of parasites was summarized using the dispersion index defined as variance divided by mean of monthly abundance values for each parasite at each site, which is a commonly used descriptor

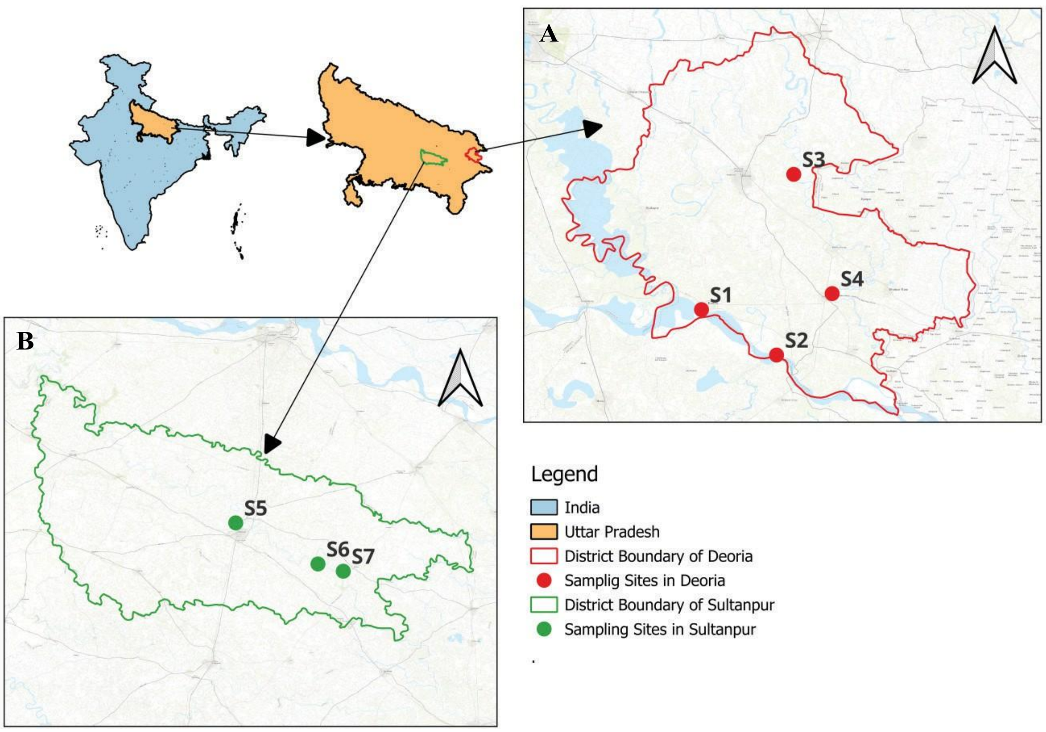

Fig. 1. Sampling sites of the *C. punctatus* with their Geo-coordinates for: (A) District Deoria locations as site (S)1; 26.27440 and 83.71060, S2; 26.19641 and 83.83964, S3; 26.50653 and 83.86919, S4; 26.30190 and 83.93522 and (B) District Sultanpur as S5; 26.28390 and 82.06295, S6; 26.17634 and 82.27854, S7; 26.15737 and 82.34478, as their Latitude (DD) and Longitude (DD), respectively.

in parasitology alongside alternative aggregation measures and indices of discrepancy (Rózsa *et al.* 2000, Morrill *et al*. 2022). All hypothesis tests were two sided with α set at 0.05 and statistical outputs reported include χ^2, H, V, the dispersion index and exact p values in accordance with current quantitative parasitology practice (Reiczigel *et al.* 2019). All statistical analyses were performed using R (R Core Team 2016).

RESULTS AND DISCUSSION

Parasite identification: Parasites were identified to genus level using stable morphological traits. *Pallisentis* was recognized by a cylindrical proboscis with four circles of ten hooks that decrease posteriorly into a single walled proboscis receptacle having eight cement glands in males and trunk spination in females (Fig. 2K–R) (Rana and Kaur 2021). *Clinostomum* metacercariae were dorsoventrally flattened with a subterminal oral sucker along with a large ventral sucker anterior to midbody, a short prepharynx, a muscular pharynx posteriorly extending caeca and encystment in the buccal cavity or musculature (Fig. 2G–J) (Won *et al.* 2020). *Genarchopsis* adults showed an elongated body, a subterminal oral sucker, a short oesophagus caecum near the testis, tandem testes in the posterior half along with a median ovary vitellaria in lateral fields and thin-shelled eggs (Rajput and Langer 2022). *Neocamallanus* displayed a bilobed buccal capsule with longitudinal ridges and trident-like basal armature deirids

Fig. 2. Scanning electron micrographs (SEM) and light micrographs of helminth parasites. Nematode: Neocamallanus sp. (A) SEM of anterior end showing oral lips; (B) SEM of female posterior end showing vulval aperture; (C-D) anterior and posterior ends of female; (E-F) anterior and posterior ends of male. Trematodes: Clinostomum sp. (G) SEM of oral sucker; (H) SEM of ventral sucker; (I) anterior part; (J) posterior part. Acanthocephalan: Pallisentis sp. (K) SEM of male scolex with spines; (L) SEM of male posterior part with spicules; (M) male anterior part; (N) male posterior part; (O) SEM of female scolex with thorny spines; (P) SEM of female posterior part with vulva; (Q) female anterior part; (R) female posterior part. Cestode: *Senga* sp. (S) Showing various body segments.

near the nerve ring unequal spicules a gubernaculum and diagnostic caudal papillae (Fig. 2A–F) (Ailán-Choke and Pereira 2021, Moravec and Justine 2019). *Senga* was identified by a scolex with two elongated bothria, a short neck, numerous proglottids, medullary testes, a median uterus with diverticula and alternating lateral genital pores (Fig. 2S) (Scholz and Kuchta 2022). These combined characters confirmed reliable genus level identification of the examined helminths.

Indices: Prevalence was calculated as infected hosts divided by examined hosts which describes proportion of hosts with infection in the target population sample. Abundance was calculated as total parasites divided by examined hosts to describe mean parasite burden across all sampled hosts. Mean intensity was total parasites divided by infected hosts to describe average burden among infected hosts only. These methods followed standard ecological parasitology usage and supported comparison across sites and months in a consistent manner for reviewer reproducibility (Poulin and Presswell 2022, Morrill *et al.* 2023, Ieshko *et al*. 2024).

Prevalence: In total, 200 *C. punctatus* were examined from each district. In Deoria, 157 individuals were infected (78.5%) while in Sultanpur, 152 were infected (76.0%). The chi-square test comparing pooled prevalence yielded χ^2 = 0.228, df = 1, p = 0.633, showing no significant difference between districts. Monthly Fisher's exact tests likewise detected no significant contrasts, indicating that infection proportions were consistently similar between sites throughout the year. These results demonstrated that overall infection probability was stable and not influenced by locality. Parasite-level details, including infected host counts, total parasites recovered, abundance per host and mean intensity, are summarized in Table 1.

Abundance and intensity comparisons across sites: Paired Wilcoxon signed rank tests across matched months for each parasite yielded no statistically significant site differences for either abundance or mean intensity. Wilcoxon signed rank V values ranged from 20 to 32 and all associated p values were greater than 0.05, indicating no differences between Deoria and Sultanpur. Similar parasite loads and intensities were across within taxon the two districts over the annual cycle (Poulin and Presswell 2022, Morrill *et al*. 2023, Lester and Blomberg 2021). This concordance aligns with the consistent rank order of abundance observed at both localities, which further supports the inference of comparable transmission pressure across sites under the sampled conditions (Horn and Luong 2023, Novoa and Hechinger 2024, Ieshko *et al*. 2024). The paired month-wise design enhanced sensitivity by controlling seasonal structure that could obscure spatial contrasts in unpaired comparisons and it remained as an appropriate choice for ecological parasitology time series in working fisheries and river systems (Poulin and Presswell 2022, Morrill *et al*. 2023, Horn and Luong 2023).

Within site variation of mean intensity: Kruskal-Wallis tests did not detect strong month effects on mean intensity of individual parasite within each site. These results suggested relative stability of average burdens among infected hosts across the study year (Morrill *et al*. 2023, Ieshko *et al*. 2024, Horn and Luong 2023). Some taxa had limited non-missing monthly values, and test power was constrained, which motivated longer temporal series or pooled multi-year monitoring to improve detection of subtle seasonal patterns in similar field programs (Novoa and Hechinger 2024, Morrill *et al*. 2023, Poulin and Presswell 2022). The combination of nonparametric tests and synchronized monthly sampling provides a robust framework for assessing intensity dynamics when distributional assumptions are not met and when sample sizes are modest in applied settings (Lester and Blomberg 2021, Ieshko *et al*. 2024).

Aggregation and dispersion: Variance to mean ratios from monthly abundance values were mostly less than one at the month by site level which reflects averaging parasite counts across infected and uninfected hosts and reduces dispersion at that scale (Reiczigel *et al*. 2019). At the individual host level, helminth burdens are typically right skewed with many lightly infected fish and a few heavy carriers which produces aggregation (Morrill *et al*. 2023, Ieshko *et al*. 2024, Horn and Luong 2023). These patterns supported model choices that allow extra variance and excess zeros such as negative binomial and zero inflated families when host level data are available (Lester and Blomberg 2021, Reiczigel *et al*. 2019). In this dataset, the combination of dispersion summaries and paired nonparametric comparisons provided a defensible view of spatial similarity while acknowledging the usual host level aggregation in freshwater fish helminth communities (Novoa and Hechinger 2024, Poulin and Presswell 2022).

Table 1. Parasite community summary by site

Parasite	Deoria infected	Deoria total parasites	Deoria abundance per host	Deoria mean intensity	Sultanpur infected	Sultanpur total parasites	Sultanpur abundance per host	Sultanpur mean intensity
Pallisentis sp.	79	240	1.200	3.038	78	230	1.150	2.949
Clinostomum sp.	58	168	0.840	2.897	57	173	0.865	3.035
Genarchopsis sp.	48	148	0.740	3.083	43	130	0.650	3.023
Neocamallanus sp.	32	95	0.475	2.969	32	98	0.490	3.062
Senga sp.	23	71	0.355	3.087	18	54	0.270	3.000

The similarity in prevalence, abundance and intensity across sites suggested comparable transmission drivers across the two localities during the year and implied that management decisions built on these indices could be applied consistently across both areas with appropriate local adaptation for operational details and logistics. *Pallisentis* sp. as the dominant taxon remained at both th e sites which suggest targeted life cycle studies and focused monitoring of intermediate host pools to better understand ecological leverage points for risk management and early warning in future fisheries health programs.

Prevalence, abundance and mean intensity of helminth parasites in the studied freshwater fish were similar across Deoria and Sultanpur over the annual cycle with no significant site differences detected in pooled prevalence or in paired tests of abundance and intensity of parasite. The same rank order of taxa appeared in both sites and dispersion patterns supported the expectation of aggregation at the host level. The findings provide a reproducible baseline for monitoring and decision support. It also demonstrated the utility of paired nonparametric comparisons and dispersion summaries for comparative parasitology in working fisheries contexts.

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