Ecological morphotaxometry of trematodes of garfish (Teleostomi: Belonidae) from Gangetic riverine ecosystem in India. III. Principal component analysis in the dynamics of *Cephalogonimus yamunii* (Upadhyay, Jaiswal, Malhotra and Malhotra, 2012)

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Abstract The attributes of multivariate analyses were applied to infer peculiarity in distribution dynamics of *Cephalogonimus yamunii* Upadhyay et al. (J Parasit, 2012) in *Xenentodon cancilla* under influence of interionic interactions amongst hydrobiological factors. Non-parametric Mann–Whitney's Test χ^2 statistic was significant for the effect of Dissolved Oxygen and magnesium. The temperature optimum 23–27 °C was concluded for expression of peak prevalence and mean intensity during change in water temperature between autumn and winter periods. The dominant 1st component (PC1st) from Principal Component Analysis of monthwise response of infection data by *C. yamunii* in *X. cancilla* was further confirmed by Scree Plot of Eigenvalues and Factor Loadings Plot to identify the critical impact of hardness of water on infection prevalence and mean intensity. The findings of larger PC1st positive coefficients comprehensively substantiated predominating hardness factor, under the influence of enhanced Dissolved Oxygen and optimum thermal effect. Therefore, the role of multifactorial etiology is a definite possibility.

Keywords *Xenentodon cancilla* ·
Cephalogonimus yamunii · Interionic interaction ·
Poisson series · Overdispersion

Introduction

The components of aquatic ecosystem have potential to influence helminth distribution patterns in an indirect manner (Thomas 1958; Malhotra and Nanda 1989; Kennedy and Bush 1992; Kennedy and Hartvigsen 2000; Kennedy 2009). The helminth fauna of bottom dweller Indian carp, *Xenentodon cancilla* is poorly known in India. The authors have made efforts recently to work out zoonotic interactions in helminth parasites infesting this gar fish (Teleostomi: Belonidae) (Upadhyay 2012). The non-parametric Mann–Whitney test has provided evidence of significant impact of Dissolved Oxygen and hardness due to magnesium on the infrapopulation dynamics of fish fluke, *Cephalogonimus yamunii*. The interionic interactions have been of special interest to work out predominant effect of specific attribute by multivariate analysis to identify its noticeable role in helminth's population dynamics. The present study has employed advanced ecological tools like Principal component analysis to substantiate impact of hydrobiological and other environmental parameters on the distribution pattern of *C. yamunii*.

Materials and methods

The fish were collected and worms gathered from intestine were isolated and processed for morphometric analysis as mentioned in Upadhyay et al. (2012). The fortnightly

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qualitative estimation of Dissolved Oxygen (heretofore mentioned as DO in text) in water was conducted at the sites of investigation at the banks of River Yamuna (Kakraha Ghat) at Allahabad, U.P. (81°49'06.28"E (Lon)) (25°24'53.24"N (Lat), 74 m (Alt)) after APHA (1998). Water samples were collected in Torson's plastic samplers to conduct analyses of physico-chemical parameters viz., hardness (mg/l), alkalinity (mg/l), acidity (mg/l), chloride (mg/l), calcium (mg/l) and magnesium (mg/l) after APHA (1998). Biostatistical correlations to demonstrate Linear regression, Multivariate analysis (Table 5), Principal component analysis, Pearson correlation matrix (heretofore mentioned as PCM in the following text), Students' *t* test, the non-parametric Mann–Whitney's test, and Poisson distribution were worked out by using SYSTAT11 software.

Results

Hydrobiological parameters

Monthwise association of combined influence of hydrobiological factors on infection prevalence and mean intensity by *C. yamunii* in *X. canicilla* was strongest in May and June during 2008–2009 and during November in 2009–2010. The functional linear regression trends illustrating effect of individual hydrobiological factors on infection prevalence and mean intensity in either sex of fish during 2008–2009 and 2009–2010 are presented in Table 1. The Linear regression curves depicting varied influence of hydrobiological parameters on infection prevalence and mean intensity by *C. yamunii* in both sexes of fish during 2008–2009 (Fig. 1; Table 2) and during 2009–2010 (Figs. 2, 3, 4; Table 2) were computed. The break-up of illustrations for alkalinity (Table 2), acidity, DO (Fig. 1), chloride (Table 2) and water temperature (Table 2) during 2008–2009 and hardness (Table 2), alkalinity (Table 2), acidity (Table 2), calcium (Table 2), DO (Figs. 2, 3, 4; Table 2), chloride (Table 2), magnesium (Tables 2, 3) and water temperature (Table 2), during 2009–2010, have been represented.

Pearson's correlation matrix

The significant impact of alkalinity for infection prevalence (PCM, 0.568), mean intensity (PCM, 0.517) in female fish, infection prevalence (PCM, –0.509) in male fish; DO for infection prevalence (PCM, 0.587), mean intensity (PCM, 0.50) in female fish, mean intensity (PCM, 0.570) in male fish; calcium for infection prevalence (PCM, –0.50) in male fish; Chloride for mean intensity (PCM, –0.50) in female fish, infection prevalence (PCM, 0.653) in male

fish, and water temperature for mean intensity (PCM, –0.50) in female fish, mean intensity (PCM, –0.542) in male fish were substantiated by Pearson's correlation matrix.

Mann–Whitney's test

The Non-Parametric Mann–Whitney's Test were found significant for Dissolved oxygen on infection prevalence in male fish ($\chi^2 = 0.55$) and magnesium on mean intensity of male ($\chi^2 = 0.50$) and female ($\chi^2 = 0.50$) fish during 2009–2010 (Table 3).

Principal component analysis

The biostatistical evaluation, particularly of Pearson's coefficient matrix affirmed dominant 1st component (PC1^P) form Principal component analysis of infection data during 2008–2010 (Table 4) by *C. yamunii* in *X. canicilla* during 24 months' study. The predominance of the 1st component was also reflected in the Scree plot and factor loadings plot of Eigenvalues as depicted in Figs. 5, 6, 7, and 8 (2008–2009).

Multivariate analysis

The interrelationship of multiple hydrobiological parameters reacting together in the same aquatic body during 2 years of observations were described by Multivariate analysis depicted in Table 5.

Discussion

Hydrobiological parameters

The uniform trend of decline in infection prevalence in both sexes of fish with increase in hardness during 2009–2010 (Table 2), and decline in infection prevalence in both sexes of fish with corresponding decrease in calcium during 2009–2010, that was significant in males (Table 2; PCM, –0.50) during 2008–2009, as well as magnesium content of riverine water during 2009–2010 in male (Mann–Whitney's Test Statistic $\chi^2 = 0.50$) and female fish (Mann–Whitney's Test Statistic $\chi^2 = 0.50$; Table 2), were the remarkable findings. This corroborated evidence of consistent decrease in infection prevalence along with the decrease in the contributory factors to hardness i.e. calcium and magnesium in the water body. The consistently increasing effect of alkalinity of water on the infection prevalence in female fish (PCM, 0.568; Table 2) and mean intensity (PCM, 0.517; Table 2) in female fish, by *C. yamunii* was a noticeable finding during 2008–2009. The infection prevalence, however, declined at

Table 1 Linear regression trends depicting correlation of infection by *C. yamunii* in *X. cancella* with various hydrobiological parameters in river Yamuna at Allahabad during 2008–2010

Parameters	2008–2009		2009–2010	
	Male fish	Female fish	Male fish	Female fish
Hardness versus IP	$Y = 53.11 - 0.19X$ $r = 0.40$ $P < 0.40$	NS	NS	NS
Hardness versus MI	NS	$Y = 15.12 - 0.05X$ $r = 0.30$ $P < 0.50$	$Y = -2.92 + 0.06X$ $r = 0.30$ $P < 0.40$	NS
Alkalinity versus IP	$Y = 18.00 + 0.38X$ $r = 0.30$ $P < 0.50$	NS	NS	NS
Acidity versus IP	NS	$Y = 51.01 - 0.94X$ $r = 0.40$ $P < 0.40$	NS	NS
Acidity versus MI	NS	$Y = 3.13 + 0.25X$ $r = 0.40$ $P < 0.40$	$Y = 2.54 + 0.48X$ $r = 0.40$ $P < 0.40$	NS
Chloride versus MI	$Y = 22.96 - 0.21X$ $r = 0.40$ $P < 0.40$	NS	NS	$Y = 15.78 - 0.14X$ $r = 0.30$ $P < 0.50$
Calcium versus IP	NS	$Y = 47.29 - 0.62X$ $r = 0.34$ $P < 0.40$	NS	$Y = 45.44 - 0.33X$ $r = 0.40$ $P < 0.40$
Magnesium versus IP	$Y = 42.39 - 0.82X$ $r = 0.40$ $P < 0.40$	NS	NS	$Y = 39.52 - 0.23X$ $r = 0.30$ $P < 0.50$

IP infection prevalence, MI mean intensity, NS nonsignificant, DO dissolved oxygen, Temp water temperature

Fig. 1 Correlation of mean intensity by *C. yamunii* in male *X. cancella* with dissolved oxygen (mg/l) during 2008–2009

enhanced alkalinity in male fish during 2009–2010 (PCM, -0.509 ; Table 2). The infection prevalence increased at enhanced acidity, in females (Table 2) during 2009–2010. A uniform positive correlation of enhanced DO with infection prevalence in female (Fig. 2, PCM, 0.587 ; Table 2) and male fish (Mann–Whitney's Test Statistic $\chi^2 = 0.55$) during 2009–2010, as well as with mean intensity in both sexes during 2008–2009 (Female, PCM, 0.552 ; male, Fig. 1) and 2009–2010 (male, PCM, 0.570 , Fig. 3, Table 2; female, PCM, 0.50 , Table 2, Fig. 4) was observed. The mean intensity of trematodes declined at enhanced thermal regime during 2008–2009 in female fish (PCM, 0.50 , Table 2), as

well as in female fish during 2009–2010 (Table 2). The mean intensity too declined at augmented chloride content in female fish during 2008–2009 (PCM, -0.50 , Table 2), and infection prevalence also followed the similar pattern in male fish (PCM, -0.653 , Table 2).

The temperature optimum $23\text{--}27\text{ }^{\circ}\text{C}$ was concluded in the investigations conducted on edible fish in the wild. There was a spurt in the mean intensity (Table 2) in male fish at increased temperatures during 2009–2010. The trematode recruitment was effectively under influence of spatial and temporal variations (Sausa 1990). The influence of temporal factors on the community dynamics of trematodes in the snails have vastly been worked out in temperate climates (Esch and Fernandez 1993). The regular cyclic fluctuations in the prevalence and intensity of infections by trematodes under influence of temperature changes in the water bodies have been well documented (Al-Kandari et al. 2000). The contribution of water temperature in the progression of helminthes parasites was concluded in the present study. The temperature increased the growth period of parasites and shortened their generation time (Chubb 1980; Ernst et al. 2005). Several earlier studies have also demonstrated that numerous parasite life cycles, through developmental stage, were affected by changes in temperature (Salvati et al. 2002; Cattadori et al. 2005). The abundance of *Dactylogyrus* sp. was shown to increase at higher water temperature of the water body by Koskivaara (1992).

Table 2 One way ANOVA depicting biostatistical significance of infection prevalence (IP) and mean intensity (MI) of *C. yamunii* in both sexes of *X. cancella*

Source	Sum-of-squares	df	Mean-square	F-ratio <i>P</i>
<i>2008–2009</i>				
IP in female fish versus alkalinity				
Regression	1,920.710	1	1,920.710	$F_{1,10} = 4.754^*$
Residual	4,040.529	10	404.053	
MI in female fish versus water temperature				
Regression	101.160	1	101.160	$F_{1,10} = 2.538^*$
Residual	4,040.529	10	404.053	
MI in female fish versus alkalinity				
Regression	133.523	1	133.523	$F_{1,10} = 3.646^*$
Residual	366.241	10	36.624	
MI female versus chloride				
Regression	116.248	1	116.248	$F_{1,10} = 3.031^{\wedge}$
Residual	383.516	10	38.352	
IP in male versus hardness				
Regression	1,623.363	1	1,623.363	$F_{1,10} = 10.02^{\#}$
Residual	1,619.864	10	161.986	
IP in male versus alkalinity				
Regression	840.607	1	840.607	$F_{1,10} = 3.499^*$
Residual	2,402.620	10	240.262	
<i>2009–2010</i>				
IP in male versus chloride				
Regression	1,381.399	1	1,381.399	$F_{1,10} = 7.42^{\#}$
Residual	1,861.828	10	186.183	
IP in male versus calcium				
Regression	629.948	1	629.948	$F_{1,10} = 2.41^{**}$
Residual	2,613.280	10	261.328	
IP in female versus DO				
Regression	391.812	1	391.812	$F_{1,10} = 5.269^*$
Residual	743.666	10	74.367	
IP in female versus hardness				
Regression	122.683	1	122.683	$F_{1,10} = 1.211^{\wedge}$
Residual	1,012.796	10	101.280	
IP in female versus acidity				
Regression	82.015	1	82.015	$F_{1,10} = 0.779^{\wedge}$
Residual	1,053.464	10	105.346	
IP in female versus magnesium				
Regression	79.400	1	79.400	$F_{1,10} = 0.752^{\wedge}$
Residual	1,056.079	10	105.608	
MI in male versus water temperature				
Regression	155.063	1	155.063	$F_{1,10} = 4.153^*$
Residual	373.337	10	37.334	
MI in male versus DO				
Regression	171.597	1	171.597	$F_{1,10} = 4.809^*$
Residual	356.803	10	35.680	
MI in female versus water temperature				
Regression	49.304	1	49.304	$F_{1,10} = 1.85^{**}$
Residual	65.206	10	26.521	
MI in female versus DO				
Regression	69.928	1	69.928	$F_{1,10} = 2.85^{**}$
Residual	244.582	10	24.458	

Significant at * 0.025, $\wedge$ 0.250,
 $\#$ 0.005, and ** 0.100

Fig. 2 Correlation of infection prevalence by *C. yamunii* in female *X. cancilla* with dissolved oxygen (mg/l) during 2009–2010

Fig. 4 Correlation of mean intensity by *C. yamunii* in female *X. cancilla* with dissolved oxygen (mg/l) during 2009–2010

Fig. 3 Correlation of mean intensity by *C. yamunii* in male *X. cancilla* with dissolved oxygen (mg/l) during 2009–2010

The temperature optimum in this study, 23–27 °C was closely related to 26–27 °C as reported by Khan et al. (2003) for parasites of fishes in Meinhart and Manginia dam in Pakistan. The association of larval stages of trematodes with summer season in Pakistan fishes indicated that high water temperature revealed suitable conditions for their reproduction. The report by De et al. (1986) for development of *Procamallanus spiculogubernaculus* in the intermediate copepod hosts *Mesocyclops obsolatus* and *M. oithoruoides* to conclude 20–28 °C as the best temperature regime than the other two, 29–35 °C and 35–37 °C for the completion of larval stage between 3 and 8 days P.I. Therefore, the association of water temperature with the development stages of variety of helminthes is well established in literature.

The conclusions of peak infection by mature flukes in late summer and spring were in conformity with report of Skorping (1981) on *Bucephalopsis luciopercae* infections of *Perca fluviatilis* and Boxshall (1974) on *Lepeophtheirus pectoralis* infesting flat fishes in Great Britain. These

results were also closer to the conclusions of Gairola and Malhotra (1988) on the infections by *D. fusiformis* in *Eutropiichthys vacha*. The closest resemblance occurred with the spring and winter peak by trematode *Accacoeilium garuensis* in *Clupeisoma garua* in the same area of study by Jaiswal (2006).

It was concluded that DO and Magnesium play a key role in the dynamics of *C. yamunii*, that verified by the multivariate analysis, but the other extrinsic individual environmental factors such as hardness and alkalinity, enhanced due to the man made alterations in the natural water also play a significant impact on the establishment and abundance of the helminthes parasites in the freshwater aquatic fauna (Aznar et al. 1994; Krasnov et al. 2006).

Principal component analysis

A dominant 1st component ($PC1^P$) was observed from Principal Component Analysis of monthwise response of infection data by *C. yamunii* in *X. cancilla* (Figs. 5, 6, 7, and 8; Table 4). The Scree plot of Principal Component Analysis Eigenvalues (Fig. 5) and Factor Loadings plot (Figs. 6, 7, 8) also confirmed the predominance of the 1st component (Table 4). The influence of certain pollution parameters, viz. hardness (2009–2010, +0.815; Table 4; Figs. 6, 7) had a critical impact on the oscillations in monthly and seasonal cycle of helminth prevalence and mean intensity. Principal component analysis of infection variables of mean intensity (2008–2009: female, 0.834; 2009–2010: male, 0.737, Table 4) by *C. yamunii* in *X. cancilla* showed a first component ($PC1^P$) accounting for 27.074% (2008–2009, Table 4) and 28.022% (2009–2010, Table 4) of total variation during the period of maximum change in DO (2008–2009, 0.851, Table 4) and hardness (2009–2010, 0.815, Table 4). Simultaneously, bio-ecological significance of significant variations in larger values of water temperature

Table 3 Mann–Whitney test one-way ANOVA, to substantiate correlation of physico-chemical parameters with infectivity attributes during 2009–2010

Group	Count	Rank sum	
IP male versus DO			
4.7	1	2.000	Mann-Whitney test χ^2 statistic = 7.872 $P < 0.55$ df 9
5.4	1	6.000	
5.5	1	5.000	
5.9	1	11.000	
6	3	23.000	
6.3	1	4.000	
6.4	1	10.000	
6.5	1	1.000	
6.7	1	9.000	
7.7	1	7.000	
7.4	1	6.00	
MI male versus Mg ⁺⁺			
5.41	1	2.000	Mann-Whitney test χ^2 statistic = 9.611 $P < 0.50$ df 10
8.74	1	6.000	
9.87	1	1.000	
12.54	1	9.000	
14.56	1	3.000	
15.62	1	8.000	
18.3	1	11.500	
21.08	1	11.500	
24.3	2	14.000	
30.5	1	7.000	
47.4	1	5.000	
MI female versus Mg ⁺⁺			
5.41	1	6.000	Mann-Whitney test χ^2 statistic = 9.615 $P < 0.50$ df 10
8.74	1	4.000	
9.87	1	2.000	
12.54	1	8.000	
14.56	1	1.000	
15.62	1	9.000	
18.3	1	10.000	
21.08	1	12.000	
24.3	2	16.000	
30.5	1	3.000	
47.4	1	7.000	

(2008–2009, -0.670 ; 2009–2010, -0.501 , Table 4) could not be ruled out. Thereby, the temperature optimum 20°C as well supported by Principal component analysis (2008–2009, -0.670 ; 2009–2010, -0.501 , Table 4) and other substantiating inferences concluded by multivariate analysis, the negative fluctuations monthly values of infection prevalence in male *X. canicilla* ($Y = 45.340 + 0.560X_1 - 2.871X_2 - 0.148X_3 + 0.056X_4 - 0.762X_5 + 0.358X_6 + 0.045X_7 - 0.688X_8$), however, female *X. canicilla* ($Y = 170.859 - 2.058X_1 - 11.263X_2 + 0.367X_3 + 0.141X_4 - 1.286X_5 -$

$0.657X_6 - 0.723X_7 - .534X_8$), as well as mean intensity in male ($Y = 3.359 - 0.601X_1 + 9.154X_2 + 0.224X_3 - 0.032X_4 - 0.757X_5 - 0.605X_6 + 0.253X_7 - 0.738X_8$) and female ($Y = 38.539 - 0.730X_1 - 1.462X_2 - 0.093X_3 + 0.027X_4 + 0.225X_5 - 0.179X_6 + 0.113X_7 + 0.344X_8$) were apparently independent of rapid changes in DO of the water body. These findings of larger $PC1^P$ positive coefficients comprehensively substantiated predominating hardness factor, under the influence of enhanced DO and optimum thermal effect. The high level of significance depicted by Pearson's correlation matrix for effect of hardness on infection prevalence in male fish (2009–2010, PCM, -0.707), of DO in infection prevalence in female fish (2009–2010, PCM, 0.587) and of DO on mean intensity in female (2009–2010, PCM, 0.552), further strengthened the viewpoint that the physico-chemical factors did not operate in isolation (Aznar et al. 1994; Combes 2001; Krasnov et al. 2005; Fenton 2007; Poulin and Morand 2004; Krasnov et al. 2008; Barrett 2009; Kennedy 2009). The evidence therefore, is available in this study to indicate that the association of infection prevalence and mean intensity was independent of fluctuations in DO of water, particularly because of the continuous availability of infective larvae of trematodes in the area of study that lead ultimately two separate waves of infection in summer and winter periods (Kennedy 1968). Therefore, the evidence of multifactorial etiology is available. And thus no single factor could be segregated for typical twin peaks of infections during late summer and winter periods. The multivariate analysis, however, confirmed dominating influence of Dissolved Oxygen (Figs. 1, 2, 3, 4) as well as Magnesium (Tables 2, 3), as also verified by high levels of significance obtained on Mann–Whitney's test (2009–2010: DO—infection prevalence male, $\chi^2 = 0.547$; Mg^{++} —mean intensity male, $\chi^2 = 0.50$; mean intensity female, $\chi^2 = 0.50$) and on Pearson correlation matrix (2008–2009: DO vs mean intensity female, PCM, 0.552 ; alkalinity vs infection prevalence female, PCM, -0.568 and mean intensity female, PCM, 0.517 ; free CO_2 vs infection prevalence male, PCM, -0.575 ; 2009–2010: Water temperature vs mean intensity male, PCM, -0.542 ; DO vs infection prevalence female, PCM, 0.587 and mean intensity male, PCM, 0.570 ; Hardness vs infection prevalence male, PCM = -0.707 ; Alkalinity vs infection prevalence male, PCM, -0.509 and Chloride vs infection prevalence male, PCM, -0.653).

Several parasite ecologists have opined that the complete life cycle of helminthes necessitated trophic transmission from one host to other, by the consumption of infected intermediate hosts (Genc et al. 2005). The parasite carrying capacity of predator fish might essentially be higher (Amundsen et al. 2003). Since *X. canicilla* (Belonidae) were predators, the prevalence of infestations of gar fish are worth being taken into account.

Table 4 The magnitude of pattern of PC1^P coefficient for sexwise infection by *C. yamunii* in *X. cancella* during 2008–2009 and 2009–2010

1	2	3	4	5	6	7	8	9	10	11	12
<i>2008–2009</i>											
Latent roots (Eigenvalues)											
3.249	2.532	2.107	1.576	1.180	0.506	0.379	0.228	0.156	0.082	0.005	0.000
		1		2		3		4		5	
<i>Component loadings</i>											
WATTEMP		–0.670		0.028		0.115		0.655		–0.023	
DO		0.851		0.102		0.139		–0.165		–0.046	
HARDNESS		0.081		0.759		0.447		0.062		0.357	
ALKALINITY		0.534		–0.315		0.658		–0.147		0.223	
ACIDITY		0.527		0.290		–0.571		–0.290		0.336	
CHLORIDE		–0.134		0.530		0.495		–0.578		–0.200	
CALCIUM		0.443		0.343		0.351		0.130		–0.630	
MAGNESIUM		0.218		0.570		0.418		0.590		0.169	
IPMALE		–0.341		–0.594		0.502		–0.281		–0.289	
IPFEMALE		–0.108		–0.615		0.562		0.031		0.497	
MIMALE		0.650		–0.335		–0.049		0.439		–0.292	
MIFEMALE		0.834		–0.443		–0.052		0.197		0.070	
1		2		3		4		5			
<i>Variance explained by components</i>											
3.249		2.532		2.107		1.576				1.180	
<i>Percent of total variance explained</i>											
27.074		21.103		17.561		13.132				9.835	
1	2	3	4	5	6	7	8	9	10	11	12
<i>2009–2010</i>											
Latent roots (Eigenvalues)											
3.363	2.993	2.171	1.163	0.849	0.604	0.419	0.280	0.077	0.066	0.016	0.000
		1		2		3		4			
<i>Component loadings</i>											
WATTEMP		–0.501		–0.506		0.411				0.376	
DO		0.333		0.681		0.253				0.089	
HARDNESS		0.815		–0.189		–0.469				–0.103	
ALKALINITY		0.455		–0.606		0.452				0.277	
ACIDITY		0.431		–0.156		0.523				–0.012	
CHLORIDE		0.353		–0.590		0.243				–0.593	
CALCIUM		0.550		–0.454		–0.137				0.592	
MAGNESIUM		0.177		0.325		–0.807				0.066	
IPMALE		–0.731		0.476		0.095				0.277	
IPFEMALE		0.014		0.599		0.652				–0.210	
MIMALE		0.737		0.564		0.237				–0.000	
MIFEMALE		0.638		0.533		0.143				0.313	
1		2		3		4					
<i>Variance explained by components</i>											
3.363		2.993		2.171		1.163					
<i>Percent of total variance explained</i>											
28.022		24.939		18.091		9.691

Fig. 5 Screen plot of principal component analysis of *C. yamunii* in *X. cancella* during 2008–2009

Fig. 7 The factor loadings plot of infection prevalence of *C. yamunii* in female during 2008–2009

Fig. 6 The factor loadings plot of infection prevalence of *C. yamunii* in male during 2008–2009

Fig. 8 *X. cancella* vis-à-vis water temperature, dissolved oxygen, alkalinity, hardness, acidity and chloride, and mean intensity in male and female fish vis-à-vis magnesium during 2008–2009

Table 5 Multivariate analysis data to conclude predominant interrelationships of hydrobiological factors

2008-2009:								
IPMALE- $r=0.791$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg*	
$Y=45.340+0.560X_1-2.871X_2-0.148X_3+0.056X_4-0.762X_5+0.358X_6+0.045X_7-0.688X_8$								
IPFEMALE- $r=0.943$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg	
$Y=170.859-2.058X_1-11.263X_2+0.367X_3+0.141X_4-1.286X_5-0.657X_6-0.723X_7-0.534X_8$								
MIMALE- $r=0.777$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg	
$Y=3.359-0.601X_1+9.154X_2+0.224X_3-0.032X_4-0.757X_5-0.605X_6+0.253X_7-0.738X_8$								
MIFEMALE- $r=0.897$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg	
$Y=38.539-0.730X_1-1.462X_2-0.093X_3+0.027X_4+0.225X_5-0.179X_6+0.113X_7+0.344X_8$								
2009-2010:								
IPMALE- $r=0.906$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg	
$Y=133.282-0.678X_1+1.876X_2-0.499X_3-0.037X_4+1.048X_5-0.348X_6+0.271X_7+0.625X_8$								
IPFEMALE- $r=0.843$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg	
$Y=-41.650+1.120X_1+9.510X_2+0.106X_3-0.082X_4+0.547X_5-0.048X_6-0.359X_7-0.436X_8$								
MIMALE- $r=0.828$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg	
$Y=39.968-1.892X_1+2.311X_2-0.201X_3+0.079X_4+0.612X_5+0.154X_6+0.162X_7+0.401X_8$								
MIFEMALE- $r=0.732$								
°C	DO	Hardness	Alk	Acidity	Cl	Ca	Mg	
$Y=34.364-1.190X_1+1.356X_2-0.123X_3+0.068X_4+0.183X_5-0.077X_6+0.146X_7+0.238X_8$								

°C water temperature, DO dissolved oxygen, Alk alkalinity, Cl chloride, Ca calcium, Mg magnesium

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