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Municipal wastewater can result in a dramatic decline in freshwater fishes: a lesson from a developing country

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Abstract – Impacts of ineffective wastewater management on the biodiversity of receiving waters in developing countries are poorly documented. Using a before-after-control-impact methodology, we measured the effects of untreated wastewater release on the fish community in the Barnoi River, Bangladesh. In 2006, prior to untreated wastewater discharge, fish abundance, species richness and water quality were similar across sampling sites. In 2016, after 8 years of wastewater release to the downstream reach, fish abundance and species richness were reduced by >47% and >35% respectively at downstream sites compared to unaffected upstream sites and >51% and >41% lower respectively compared to the pre-wastewater discharge period. The wastewater impact was particularly severe during months of low discharge (October–December). Water transparency, dissolved oxygen and pH were lower ($P < 0.001$) at impacted downstream sites compared to upstream sites. Nineteen species (41.3% of all species we recorded) are threatened in Bangladesh and the abundance of these species, except one, decreased significantly ($P < 0.05$) at the impacted sites. We recommend improved wastewater management by applying primary treatment facilities and incorporating reedbed filtration as a mean of biological treatment, into the canals carrying wastewaters. The success of such measures should be tested with fish species that were most responsive to wastewater, using the indicator species concept.

Keywords: Wastewater / water pollution / biodiversity loss / threatened fish / BACI

Résumé – Les eaux usées municipales peuvent entraîner une baisse spectaculaire du nombre de poissons: un exemple d'un pays en développement. Les impacts d'une gestion inefficace des eaux usées sur la biodiversité des eaux réceptrices dans les pays en développement sont mal documentés. En utilisant une méthodologie BACI, nous avons mesuré les effets des rejets d'eaux usées non traitées sur la communauté de poissons de la rivière Barnoi, au Bangladesh. En 2006, avant le rejet des eaux usées non traitées, l'abondance des poissons, la richesse en espèces et la qualité de l'eau étaient semblables dans tous les sites d'échantillonnage. En 2016, après 8 ans de rejet d'eaux usées dans le bras aval, l'abondance des poissons et la richesse en espèces ont été réduites de >47% et >35% respectivement sur les sites en aval par rapport aux sites en amont non affectés et >51% et >41% respectivement par rapport à la période de rejet avant le rejet des eaux usées. L'impact des eaux usées a été particulièrement grave pendant les mois de faible débit (octobre-décembre). La transparence de l'eau, l'oxygène dissous et le pH étaient inférieurs ($P < 0,001$) aux sites impactés en aval par rapport aux sites en amont. Dix-neuf espèces (41,3% de toutes les espèces que nous avons recensées) sont menacées au Bangladesh et l'abondance de ces espèces, sauf une, a diminué de façon significative ($P < 0,05$) dans les sites touchés. Nous recommandons d'améliorer la gestion des eaux usées en appliquant des

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installations de traitement primaire et en incorporant la filtration sur lit de roseaux comme moyen de traitement biologique dans les canaux transportant les eaux usées. Le succès de ces mesures devrait être testé avec les espèces de poissons les plus sensibles aux eaux usées, en utilisant le concept d'espèces indicatrices.

Mots-clés : Eaux usées / pollution de l'eau / perte de biodiversité / poissons menacés / BACI

1 Introduction

Worldwide, freshwater habitats are being exposed to a complex mixture of stressors including water pollution and habitat degradation (Dudgeon *et al.*, 2006; Dirzo *et al.*, 2014), threatening the diversity of life (Moss, 2008; Vorosmarty *et al.*, 2010). Rivers can be particularly vulnerable to receiving chemical stressors from anthropogenic origin, including organic matter and inorganic nutrients (Moss, 1998), and micropollutants such as pesticides or industrial products (Meybeck, 2004; Sabater *et al.*, 2016). Part of the reason for this is that rivers are employed to dilute and transport treated wastes away from human populations. The impacts of pollution and other stressors on freshwater ecosystems can be detrimental because decreasing biodiversity reduces the ability of ecological communities to provide many fundamental ecosystem services (Keesing *et al.*, 2010). However, the intensity of threats to freshwater biodiversity in tropical Asia is exceptional compared to other parts of the world because of a complex mixture of different factors (*e.g.* dense population, deforestation, and flow regulation; see Dudgeon, 2005 for details).

While the impacts and remediation of freshwater pollution, especially organic pollution, have a long and well-recorded history in the developed world (Hynes, 1963; Mason, 2001), wastewater is a serious and emerging problem in the majority of developing countries (*e.g.* Girija *et al.*, 2007; Omosa *et al.*, 2012; Haque, 2017). This is primarily because industrial, agricultural, municipal and sewage discharge cause deterioration of receiving water quality (Dudgeon, 1992; Vaseem and Banerjee, 2013; Paul, 2017) and make freshwater habitats unfavourable for natural communities of micro and macro-organisms living in the system (Karaouzas *et al.*, 2018). The majority of research carried out on wastewater pollution in Asia focuses on changes in the receiving water's physico-chemical quality and sediments (*e.g.* Kumar and Reddy, 2009; Dai *et al.*, 2018; Rajeshkumar *et al.*, 2018; Ramachandra *et al.*, 2018); impacts on invertebrate communities (*e.g.* Morse *et al.*, 2007 summarised the status of East Asian countries) or impacts on the physiology of individual fish species (*e.g.* Sanchez *et al.*, 2011; Vaseem and Banerjee, 2013; Yazıcı and Sisman, 2014). Few studies have reported impacts of water pollution on fish communities in tropical Asia (except see Dudgeon, 1992, 2002; Mulk *et al.*, 2016). However, these fish studies often lack long-term data that can explain responses of aquatic biota to stressors and that can quantify changes in the target community over time and space.

In Bangladesh, water pollution has resulted from unplanned urbanization and industrialization and is of high concern (Ali *et al.*, 2016; Kibria *et al.*, 2016; Haque, 2017). Primarily with a perspective of human health risks, several studies have been conducted with a view to assessing metal concentrations in waters (*e.g.* Ali *et al.*, 2016; Kibria *et al.*, 2016; Bhuyan and Bakar, 2017) and also in the bodies of aquatic animals living in polluted waters (*e.g.* Ahmed *et al.*, 2015; Islam *et al.*, 2015). Although it is believed that freshwater fishes in inland waters of Bangladesh have declined markedly because of pollution (*e.g.* Hussain, 2010), no studies have been carried out to date to address its actual impacts on fish communities. Although elevated levels of heavy metals are common in urban or industrial wastewaters, oxygen-demanding organic waste is often the predominant polluting component and has the potential to cause dramatic changes in biodiversity in tropical freshwaters (Dudgeon, 2002). Therefore, we sought to determine the extent of degradation to natural biota due to long-term discharge of wastewaters from nearby municipal, agricultural and small-scale industrial sources, using the Barnoi River in northern Bangladesh as a case study.

In this study, we analysed changes in fish abundance, species richness and water quality parameters over time and space in relation to current wastewater management practice using a before-after-control-impact (BACI) approach. We hypothesized that wastewater disposal would have negative impacts on the fish abundance, species richness and water quality at the sampling sites receiving wastewaters compared to unaffected sites in the Barnoi River.

2 Methods

2.1 Study area

The Barnoi River (Fig. 1), whose upstream part is known as the Shiva River, originates from the Manda floodplain in the Tanore sub-district of Rajshahi district (Bashar *et al.*, 2009), in north-west Bangladesh. The Barnoi channel can be up to 25 m wide and 3 m deep during the wet season (June–September). This river joins the Gumani River (at $24°33'27''$ N $88°48'34''$ E) at Bagmara Upazila (sub-district) of Rajshahi district.

In 1994, the Rajshahi City Corporation (local municipality) passed a plan for the construction of two canals that would drain municipal wastewaters into the Barnoi River. The construction works finished in December 2008 and became operational immediately and since then the river has continuously been receiving wastewaters from Rajshahi City Corporation through two canals (each 5.1 m wide and 2.4 m high; Fig. 1). These wastewaters contain untreated kitchen wastes, water containing detergent from households, run-off from streets and often faeces and urine (this is not legal but many households illegally installed underground pipes between sewage tanks and drains; also when sewage tanks become full many residents empty their tanks by draining the faeces into nearby drains). Currently, there is no
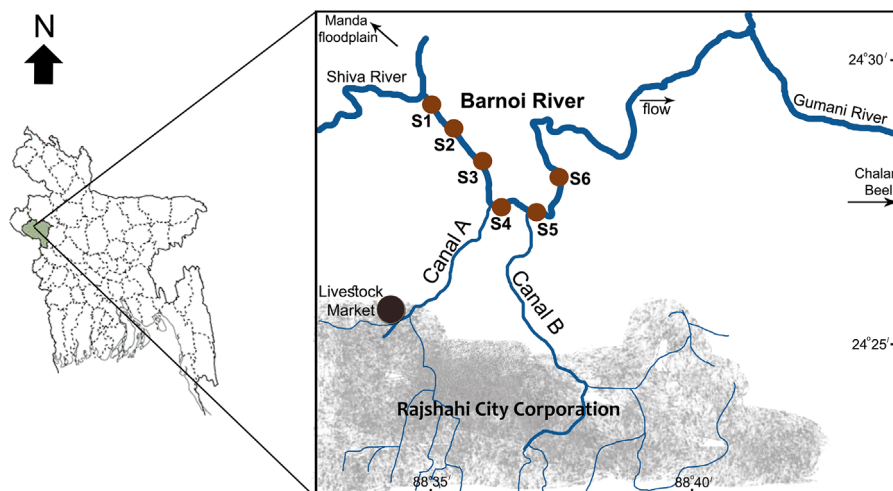
Fig. 1. Map of the Barnoi River showing sampling sites (S_1–S_6) and the two major canals (A and B) that carry untreated wastewater to the river.

wastewater-treatment facility in the study area and thus untreated wastewaters are being drained directly into the river. Moreover, a large livestock wholesale market (locally know as City Hat) is situated by one of these canals (canal A; Fig. 1) and dead animals (primarily cattle, goats and buffalo) and organic waste products from slaughtered animals from this market are often thrown into the canal. Canal A also receives pharmaceutical and clinical waste (used medicine bottles, syringes, bandages, etc.; Fig. S1). These two canals join the Barnoi near Duari and Nowhata points (sampling points S_4 and S_5 in this study, Fig. 1) and there has been a concern for its impact on aquatic biodiversity and riverine habitat among local people since the wastewater canals opened. Reduced fish catch and bad odour from harvested fish have been reported by the local people in these areas. Local fishermen also reported complete loss of a fish breeding ground located between sampling site S_5 and S_6 due to untreated wastewater mixing with the river water (T. Pervez and S. Arnob, pers. obs.).

Although two sluices have been built to regulate the waters in the canals joining the Barnoi the untreated wastewater has continuously flowed into the river through broken and partially opened gates (Fig. S2). These sluices were once managed by the government officials but since 2015 no personnel have been appointed for this purpose and they were being operated (*i.e.* fully opened) by local people whenever needed depending on the accumulation of wastewater. Although there are no statistics of wastewater flowing into the Barnoi River, during the dry season when the river water becomes almost stagnant, the wastewater from these two canals constitutes the only water flow.

2.2 Fish sampling and study approach

Fish sampling at six sites (S_1–S_6; Fig. 1) in the Barnoi River was conducted on eight occasions between July and December in each of 2 years, 2006 (before the wastewater canals opened) and 2016 (8 years after the wastewater canals opened). Each sampling site was ~3 km distant from neighbouring sites. From January to June the water depth in the river became too shallow for the operation of fishing gears and thus it was not possible to carry out sampling. Sampling dates were not the same in both years (varied by ±3 days) but the number of samples in each month in both years were the same. All samples at all sites in 2006 represent pre-waste outflow conditions. In 2016, three sampling sites (S_4–S_6) were affected by the wastewater carried through two channels as these sites were located downstream of the outlets (Fig. 1) and samplings carried out that year represent post-waste outflow samples. This setup enabled a balanced BACI analysis approach (Downes *et al.*, 2002; Boys *et al.*, 2012) to be used in this study. In this study, sampling years (2006 *vs.* 2016) represents "before–after (BA)", and location (upstream [S_1–S_3]/downstream [S_4–S_6]) resembles "control-impact (CI)" sites of a BACI design.

All sampling sites appeared similar in habitat with a sand and mud bottom (about 50:50 ratio), with a channel width of ~30 m and gently shelving banks to a maximum depth of 4–6 m at the peak of the flood. In 2006, the water depth was quite similar between upstream and downstream sites over the sampling period. In 2016, although the water depth was similar (4–6 m) between upstream and downstream sites during the wet period (July–September) it was slightly greater at downstream sites (2.5 ± 0.2 m) during the dry season (October–December) compared to upstream sites (2.2 ± 0.2 m). No water flow measurements were taken but it was noticed that there was almost no flow at the upstream sites over the drying months whereas slow-flowing water (<0.05 ms^{-1}) was noticed at the downstream sites. It may be assumed that these differences in water depth and flow at downstream sites were due to wastewater addition to sites located downstream. Vegetation on both banks of the river was similar at all the sampling sites in both years. In 2006, the water surface near both banks at all the sampling sites were partially covered (~10%) by marginal aquatic macrophytes, *i.e. Alternanthere*, *Ipomoea*, and *Marsilea* spp. But marginal macrophyte vegetation reduced to below 5% at the downstream sites in 2016 with a blackish/greyish layer of silt on the macrophyte leaves, whereas the upstream part remained almost unchanged (8–10% cover).

At the sampling sites, on a given occasion, fishes were collected with three cast nets (mesh 10×10 mm; $\pi \times 1.25^2$ m = 4.9 m^2), two lift nets (mesh 10×10 mm, 5×4.5 m) and three rectangular fishing traps locally known as "Kholsun" (made of split bamboo sticks; mesh 20×20 mm, $0.8 \times 0.8 \times 0.1$ m). Fishing traps were set in the evening before the day of sampling and left overnight (approximately 15 h) in the water. The lift nets were hauled five times at each sampling site and the total fishing duration was about one hour. The cast nets were hauled 15 times at each sampling site and the fishing duration was about 1.5 h. The combination of these fishing gears was effective in sampling fishes of a wide range of species and sizes from different depth strata (Galib *et al.*, 2018). All the fishing gears were operated over the same time on every sampling date to standardize the sampling effort. The fish abundance data gathered therefore represent Catch Per Unit Effort (CPUE), enabling the comparison of relative abundance between locations and dates. Individuals were identified to species and counted at the sampling sites. Specimens that were difficult to identify on site were preserved in 10% formaldehyde solution and brought to the laboratory for identification. Other fish sampled were returned to the river unharmed. Identification was made based on morphometric and meristic characters following Rahman (2005) and Talwar and Jhingran (1991). Scientific names and systematic positions of fishes follow those of Froese and Pauly (2017).

Reference to the global conservation status categories in this paper (*e.g.* Least Concern, Near Threatened, Vulnerable) are those in the online classification database developed by the International Union for the Conservation of Nature and Natural Resources (IUCN, 2018). National conservation categories are those published in the "Red List of Bangladesh" (IUCN Bangladesh, 2015).

Physico-chemical parameters (water temperature, water transparency, dissolved oxygen [DO], and pH) were measured at each sampling point. Water temperature was measured by using a digital thermometer (Hanna), DO and pH were measured using portable water quality test kits (HACH Kit Box, Model FF1 and FF2; USA) and water transparency was measured using a Secchi disk. pH and DO tests remained consistent against laboratory calibrations; although pH was measured only with a precision of 0.5 of a pH unit, this was adequate to detect the substantial changes evident in the polluted zone.

2.3 Data analysis

The statistical software R was used to analyse the data (version 3.4.2; R Core Team, 2017), with an α level of significance of 0.05. Linear mixed modelling (LMM) was employed to analyse repeated measures fish abundance and richness data using the "lmer" function of the "lme4" package (Bates *et al.*, 2014); *p*-values were obtained by the "lmerTest" package (Kuznetsova *et al.*, 2016). During LMM location of the sampling sites, sampling years and their interaction were tested as fixed effects, and sampling sites and time were considered random effects. Least square means (*i.e.* marginal mean; Lenth, 2017) were also calculated to estimate the BACI contrast. Least square means are useful in the analysis of data for summarizing the effects of factors, and for testing linear contrasts among predictions including interaction contrasts like BA $\times$ CI (Schwarz, 2015; Lenth, 2017).

A Non-metric Multidimensional Scaling (NMDS; Kruskal and Wish, 1978) ordination plot was generated to visualize spatial and temporal variation of fishes using the "metaMDS" function of the "vegan" package (Oksanen *et al.*, 2017). Fish CPUE abundance data were used to calculate Similarity Percentage Analysis (SIMPER), based on the decomposition of Bray-Curtis dissimilarity index (Clarke, 1993) was used to identify those native taxa that were most important in driving changes in species abundance among control and impacted sites.

Changes in water quality parameters were also analysed using LLMs outlined previously. The BIOENV procedure (Clarke and Ainsworth, 1993) was used to determine how well the patterns in water quality parameters correlated (Pearson's) with the changing assemblage patterns of fish (Boys *et al.*, 2012). We ensured the exploration of data during analyses to avoid common statistical problems (Zuur *et al.*, 2010). Before analysis data were checked for normality by Shapiro–Wilk test (Peat and Barton, 2005) and necessary transformations (square-root transformation for abundance data (McDonald, 2014) and log $(x + 1)$ transformation for water quality data (Clarke, 1993)), were made to meet the statistical assumption for the tests. Normality of the model residuals were also checked with a q–q plot and no deviation from the linearity of the observations was observed.

3 Results

3.1 Fish fauna

In total, 60 973 fish specimens belonging to 46 species, 35 genera and 18 families were collected and identified (Tab. 1). Three species (*Sisor rabdophorus*, *Chaca chaca* and *Nandus nandus*) were present in pre-waste outflow samples but were absent in 2016 samples (Tab. 1).

In the Barnoi River, the NMDS ordination plot shows similar species assemblages in the six sites sampled in 2006 (Fig. 2). In contrast, in 2016, three downstream sites (S_4–S_6) showed a notable shift in assemblage composition from before to after the starting of the wastewater release into the river. A similar shift was not observed in the upstream sites (S_1–S_3) over the same time period (Fig. 2). Although lower fish abundance and richness were observed at downstream sites in 2016, the difference was comparatively small from July to September but when the water level began to recede (September), the difference was more evident (Fig. 3).

3.2 Fish abundance, species richness and water quality parameters in relation to waste dumping

Levels of fish abundance (measured as CPUE) and fish species richness were similar between sampling sites before starting the waste dumping in the Barnoi River and reduced dramatically at the effluent-receiving downstream sites in recent times (Figs. 3 and 4). Significant variations in fish abundance and species richness (both $P \leq 0.001$) between sampling locations (upstream *vs.* downstream; CI) and period (before *vs.* after; BA) were evident from LLM results (Tab. 2). Interaction effects (BA $\times$ CI) on fish abundance and species

Table 1. Fish species caught at different locations (upstream; sites S_1–S_3; downstream; sites S_4–S_6) in the Barnoi River during the study period.

Fish species	2006 Upstream	Downstream	2016 Upstream	Downstream	Conservation BD[a]	Global[b]
Belonidae						
Xenentodon cancila	296	300	204	143	LC	LC
Clupeidae						
Gudusia chapra	403	437	456	252	VU	LC
Corica soborna	275	201	138	26	LC	LC
Engraulidae						
Setipinna phasa	281	241	137	30	LC	LC
Cyprinidae						
Amblypharyngodon mola	1145	1279	1371	779	LC	LC
Aspidoparia morar	199	224	88	3	VU	LC
Cirrhinus mrigala	109	131	54	4	NT	LC
Cirrhinus reba	170	187	147	46	NT	LC
Esomus danricus	391	401	318	43	LC	LC
Labeo bata	276	264	193	21	LC	LC
Labeo rohita	177	187	121	4	LC	LC
Puntius chola	502	494	588	152	LC	LC
Puntius sophore	2118	2136	2248	1111	LC	LC
Puntius ticto	239	267	152	56	VU	LC
Salmostoma phulo	719	796	812	445	NT	LC
Cobitidae						
Botia dario	287	225	218	42	EN	LC
Botia lohachata	80	64	47	30	EN	NE
Lepidocephalichthys guntea	567	513	515	389	LC	LC
Bagridae						
Mystus cavasius	435	444	413	282	NT	LC
Mystus tengara	1057	1046	1018	772	LC	LC
Mystus vittatus	283	282	179	57	LC	LC
Rita rita	269	268	239	48	EN	LC
Sperata seenghala	238	235	75	20	VU	LC
Chacidae						
Chaca chaca	21	9	0	0	EN	LC
Schilbeidae						
Ailia coila	553	643	544	111	LC	NT
Clupisoma garua	345	329	210	19	EN	LC
Eutropiichthys vacha	322	327	215	7	LC	LC
Pachypterus atherinoides	438	404	276	102	LC	NE
Siluridae						
Ompok bimaculatus	170	158	131	5	EN	NT
Wallago attu	71	59	24	3	VU	NT
Sisoridae						
Gagata cenia	410	441	512	91	LC	LC
Sisor rabdophorus	8	4	0	0	CR	LC
Ambassidae						
Chanda nama	1053	977	1377	1104	LC	LC
Parambassis lala	92	79	90	129	LC	NT
Parambassis ranga	1094	1009	1189	949	LC	LC
Anabantidae						
Anabas testudineus	191	214	103	73	LC	DD
Channidae						
Channa punctata	197	209	92	59	LC	LC
Channa striata	56	83	12	2	LC	LC

Table 1. (continued).

| Fish species | 2006 | | 2016 | | Conservation | |
	Upstream	Downstream	Upstream	Downstream	BD[a]	Global[b]
Gobiidae						
Glossogobius giuris	836	876	872	329	LC	LC
Nandidae						
Nandus nandus	74	52	0	0	NT	LC
Osphronemidae						
Trichogaster fasciata	499	541	440	560	LC	LC
Trichogaster lalius	126	147	91	205	LC	LC
Sciaenidae						
Otolithoides pama	66	62	43	0	LC	NE
Mastacembelidae						
Macrognathus aculeatus	109	100	30	2	NT	NE
Macrognathus pancalus	347	353	262	116	LC	LC
Mastacembelus armatus	282	270	206	58	EN	LC

BD, Bangladesh; CR, critically endangered; EN, endangered; LC, least concern; NE, not evaluated; NT, near threatened; VU, vulnerable.
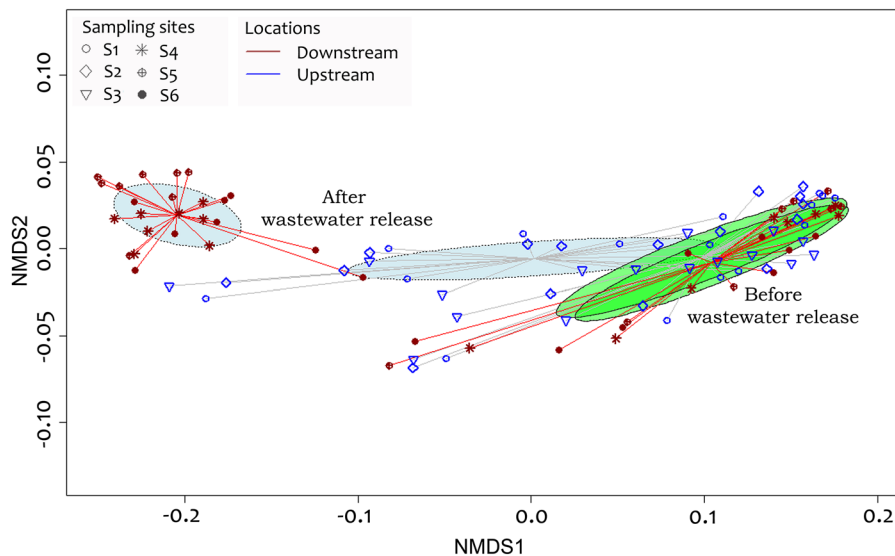[a] as per IUCN Bangladesh (2015).
[b] IUCN (2018).

Fig. 2. Non-metric multidimensional scaling (NMDS) ordination plot showing spatial and temporal variations of fish abundance in relation to wastewater release into the Barnoi River.

richness were also significant (all $P < 0.001$; Tab. 2). Least square means also indicated that both fish species richness and their abundance reduced at the downstream sites in recent times compared to other combinations (*i.e. vs.* CB or IB or CA; Tab. 2).

Similar to fish abundance and species richness, patterns of different physico-chemical parameters of water, except water temperature, also varied significantly over time (before *vs.* after) and space (upstream *vs.* downstream) (all $P < 0.01$; Fig. 5; Tab. 2). Least square means also indicated that pH, DO and water transparency reduced at the downstream sites in recent times compared to other combinations (*i.e. vs.* CB or IB or CA Tab. 2).

BIOENV analysis revealed that DO and pH collectively were strongly correlated with the fish assemblage patterns observed in the Barnoi River ($\rho = 0.731$), and showed a significant response to waste dumping practices in the river (Tab. 2). There was an overall decrease in both DO and pH from before to after wastewater release across all sampling sites receiving waste waters (S_4–S_6) when compared to upstream sites (S_1–S_3).

SIMPER test results (Tabs. S1–S4) revealed that the CPUE of only four native fish species (*Wallago attu*, *C. chaca*, *S. rabdophorus* and *N. nandus*) varied significantly ($P < 0.05$) between upstream and downstream sites before wastewater releases (Tab. S1). Subsequent to wastewater releases, the
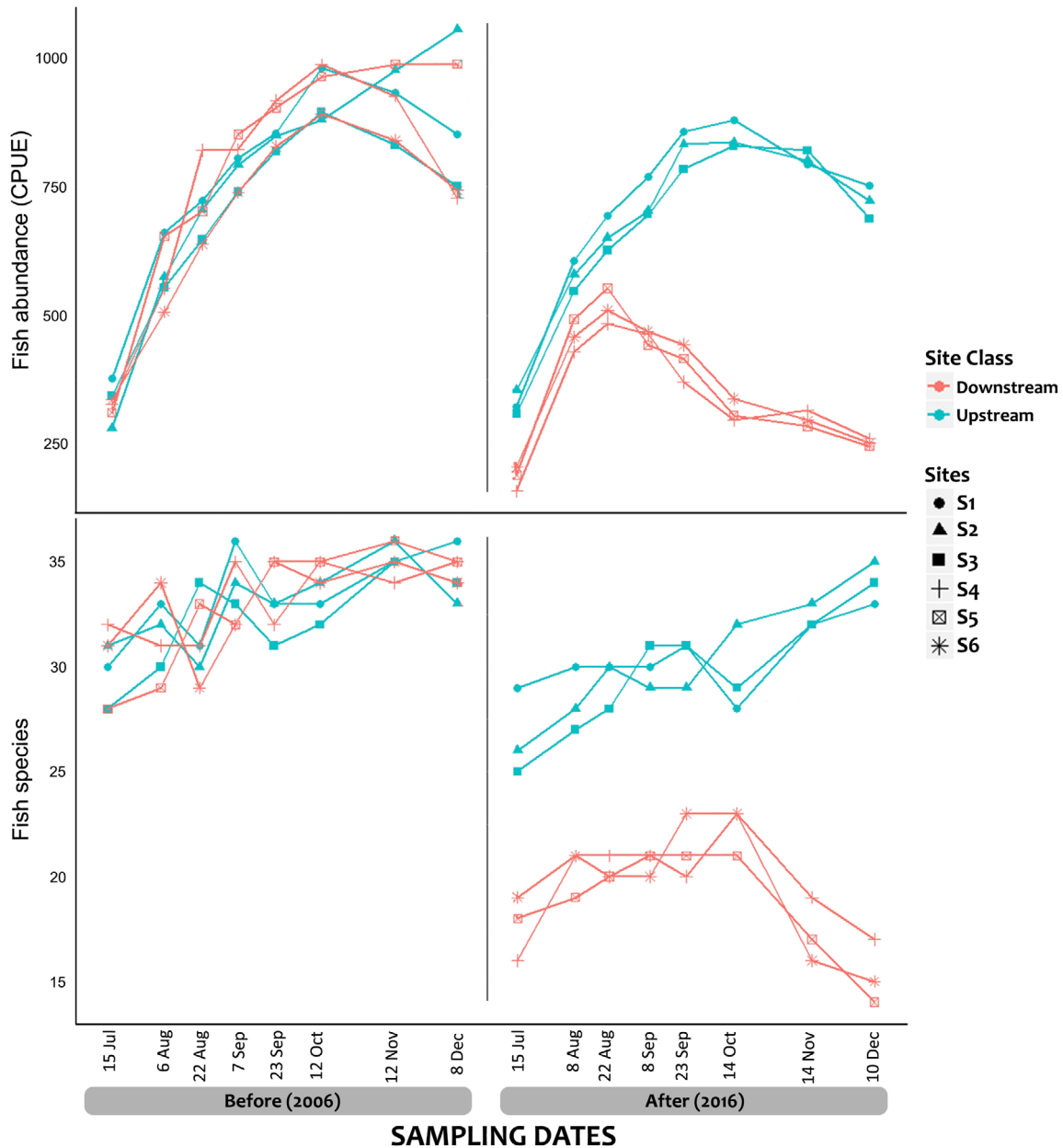
Fig. 3. Fish abundance (as CPUE) and species richness over time at different sites in the Barnoi River. Before and After refer to data gathered prior to and after the commencement of wastewater release in the middle part of the study section.

abundance of 25 species varied significantly ($P < 0.05$) between upstream and downstream sites and 16 species contributed >70% of the dissimilarity in fish assemblages (Tab. S2). Again, assemblages at the downstream sites (S_4–S_6) varied widely between time periods where 39 species (84.8% of the total species) varied significantly ($P < 0.05$; Tab. S3) whereas no significant temporal changes, except for one species (*N. nandus*), were observed in upstream sites (Tab. S4). In this case 21 native taxa contributed >70% of the dissimilarity in fish abundance. The majority of the indigenous taxa contributing to the dissimilarity among upstream *vs.* downstream [in 2016] and between downstream sites over time (2006 *vs.* 2016) were small indigenous species (Tabs. S2–S4).

3.3 Threatened taxa

Nineteen species (41.3% of all species) recorded in the present study are classed as of conservation concern in Bangladesh (critically endangered, one sp.; endangered, seven spp.; vulnerable, five spp.; near threatened, six spp.; Tab. 1). Among these, three species, between time periods, were absent in post-wastewater release (2016) catches, both downstream and upstream of the wastewater inputs. *Otolithoides pama* was absent downstream of the wastewater input in 2016, but present upstream. Among remaining threatened fish species ($n = 16$), abundance of all but one species (*Botia lohachata*) decreased significantly ($P < 0.05$) at the downstream sites following wastewater releases (Tab. S3). Similar to overall fish
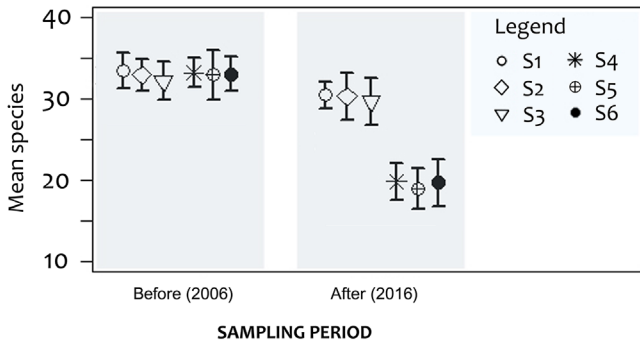
Fig. 4. Number of fish species (mean ± SD) occurring at each sampling site in relation to wastewater release (before, 2006, during, 2016) in the Barnoi River.

Table 2. BACI comparisons of fish assemblages and physico-chemical parameters of water in the Barnoi River at various times from before to after waste dumping, obtained through linear mixed-effects modelling.

Factors	*F*	*P*	LSmeans
Fish abundance			
Before–After (BA)	6.60	0.022	
Upstream–Downstream (CI)	94.0	0.001	
BA × CI	119.61	< 0.001	
Upstream–Before (CB)			5.181
Downstream–Before (IB)			5.182
Upstream–After (CA)			5.083
Downstream–After (IA)			4.319
Fish species richness			
Before–After (BA)	123.84	<0.001	
Upstream-Downstream (CI)	170.27	<0.001	
BA × CI	181.80	<0.001	
Upstream–Before (CB)			5.723
Downstream–Before (IB)			5.741
Upstream–After (CA)			5.480
Downstream–After (IA)			4.397
Abundance of locally threatened spp.			
Before–After (BA)	18.42	<0.001	
Upstream–Downstream (CI)	72.09	<0.001	
BA × CI	19.60	<0.001	
Upstream–Before (CB)			222.526
Downstream–Before (IB)			224.158
Upstream–After (CA)			173.789
Downstream–After (IA)			69.211
pH			
Before–After (BA)	183.54	<0.001	
Upstream–Downstream (CI)	63.21	0.001	
BA × CI	370.50	<0.001	
Upstream–Before (CB)			6.938
Downstream–Before (IB)			7.083
Upstream–After (CA)			6.854
Downstream–After (IA)			4.813

Table 2. (continued).

Factors	*F*	*P*	LSmeans
Dissolved oxygen			
Before–After (BA)	61.40	<0.001	
Upstream–Downstream (CI)	1194.90	<0.001	
BA × CI	1260.30	<0.001	
Upstream–Before (CB)			5.168
Downstream–Before (IB)			5.194
Upstream–After (CA)			5.045
Downstream–After (IA)			3.106
Water temperature			
Before–After (BA)	1.72	0.211	
Upstream–Downstream (CI)	0.46	0.501	
BA × CI	0.47	0.499	
Upstream–Before (CB)			24.646
Downstream–Before (IB)			24.500
Upstream–After (CA)			26.209
Downstream–After (IA)			26.208
Water transparency			
Before–After (BA)	11.28	0.005	
Upstream–Downstream (CI)	170.34	<0.001	
BA × CI	203.10	<0.001	
Upstream–Before (CB)			31.500
Downstream–Before (IB)			32.167
Upstream–After (CA)			33.083
Downstream–After (IA)			17.917

abundance and species richness patterns, abundance of species considered threatened in Bangladesh followed the same temporal and spatial variations, with a lower number of individuals at impacted sites (Tab. 2). Four species (*Ailia coila*, *Ompok bimaculatus*, *W. attu* and *Parambassis lala*) were also on the IUCN redlist of globally threatened species (ranked as Near Threatened; Tab. 1). However, out of 27 non threatened species, the abundance of 24 also decreased significantly ($P < 0.05$) at downstream sites following wastewater releases (Tab. S3).

4 Discussion

4.1 Spatial and temporal changes in fish community and water quality

This study provides evidence of the impact of wastewater on the fish community and some water quality parameters in the Barnoi River. As predicted, in sites receiving wastewater declines in species abundance (>51%) and species richness (>41%) were recorded. Over the same time, fish abundance and richness at upstream sites declined slightly by ~8%. Downstream wastewater-impacted sites supported much lower fish abundance (by >47%) and species richness (by >35%) compared to unaffected upstream sites. Similar significant changes in pH, DO and water transparency were also observed.
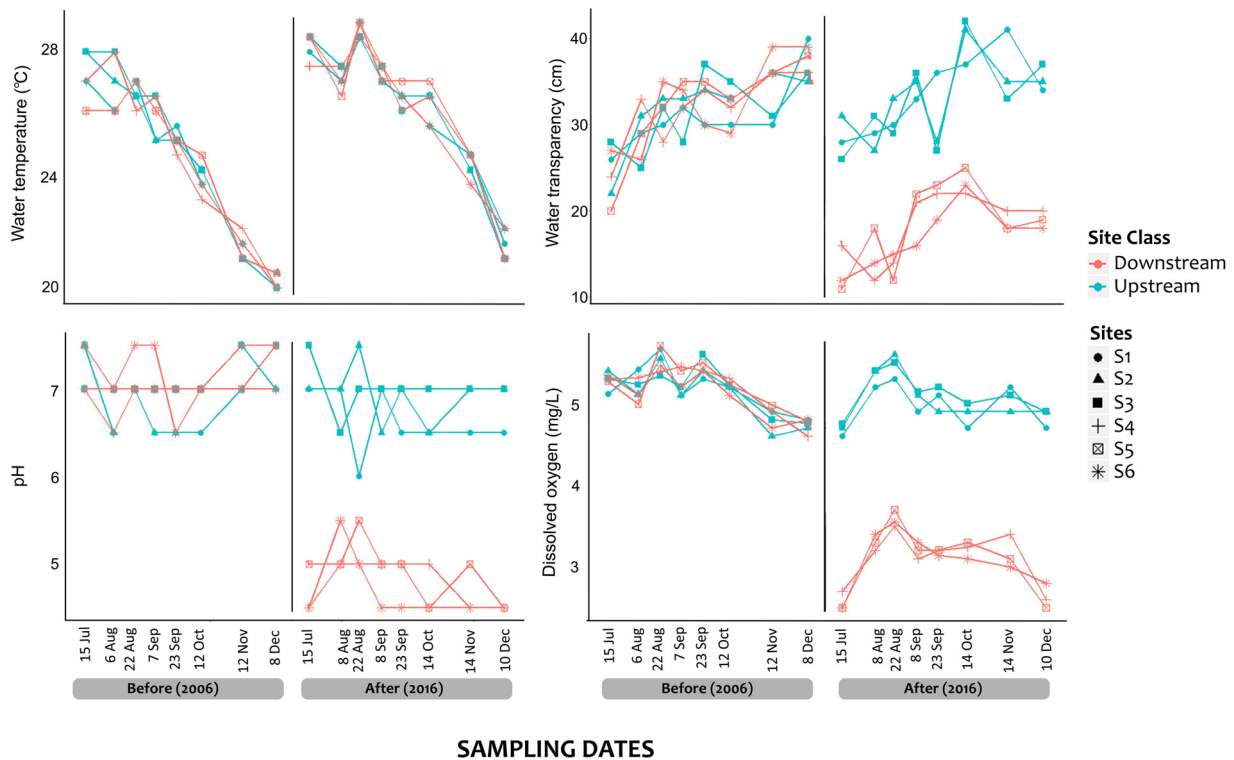
Fig. 5. Spatial and temporal variations in different physico-chemical parameters over time and space in the Barnoi River.

These changes were clearly a result of wastewater discharge, as similar changes were not observed in un-affected upstream sites.

Severe organic pollution can result in an elimination of fishes for long distances but they often disappear from the impacted area without apparently being killed (Hynes, 1963). As fishes are extremely mobile (Boys *et al.*, 2012; Cooke *et al.*, 2016) and can move to new localities rapidly (Peterson and Bayley, 1993; Sheldon and Meffe, 1995), fish populations in the downstream sites of the river might have moved to un-impacted upstream sites or further downstream to a more suitable area. However, some species, especially those with accessory respiratory organs (*i.e.* air-breathing fishes) may still survive in habitats with poor water quality (Ip *et al.*, 2004; Lefevre *et al.*, 2014). In Bangladesh air-breathing fishes include catfishes (Siluriformes), labyrinth fishes (Anabantoidea), spiny eels (Mastacelembidae) and snakeheads (Channidae) (Rahman, 2005; Hasan and Mohsin, 2010) and these species (*e.g. Anabas testudineus*, *Channa punctata* and *Channa striata*) were among the few species whose abundance did not vary significantly ($P > 0.05$) between upstream and downstream sites in 2016 despite wastewater release (Tab. S2).

Although lower numbers of individuals and species were observed in downstream sites in 2016 this difference was comparatively smaller from July to September than in other months. This might be due to the rise in water level associated with heavy rains during the rainy season (Galib *et al.*, 2016, 2018) that may reduce the level of unfavourable physico-chemical properties of water through pollutant dilution (Kumar and Reddy, 2009). When the water level began to recede, the impact of wastewater pollution on fish abundance

and species richness became much more apparent. Evidence of similar impacts of wastewater on zooplankton, the key natural food for many of the fishes, during low water level is available (Deksne, 2011).

Observed patterns of fish population abundance in the Barnoi were strongly correlated with DO and pH − two vital physico-chemical parameters for aquatic life. The influences of wastewater on these parameters are well documented (*e.g.* Hynes, 1963; Mason, 2001; Sepulveda-Jauregui *et al.*, 2013). Temporal patterns of DO, pH and water transparency in this study are clear signs of organic pollution (Allan *et al.*, 1958; Hynes, 1963). Although BIOENV results showed that the DO and pH collectively were strongly correlated with the changing patterns of the fish assemblage, reduced water transparency can also impact fish populations in various ways. Spawning and feeding efficiencies of fishes may be seriously affected by suspended matter produced through organic pollution in water (Hynes, 1963) as reported by the fishermen in the study area. In addition, it also reduces light availability to photosynthetic organisms that may result in reduced primary productivity of the habitat (Mason, 2001). However, accumulation of fine, grey sludge on the leaves of aquatic macrophyte in the impacted downstream sites also indicates effects of organic pollution that may seriously impact the aquatic vegetation and thus may make the habitat less suitable for other aquatic animals. The overall impacts of untreated wastewater release on fishes in the Barnoi are therefore likely a combination of direct water quality effects and indirect effects on the habitat, particularly on vegetation and food sources (Hynes, 1963; Mason, 2001).

4.2 Conservation implications

Serving as a channel that connects with several water bodies (Manda floodplain, Atrai River, Baral River, Chalan Beel and others) the Barnoi is ecologically vital for sustaining not only fishes but also other organisms. But wastewater releases in the impacted location (*i.e.* downstream sites) may have caused a functional disconnection by making the habitat unfavourable, reducing longitudinal movement of fish and leading to local extinction of migratory aquatic organisms (Warren and Pardew, 1998; Cooke *et al.*, 2016). Fish abundance and diversity were greater in upstream sites; however, the effect of wastewater outflow on river water quality has virtually isolated these habitats (primarily its origin, Manda floodplain) from other floodplains and rivers including the country's largest wetland. This imposes a serious threat to biota in isolated parts of the river system making them more susceptible to local extinctions.

The complete absence of three species in 2016 sampling (both upstream and downstream of the wastewater outflow) indicates that these species (*C. chaca*, *S. rabdophorus* and *N. nandus*), all bottom dwellers and of high conservation importance in Bangladesh (IUCN Bangladesh, 2015), have possibly become extinct from the study area. This might be due, in part, to existing wastewater releases in the Barnoi. Bottom dwelling fishes are very susceptible to anthropogenic river pollution that can damage their DNA and fatty acid profiles (Hussain *et al.*, 2017) and other physiological functions including reproduction (Jessica *et al.*, 2007; Nogueira *et al.*, 2011; Palanisamy *et al.*, 2011; Lenhardt *et al.*, 2012). The absence of these species from cleaner-water sites upstream of the wastewater suggests that stochastic factors acting on the more isolated populations upstream might have depleted populations too.

4.3 Implications for current wastewater management and further research

The current management practice for wastes or industrial effluents in Bangladesh, as well as in other developing countries, is similar (*e.g.* Girija *et al.*, 2007; Omosa *et al.*, 2012; Haque, 2017) — draining them out into the nearby waters, usually rivers, usually without any prior treatment (*e.g.* Dudgeon, 2000). This is primarily because the majority of the industries do not have a proper treatment plant or plants have been kept idle to reduce operating costs (Omosa *et al.*, 2012; Haque, 2017). Thus similar results can also be expected in other developing countries where wastewaters are being drained into the nearby water bodies without any prior treatment. To minimise this impact, wastewater should be treated through establishing proper treatment plants. However, treated effluents can also be dangerous to the receiving water bodies (Kumar and Reddy, 2009) and thus multiple treatments might be considered before releasing wastewater into natural water bodies.

This study also showed that not only traditional pollutants or effluents (from industrial, agricultural, municipal etc. sources) are finding their way into the rivers with wastewaters but also waste products from pharmacies and clinics are being dumped into the waste channels. Pharmaceutical effluents can result in endocrine disruption

in fishes, reduced abundance and a lack of sensitive fish species (Sanchez *et al.*, 2011). There is a high potential for accumulation of antibiotics, chemical and biological agents (including harmful pathogens) in the river water in the study area that can be a significant threat to all animals including fish and humans (Kümmerer, 2001; Brown *et al.*, 2006; Fent *et al.*, 2006). This practice must be stopped and proper waste management (incineration of medical and pharmaceutical waste) should be introduced. Another important source of water quality degradation in the studied river was dumping of large-sized dead animals (cattle, goats and buffalo) that requires huge amounts of DO for its decomposition *i.e.* biological oxygen demand (BOD) would be much higher and results in lower DO and higher dissolved carbon dioxide (Mason, 2001). Again, regulation needs to be enforced so that these dead livestock are disposed of properly, without increased pollution risk.

The broken sluices found in this study must be repaired to stop continuous flow of wastewater into the river and natural biological treatment should be considered using locally available aquatic macrophytes (*e.g.* common reeds, water hyacinths and duckweeds) that can effectively remove nutrients and heavy metals from wastewaters (O'Hogain and Gray, 2002; Nielsen and Willoughby, 2005; Rezania *et al.*, 2015). However, care should be taken if floating macrophytes are used as these may cover the water surface and restrict sunlight penetration. A reed (*Phragmites* spp.) bed treatment approach is likely to be the best option, provided that screens are used to remove large debris and most solid organic waste first. Reed bed treatment is a common, cost-effective and environmentally friendly method used for filtering water and removing suspended sediments, organic materials and metals worldwide (O'Hogain and Gray, 2002; Nielsen and Willoughby, 2005; Uggetti *et al.*, 2012). Considering the floodplain topography of the study area, a horizontal reed-bed system (into which the canals run, before release to the river) is suggested.

If in future, wastewater is treated, research should be carried out to determine the success from a biodiversity standpoint using fish species that are most responsive (*e.g. Puntius sophore*, *Chanda nama*, *Amblypharyngodon mola* and *Parambassis ranga*, *Glossogobius giuris*; a list of more responsive species is available in Tab. S2) to wastewater in the Barnoi River. Alongside this, improvements in physico-chemical parameters of river water should be monitored continuously.

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