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PLATINUM
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Baseline biodiversity and physiochemical survey in Parvati Kunda and surrounding area in Rasuwa, Nepal

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Abstract: Parvati Kunda, a small, alpine wetland located near the village of Gatlang in Rasuwa, Nepal, is a major source of drinking water for the village, possesses spiritual significance, and is a reservoir of local biodiversity. This study presents the first scientifically conducted biodiversity survey of the wetland. Here, biodiversity data (wetland plants, birds, mammals, aquatic insects), basic water chemistry (nutrients, pH, dissolved oxygen, conductivity), and basic bacterial tests (total coliform, *Escherichia coli*, Giardia, Salmonella, Shigella) for the Parvati Kunda wetland is presented from November 2016 and February and May 2017. Parvati Kunda, two of three alternate village water sources, and several village taps were found to be contaminated with *E. coli* bacteria. Within and around the wetland, 25 species of wetland plants, nine tree species, 10 macroinvertebrate taxa, 37 bird species, and at least six mammal species were documented. *Acorus calamus* was the dominant wetland plant and the rapid proliferation of this species over the past twenty years has been reported by community members. Future studies that further document and monitor wetland biodiversity are necessary. This study provides a valuable baseline for future research in this culturally and ecologically important wetland.

Keywords: *Acorus calamus*, *Escherichia coli*, eutrophication, Himalaya, wetland monitoring.

Nepalese Abstract: सारस पावर्तीकुण्ड एक सानो तर सांस्कृतिक र पारिस्थितिक रुपमा महत्वपूर्ण उच्च पहाडी क्षेत्रमा अवस्थित सिमसार हो । यो कुण्ड रसुवा जिल्लाको स्थित गत्लाङ गाउँको नजिक रहेको छ । यो गत्लाङ गाउँको लागि पीउने पानीको मुख्य स्रोत हो र यसको स्थानीय जैविक विविधतामा महत्वपूर्ण योगदान भएतापनि त्यसको अभिलेख राखिएको पाईएन । त्यसकारण नोभेम्बर २०१६, फेब्रुअरी २०१७ र मे २०१७ मा यस अध्ययनका विज्ञहरुको टोलीद्धारा चरणबद्ध रुपमा गरिएको सर्वेक्षण र स्थानीय मानिसहरुसँगको अन्तरक्रियाबाट आएको जान्कारीको आधारमा गत्लाङ पावर्तीकुण्ड क्षेत्रमा भएका रुख तथा वनस्पति, स्तनधारी, पंक्षी, शुक्ष्म जीवाणुहरु, जलीय जीवहरुको पहिलो बिस्तृत सूचीको बिकास भएको छ । यसका साथै पावर्तीकुण्ड र आसपासमा भएको पानीको भौतिक–रासायनिक गुण, पानीमा भएका पोषक तत्वहरु तथा पिएच, घुलित अकिसजनको मात्रा, विद्युतिय लचकता जस्ता कुराहरुको वर्तमान मापनको अवस्था थाह भएको छ । गत्लाङ गाउँमा उपयोग हुने पानीका तीनवटा बैकल्पिक स्रोतहरु मध्ये २ वटामा र गाउँमा भएका ५ वटा धाराहरुमा इकोली जीवाणुबाट पानी दुषित भएको पाइएको छे । सिमसार क्षेत्रको वरिपरी २५ प्रजातिका जलीय वनस्पतिहरु, ९ प्रजातिका रुखहरु, १० प्रजातिका शुक्ष्मजीवाणुहरु, ३७ प्रजातिका चराहरु र १० प्रजातिका स्तनधारी जनावरहरुको अभिलेख गरिएको छ । बोझो (*Acorus calamus*) प्रजातिको वनस्पति सिमसार क्षेत्रमा सबभन्दा बढी मात्रामा रहेको पाइयो, जुन बिगत २० वर्षदेखि व्यापक रुपमा बृद्धि भइरहेको छ । यस सर्वेक्षणको अभिलेखले भविष्यमा यस सम्बन्धी हुने अध्ययन अनुसन्धानमा तथा परिवर्तनका प्रवृतिहरुको तुलना गर्न र बुझ्न आधारभूत तथ्याङ्कहरु प्रदान गर्नेछ । पार्वतीकुण्ड सिमसार क्षेत्रको पर्यावरणीय परिमाण/वस्तु/सूचकहरुको निरन्तर रुपमा निरीक्षण र लेखाजोखा गरिरहन सुझाव दिइन्छ ।

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Author contribution: JAM organized and conducted field data collection (plants, trees, macroinvertebrates, water chemistry) and wrote the manuscript. MBS organized field logistics, conducted field data collection (trees, birds, mammals), and consulted on the manuscript. SY acted as project supervisor and consulted on the manuscript.

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INTRODUCTION

The Himalaya, often considered the "water towers of Asia", provide water for almost 1.3 billion people living downstream (Xu et al. 2007). Nowhere, however, are the Himalayan water resources as critical as in the mountains themselves. Communities throughout the Himalaya depend on alpine wetlands for drinking water, rely on wetland flora and fauna for food and medicine, and attach spiritual significance to bodies of water (Pandit 1999; Xu et al. 2009). Furthermore, the Himalayan wetlands provide pristine, favourable habitats for flora and fauna in otherwise harsh mountain environments and are therefore important reservoirs of biodiversity (Murray 2009).

The Himalayan wetlands face many threats. Climate change already causes rapid glacial reduction in many areas and the ongoing rise in temperature will likely impact rain patterns, wetland thermal regimes, and habitat for flora and fauna, jeopardizing sensitive wetland ecosystems and threatening critical water sources (Yao et al. 2004; IPCC 2007; Tse-ring et al. 2010; Gerlitz et al. 2015). Another major threat to the Himalayan wetlands is eutrophication. Eutrophication is characterized by excessive plant and algal growth in a body of water, which often results from human activities that produce excessive nitrogen and phosphorus waste, such as raising livestock or applying fertilizer (Carpenter et al. 1998; Schindler 2006; Binzer et al. 2016). Multiple studies in Himalayan wetlands identified eutrophication from human-generated pollution as a major threat to regional wetland ecosystem health (Khan et al. 2004; Romshoo & Rashid 2014).

Parvati 'Kunda' (Nepali: pond; also called 'Chhodingmo'), a small wetland in the Rasuwa District of Nepal, provides drinking water to the nearby village of Gatlang that consists of about 400 households (Merrey et al. 2018; Fig. 1). Parvati Kunda also holds spiritual significance to the people in Gatlang and is a harbour of local biodiversity. A brief, qualitative assessment of Parvati Kunda was performed by World Wildlife Fund in 2007 (Manandhar 2007) and surveys focussed on local livelihoods (e.g., Merrey et al. 2018). We, however, could not access any scientific studies from Parvati Kunda that may have characterized the wetland and provide data on biodiversity or water chemistry. As global temperatures rise and human development advances in Gatlang and throughout Nepal, it is critical to establish a baseline in Parvati Kunda now so as to assess the extent and consequences of future changes. Furthermore, such baseline information will characterize the contribution of Parvati Kunda to regional vegetation and bird and macroinvertebrate biodiversity, and ultimately provide information that will promote the sustainable management of the wetland ecosystem.

Study site

Parvati Kunda is in ward number three of Aama Chhodingmo Rural Municipality, about 100km north of Kathmandu in Nepal's Rasuwa District. Climactically, the region is cold and snowy in December through February and temperature maximums occur between May and July. Seasonal climate variations are dominated by the Indian monsoon that occurs between June and September, while small scale climactic variations depend on altitude and aspect (Kharel 1997).

Parvati Kunda is just outside Langtang National Park. The NP encompasses 1,710km^2 (660mi^2) area in Nuwakot, Rasuwa, and Sindhulpalchok districts in the central Himalayan region. Langtang NP and the surrounding area are home to 46 types of mammals and 250 species of birds, including several rare and protected species such as Snow Leopard *Panthera uncia*, Clouded Leopard *Neofelis nebulosi*, Leopard *Panthera pardus*, and Red Panda *Ailurus fulgens* (Fox et al. 1996; DNPWC 2019).

Gatlang Village is located at 2,300m on a trekking route called Tamang Heritage Trail and is known for its traditional Tamang architecture. Most inhabitants of Gatlang identify as Tamang and speak the Tamang language (Merrey et al. 2018). Like other parts of the Langtang region, people grow potato, maize, buckwheat, beans, millet, and barley; raise 'chauri' (Nepali: yak-cow hybrid), sheep, and goats; and run lodges, jeep businesses, or make handicrafts that cater to tourists (Merrey et al. 2018). Gatlang is accessible by road, although there is no regular bus service.

Parvati Kunda (85.261^{o}E & 28.156^{o}N) is a 1.87-hectare groundwater and rainfall/ snowmelt-fed eutrophic wetland located at 2,600m. The wetland is located 300m above Gatlang and lies on the northern edge of Bongjomane Community Forest from which the community members harvest fodder, firewood, and timber. Parvati Kunda is religiously significant and is often the subject of local religious festivals. Water from the deep centre of the wetland is connected to a system of pipes and public taps in the village. Some village taps are fed from one of the three additional springs in the vicinity. Water flows out through a small opening on the northeastern edge of the wetland, where a man-made wall confines the outflow channel to about 1m in width (Fig. 3a). The outflow descends 300m over 1km to Gatlang Village.

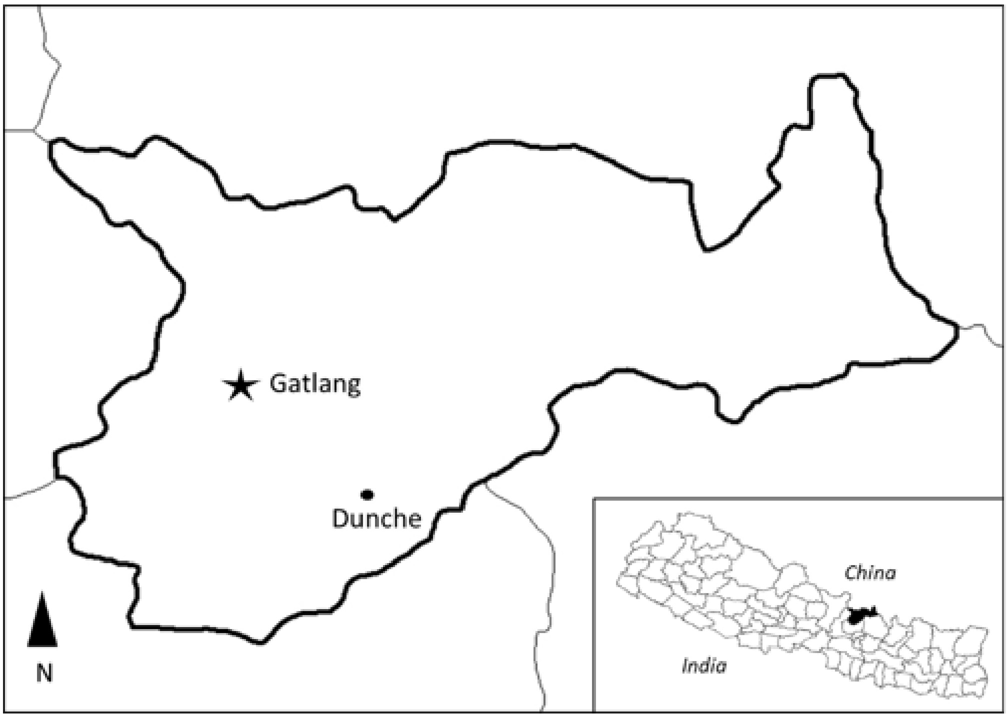

Figure 1. Village Gatlang and Dunche (Dhunche), the administrative seat of Rasuwa District in Nepal (inset).

METHODS

Parvati Kunda was surveyed three times, in November 2016 and in February and May 2017. Surveys for particular taxa groups were conducted when seasonally appropriate. Vegetation and physicochemical parameters were measured in 18 quadrats within the wetland, and others were analyzed in a subset of 10 for macroinvertebrates and 12 nutrient quadrats.

Wetland Vegetation

The locations of vegetation quadrats within Parvati Kunda included the inflow plus 17 randomly determined locations from ArcGIS (ESRI 2014; Fig. 3b). Vegetation surveys included areas that were permanently inundated with emergent or floating macrophytic vegetation, but excluded open water, i.e., the areas inundated but lacking emergent macrophytic vegetation. Open water was excluded from the location generator using satellite imagery and ArcGIS (ESRI 2014; Google Earth 2016). No survey site was more than 0.7m deep and all could be accessed by wading. Vegetation surveys were conducted in November and May only. February was excluded because there was minimal vegetation growth. Quadrat-based sampling was applied to the vegetation study using 0.5m^2 square quadrats and plants were identified using standard taxonomic keys (Malla et al. 1976). Unidentified species were collected in herbarium sheets for identification by experts at the National Herbarium in Godavari. Per cent cover of each species was visually estimated and the number of individuals of each species was counted. For rhizomous species such as *Juncus leucanthus* or *Acorus calamus*, where multiple shoots might be connected by a single root system, individual shoots were counted to avoid digging up the entire plant. In cases where moss was present, the number of individuals were counted in three 10cm x 10cm sample plots and used to calculate the average number of individuals per square metre. This was used to estimate the number of individuals in quadrats.

Specific information about the proliferation of *A. calamus* in Parvati Kunda over the last 20 years was gathered during conversations with community members. Such local accounts are considered important to understand changes in Parvati Kunda over time since prior published data on wetland vegetation does not exist to the best of our knowledge. Before commencing biodiversity surveys, the researchers interviewed seven high-profile community leaders specifically about water resources in the village. Further insight into questions about changes in Parvati Kunda could be obtained from 31 household surveys that were conducted in conjunction with another study during this time.

Riparian Trees

Four circular riparian tree plots were created on the northern, southwestern, southern, and northeastern sides of the wetland (Fig. 3c). The plots measured 5m in radius, 78.54m^2 in area, covering at least 5% of the riparian area as defined by the wetland boundary wall. Trees with a diameter at breast height greater than 10cm were identified and counted.

Aquatic Macroinvertebrate Survey

Aquatic macroinvertebrate surveys were conducted in May in 10 of the vegetation plots using the pipe method (Britton & Greeson 1989), where a sturdy plastic bucket with a height of 0.81m and diameter 0.288m and no bottom was placed firmly in the mud at each sampling location (Fig. 3d). Using a net, the water inside the bucket was agitated and all insects and loose debris were scooped out with five to six scoops of the net, ensuring that at least three scoops scraped the bottom. Contents of the net were placed in a sieve and excess debris was rinsed and removed in the field. The remaining debris and macroinvertebrates were preserved in 60% ethanol. In the laboratory, invertebrates were sorted, counted, and identified to family level. The Nepal lake biotic index (NLBI) and lake water quality class (LWQC) were calculated using family level identification according to Tachamo-Shah et al. (2011).

Bird Survey

In November, February, and May the point-count method was used for bird observations in which a single observer stayed in one fixed position during a specified period of time and recorded all birds seen or heard during that period (Ralph et al. 1995). This method was used for one hour in the morning and evening. The timings of observations were 07.00–08.00 h and 16.00–17.00 h in May and 08.00–09.00 h and 15.00–16.00 h in November and February, for two consecutive days during each survey period. A single observer stood on the northern bank of the wetland where they had a good view of the entire wetland area with the aid of binoculars.

Wildlife Observation

Data on animals were gathered from direct sighting, evidence of animal usage from faeces, wallows and walking signs, and mentions by local people. Photographic evidence of animals were collected when possible.

Physiochemical Parameters

Physical water parameters were measured in each of 18 quadrats and the outlet in November, February, and May. Measurements for dissolved oxygen, temperature, pH, and electrical conductivity were performed using handheld probes, namely, Lutron DO meter 519, Hanna pHep meter, and Hanna DiST meter. Nitrate, ammonia, and total phosphate were measured at a subset of 12 quadrats (Fig. 3e). To measure nutrients, samples were collected in 500ml clean plastic sample bottles, kept cold, and transported to Kathmandu within 24 hours. Lab work and analysis were performed by CEMAT Water Labs, Kathmandu, Nepal, the procedural references for which are presented in Table 1.

Table 1. Tests and methods used for detection and quantification of nutrients and detection of pathogens. All tests were performed by CEMAT Water Labs in Kathmandu, Nepal.

Test	Method used
Total phosphorus	ISO 6878:1998(E)
Nitrate	ISO 7890-3
Ammonia	4500-NH3 C, APHA 17th Ed.
Total coliform	9221 B, APHA 17th Ed.
E. coli	9221 F, APHA 17th Ed.
Salmonella spp.	9260 B, APHA 17th Ed.
Shigella spp.	9260 E, APHA 17th Ed.
Ova/ worms/ *Giardia*/ amoeba/ cyst	-

Biological Water Quality

Because Parvati Kunda is an important source of drinking water for the Gatlang community, we performed bacterial tests in the wetland and in several other spring-fed water sources and village taps. Using public data on water-borne illnesses from the village health centre to guide our study, we tested for multiple bacterial contaminants, including total coliform, *Escherichia coli, Salmonella, Shigella, Giardia,* and for the possible presence of any ova, worms, cysts, or amoeba. Tests for total coliform, *E. coli, Salmonella*, and *Shigella* were performed in February and May. Tests for *Giardia* and other microscopic stages were done in May. Sampling was performed at three locations in Parvati Kunda—the inlet, the outlet, and WQ1. Tests were also conducted in three additional spring sources, namely, Chyange Spring, Sanglang Ghode Spring, and Shernemba Spring, which supplied water to six village taps (Fig. 4). Water samples were collected in pre-sterilized 250ml collection bottles, kept cold, and transported to Kathmandu within 24 hours. Lab work and analysis was performed by CEMAT Water Labs (Table 1).

Data Analysis

For analysis of wetland vegetation and data from tree surveys, we used density and Simpson's reciprocal index of biodiversity,

$$D = \frac{1}{\frac{\sum n(n-1)}{N(N-1)}}$$

where n = the total number of organisms of a particular species and N = the total number of organisms of all species. The indices were calculated for each quadrat using the vegan package in R (R Development Core Team 2016). Simpson's reciprocal index was used because it tends to be less sensitive to both the shape of the abundance distribution and small sample sizes, compared to other diversity metrics (Gotelli & Chao 2013).

NLBI was calculated by assigning scores to each macroinvertebrate identified according to family. The sum of these scores divided by the number of scored taxa is equivalent to the NLBI. The NLBI score was then translated to a LWQC rating that indicates the degree of pollution likely in the waterbody (Tachamo-Shah et al. 2011).

Total phosphorus was determined using the ammonium molybdate spectrometric method. Ascorbic acid and acid molybdate were added to a 30ml filtered subsample and formed a complex with orthophosphate ions. The complex was then reacted with ascorbic acid to form a blue compound, and the absorbance of this compound was measured against a calibration curve to determine the concentration of orthophosphate in the sample (ISO 6878:1998(E)). Nitrate was measured with the spectrometric method using sulfosalicylic acid. Sodium salicylate and sulfuric acid were added to a 30ml filtered subsample and reacted with nitrate. After alkali treatment, a yellow compound formed. The absorbance of this compound was used to determine the nitrate concentration (ISO 7890-3). The nesslerization method was used to measure ammonia concentration. The water sample was filtered, buffered with a borate buffer, and then distilled into a boric acid solution. Nessler reagent was added to the distillate, reacted with ammonia to produce a yellow colour, and ammonia concentration was measured spectrophotometrically (4500-NH3 C, APHA 17th Ed.).

Total coliform was identified using a fermentation technique. Lauryl tryptose broth was inoculated in fermentation tubes and incubated for 24h at 35^{o}C. If gas or acid was produced, samples were transferred to green lactose bile and incubated for 48h at 35^{o}C. If coliform colonies were observed, samples were transferred to an agar slant and lauryl tryptose broth fermentation tube for 24h at 35^{o}C. If gas was produced at this stage, part of the agar slant growth was gram stained. If gram negative rods were present, coliform was present. Coliform density was estimated by calculating the most probable number (MPN) value from the number of positive green lactose bile tubes (9221 B, APHA 17th Ed.). *Escherichia coli* was identified using the membrane filter method. A quantity of 100mL of sample water was filtered through a 0.45µm pore size membrane filter and placed on a plate of M-endo agar. The plate was incubated at 35^{o}C for 24h and bacterial colonies were inspected for red-metallic colour indicating *E. coli*. Density was estimated with MPN (9221 F, APHA 17th Ed.).

Salmonella and *Shigella* samples were pre-enriched in peptone water, then enriched in tetrathionate broth and incubated at 35^{o}C for 24h. The samples were streaked on *Salmonella Shigella* (SS) agar. The selective solid media allowed plates to be screened for the presence or absence of both *Salmonella* and *Shigella* (9260 B and E, APHA 17th Ed.). Giardia, ova, worms, and cysts were identified by microscopic observation of 100ml of sample for presence/ absence analysis.

RESULTS

Biodiversity Surveys

Vegetation

Twenty-five species of plants were identified from surveys of wetland vegetation (Fig. 3b; Table 2). Surveys also identified a large population of *Sphagnum palustre*, a type of peat moss that is rare in Rasuwa District (Nirmala Pradhan pers. comm. December 2016).

The total area of the wetland dominated by *Acorus calamus* was demarcated by a combination of field measurements on a geographic positioning system (GPS) and visual analysis of satellite imagery in ArcGIS (ESRI 2014; Fig. 3a). *Acorus calamus* covered about 7,140m^2 of the wetland or 38% of the total wetland surface area. Within vegetation survey plots, *A. calamus* covered an average of 37% of the area within the 18 survey quadrats, verifying the visual determination of *A. calamus* coverage in ArcGIS.

Five of seven key informants identified an increase in *A. calamus* and perceived the species as a major threat to the wetland. Sixty-five per cent of respondents in household surveys also identified an increase in *A. calamus* as the most noticeable change in Parvati Kunda over the past 20 years (Yonzon unpub. data).

Nine species of trees were identified during tree

Table 2. Wetland vegetation recorded in Parvati Kunda, Nepal, in 2016–2017.

Order	Family	Scientific name
Acorales	Acoraceae	*Acorus calamus*
Apiales	Apiaceae	*Acronema tenerum*
Asparagales	Orchidaceae	*Spiranthes sinensis*
Asterales	Asteraceae	*Aster tricephalus*
Asterales	Asteraceae	*Pseudognaphalium affine*
Asterales	Asteraceae	*Senecio laetus*
Brassicales	Brassicaceae	*Cardamine flexuosa*
Brassicales	Brassicaceae	*C. impatiens*
Brassicales	Brassicaceae	*C. loxostemonoides*
Caryophyllales	Caryophyllaceae	*Arenaria debilis*
Caryophyllales	Polygonaceae	*Persicaria nepalensis*
Caryophyllales	Polygonaceae	*Persicaria* sp.
Caryophyllales	Polygonaceae	*Rumex nepalensis*
Lamiales	Lamiaceae	*Elsholtzia strobilifera*
Lamiales	Scrophulariaceae	*Hemiphragma heterophyllum*
Lycopodiales	Lycopodiaceae	*Lycopodium japonica*
Malpighiales	Hypericaceae	*Hypericum japonica*
Paeoniales	Paeoniaceae	*Rubus terutleri*
Poales	Juncaceae	*Juncus himalensis*
Poales	Juncaceae	*J. leucanthus*
Poales	Poaceae	*Calamogrostis pseudophragmites*
Poales	Poaceae	*Miscanthus nepalensis*
Ranunculales	Ranunculaceae	*Anemone rivularis*
Ranunculales	Ranunculaceae	*Ranunculus cantoniensis*
Sphagnales	Sphagnaceae	*Sphagnum palustre*

surveys in four different survey plots around the wetland (Fig. 2; Table 3). Tree diversity (Simpson's reciprocal index) ranged from 2.571 on the western side (plot 2) to 4.378 on the eastern side (plot 4) of the wetland.

Macroinvertebrate Surveys

In total, 10 aquatic macroinvertebrate taxa were identified through surveys (Table 4). The NLBI was calculated using these invertebrates to assess the overall ecological quality of the wetland. Parvati Kunda received a 'poor' LWQC rating, indicating a likely high level of nutrient pollution (Tachamo-Shah et al. 2011).

Bird and Mammal Surveys

Point-count bird surveys identified 37 species of birds in and around the wetland (Table 5). Opportunistic observations by the field research team confirmed the presence of six mammal species in or near the wetland area, within 10m from the water's edge. Community members identified four additional mammals that were not directly observed by the researchers (Table 6).

Water Properties

Ammonium and total phosphate levels were mostly negligible (<0.1mg/l). Nitrate was higher at the inlet (2.3mg/l in February and 2.9mg/l in May), but low or negligible in other locations (Table 7). Other parameters including temperature, dissolved oxygen, pH, and electrical conductivity differed over seasons. Average values are presented in Table 8.

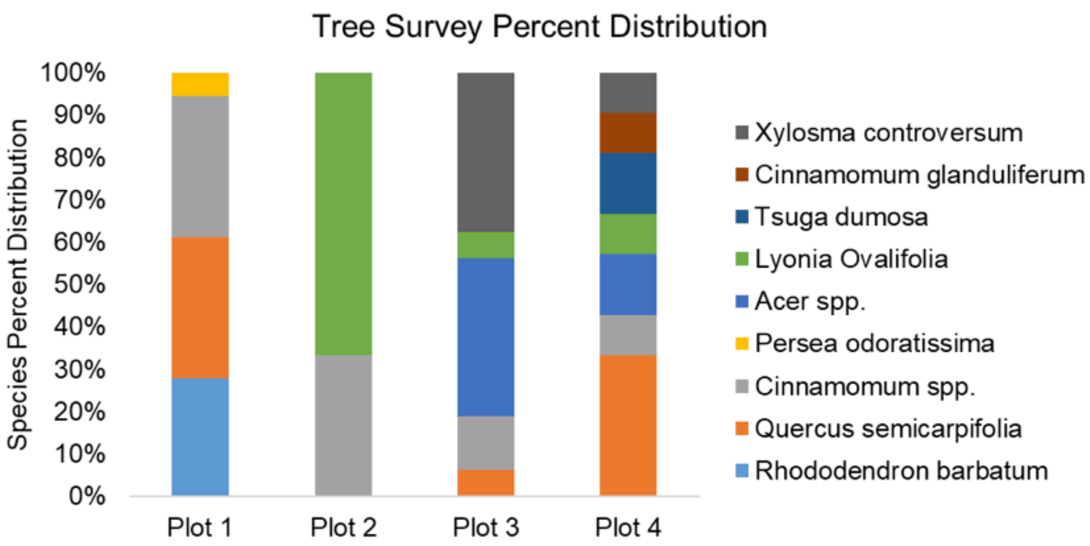

Figure 2. Percent distribution of tree species found around Parvati Kunda, Nepal.

Table 3. Trees found in riparian areas around Parvati Kunda, Nepal, in 2016–2017.

Order	Family	Scientific name	Common name	Plot 1	Plot 2	Plot 3	Plot 4
Ericales	Ericaceae	*Rhododendron barbatum*	Rhododendron	5			
Ericales	Ericaceae	*Lyonia ovalifolia*	Angeri		4	1	2
Fagales	Fagaceae	*Quercus semicarpifolia*	Oak	6		1	7
Laurales	Lauraceae	*Cinnamomum* spp.	Wild Cinnamon	6	2	2	2
Laurales	Lauraceae	*Persea odoratissima*	Pra	1			
Laurales	Lauraceae	*Cinnamomum glanduliferum*	Wild Cinnamon				2
Malpighiales	Salicaceae	*Xylosma controversum*	Willow			6	2
Pinales	Pinaceae	*Tsuga dumosa*	Himalayan Hemlock				3
Sapindales	Sapindaceae	*Acer* spp.	Maple		6	3	
Biodiversity index				**3.306**	**2.571**	**3.267**	**4.378**

Biological Contaminant Tests

Parvati Kunda was found to be contaminated with *E. coli* in both February and May and the *Giardia* trophozoite that is an active stage was found in May. Two out of the three local water sources, namely, Chyange Spring and Shernemba Spring, were contaminated with *E. coli, Giardia, Salmonella, Shigella,* and ova. Compared to water sources, taps generally contained higher levels of *E. coli* and greater variation in other tested contaminants (Table 9).

Table 4. Aquatic macroinvertebrates found in Parvati Kunda, Nepal, in 2016–2017. Two taxa are repeated twice (Haplotaxida: Tubificidae and Coleoptera: Dytiscidae). They represent different species. Identification up to the level of family was needed for NLBI calculation and family was only scored once in NLBI. Trichoptera: Polycentropodidae is not included in the NLBI index score.

	Order	Family	NLBI Score
1	Diptera	Chironomidae	1
2	Haplotaxida	Tubificidae	2
3	Haplotaxida	Tubificidae	Replicate
4	Trichoptera	Polycentropodidae	NA
5	Coleoptera	Dytiscidae	5
6	Odonata	Libellulidae	3
7	Coleoptera	Dytiscidae	Replicate
8	Hemiptera	Corixidae	2
9	Hemiptera	Gerridae	4
10	Veneroida	Sphaeriidae	5
NDBI score/ LWQC rating			3.14/ poor

DISCUSSION

Change in Vegetation Profile

Based on biodiversity surveys, the semi-aquatic plant *Acorus calamus* was by far the most dominant plant species in the wetland. *Sphagnum palustre* is the second most dominant species and is an important structural plant since it influences water chemistry and provides a platform for other plants to grow on (Glime et al. 1982). According to accounts obtained from community members during casual conversation as well as interviews of key informants, the proliferation of *A. calamus* in Parvati Kunda over the past 20 years was high and is a concern for the wetland ecosystem as a source of water for people, animals, and plants.

Monitoring changes in Parvati Kunda vegetation such as *A. calamus* is important because the species composition of wetland macrophytes often reflects water quality. For example, one study in lake Hiidenvesi in Finland identified notable changes in vegetation communities as lake eutrophication progressed—with vegetation changing from species requiring coarse bottom material and high light levels to species favouring soft bottom substrate and turbid waters (Nurminen 2003). Similarly, a study in the Tiber River basin in Italy identified a certain selection of wetland plants as bioindicators of water pollution and eutrophication (Ceschin et al. 2010). *Acorus calamus*, in particular, was identified as especially tolerant to high nitrogen levels and anoxia (Weber & Brändle 1996; Zhang et al. 2018) and therefore the alleged increase in dominance of this species over time in Parvati Kunda could be an indicator of high nitrogen levels and anoxia in the wetland. A study in the Hokersar Wetland in the Kashmir Himalaya in India, however, found that *A. calamus* nearly disappeared in the wetland, which was affected by pollution, siltation, and agriculture and had high levels of nitrogen (Khan

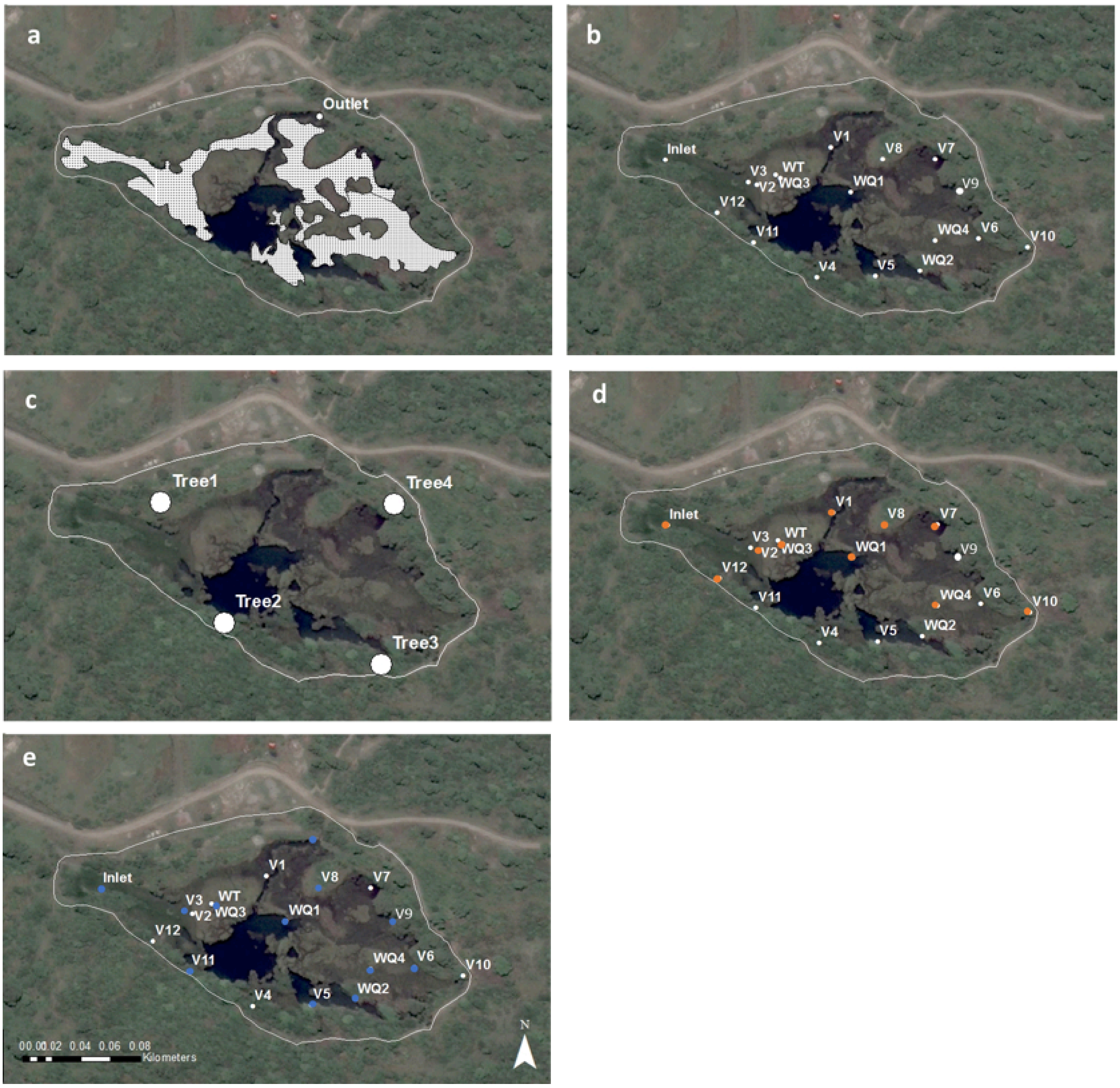

Figure 3. Sampling locations in and around Parvati Kunda, Nepal: a - outlet and *Acorus calamus* growth area | b - vegetation diversity sampling locations | c - tree diversity sampling locations | d - insect diversity sampling locations | e - nutrient sample sites with CEMAT test.

et al. 2004). It is not clear exactly what differences between Hokersar and Parvati Kunda wetlands led to different responses in the *A. calamus* population, but this could be investigated with further study.

Understanding the proliferation of *A. calamus* in Parvati Kunda is also important from the biodiversity perspective. The changes in water quality and associated changes in wetland vegetation can lead to some plant species out-competing others. For example, a long-term study in wetlands in the Jura Mountains in Switzerland found that due to N-deposition, species composition trended towards nutrient-rich flora and some species were out-competed and disappeared from the ecosystem over a period of 25 years (Rion et al. 2018). A similar trend was found at lake Hiidenvesi in Finland, where certain species requiring hard substrates were out-competed by those preferring soft substrates (Nurminen 2003). It is plausible that the proliferation of *A. calamus* identified by local community members could have similar effects on wetland plant diversity and community composition. Although our study did not examine the specific biodiversity impacts of *A. calamus*,

Table 5. Bird species of Parvati Kunda and surrounding areas, Nepal. LC = Least Concern according to IUCN (2016).

Order	Family	Scientific name	Common name	IUCN status
Accipitriformes	Accipitridae	*Spilornis cheela*	Crested Serpent-eagle	LC
Columbiformes	Columbidae	*Streptopelia orientalis*	Oriental Turtle Dove	LC
Cuculiformes	Cuculidae	*Cuculus canorus*	Common Cuckoo	LC
Cuculiformes	Cuculidae	*C. saturatus*	Oriental Cuckoo	LC
Galliformes	Phasianidae	*Francolinus francolinus*	Black Francolin	LC
Gruiformes	Rallidae	*Zapornia bicolor*	Black Tailed Crake	LC
Passeriformes	Aegithalidae	*Aegithalos concinnus*	Black-throated Tit	LC
Passeriformes	Cettiidae	*Horornis flavolivacea*	Aberrant Bush Warbler	LC
Passeriformes	Corvidae	*Corvus macrorhynchos*	Large-billed Crow	LC
Passeriformes	Corvidae	*Pyrrhocorax pyrrhocorax*	Red-billed Chough	LC
Passeriformes	Corvidae	*Urocissa flavirostris*	Yellow-billed Blue Magpie	LC
Passeriformes	Dicruridae	*Dicrurus leucophaeus*	Ashy Drongo	LC
Passeriformes	Fringillidae	*Carduelis spinoides*	Yellow-breasted Greenfinch	LC
Passeriformes	Fringillidae	*Carpodacus thura*	White-browed Rosefinch	LC
Passeriformes	Laniidae	*Lanius schach*	Long-tailed Shrike	LC
Passeriformes	Laniidae	*L. tephronotus*	Grey-backed Shrike	LC
Passeriformes	Leiothrichidae	*Trochalopteron lineatum*	Streaked Laughingthrush	LC
Passeriformes	Leiothrichidae	*T. variegatum*	Variegated Laughingthrush	LC
Passeriformes	Leiothrichidae	*Heterophasia capistrata*	Rufous Sibia	LC
Passeriformes	Motacillidae	*Anthus* spp.	Pipit spp.	-
Passeriformes	Motacillidae	*Motacilla cinerea*	Grey Wagtail	LC
Passeriformes	Muscicapidae	*Enicurus maculatus*	Spotted Forktail	LC
Passeriformes	Muscicapidae	*Ficedula westermanni*	Little Pied Flycatcher	LC
Passeriformes	Muscicapidae	*Myophonus caeruleus*	Blue Whistling-thrush	LC
Passeriformes	Muscicapidae	*Phoenicurus frontalis*	Blue-fronted Redstart	LC
Passeriformes	Muscicapidae	*Saxicola ferreus*	Grey Bushchat	LC
Passeriformes	Muscicapidae	*S. torquatus*	Common Stonechat	LC
Passeriformes	Paridae	*Lophophanes dichrous*	Grey-crested Tit	LC
Passeriformes	Paridae	*Parus monticolus*	Green-backed Tit	LC
Passeriformes	Pycnonotidae	*Pycnonotus leucogenys*	Himalayan Bulbul	LC
Passeriformes	Sittidae	*Sitta himalayensis*	White-tailed Nuthatch	LC
Passeriformes	Stenostiridae	*Culicicapa ceylonensis*	Grey-headed Canary-flycatcher	LC
Passeriformes	Sylviidae	*Fulvetta vinipectus*	White-browed Fulvetta	LC
Passeriformes	Timaliidae	*Pomatorhinus erythrogenys*	Rusty-cheeked Scimitar-babbler	LC
Passeriformes	Troglodytidae	*Troglodytes troglodytes*	Northern Wren	LC
Passeriformes	Turdidae	*Turdus unicolor*	Tickell's Thrush	LC
Piciformes	Picidae	*Picus squamatus*	Scaly-bellied Woodpecker	LC

future studies in Parvati Kunda should make use of the baseline information presenting possible threats here to understand the consequences of *A. calamus* growth within the wetland ecosystem.

Given the dominance of *A. calamus* in Parvati Kunda, we expected to find high levels of nutrients in water samples. This was not the case and nutrient levels were, in most cases, negligible. Since *A. calamus* grows both in water and on wetland edges or areas that are not permanently inundated, however, soil could be another critical source of excess nutrients. For example, a study on residual availability of nitrogen in soil following

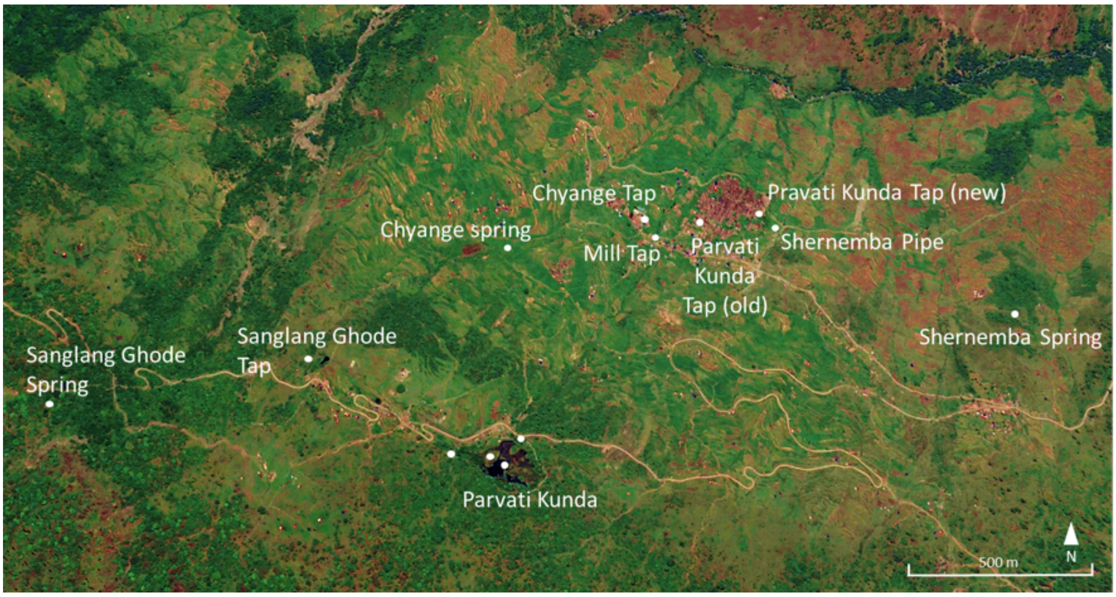

Figure 4. Four water sources tested for microorganism contamination (Sanglang Ghode, Chyange, Parvati Kunda, and Shernemba) and taps tested in the village. Three sites (inlet, outlet, and WQ1) were tested within Parvati Kunda, Nepal (Table 9).

Table 6. Mammal species occurring around Parvati Kunda, Nepal, in 2016–2017. LC = Least Concern and V = Vulnerable according to IUCN (2018).

Taxa	Scientific name	Direct observation	Secondary information	IUCN status
Nepal Grey Langur	*Semnopithecus schistaceus*	x		LC
Barking Deer	*Muntiacus muntjak*	x		LC
Royles Pika	*Ochotona roylei*	x		LC
Yellow-throated Marten	*Martes flavigula*	x		LC
Chauri	-	x		-
Goats/ Sheep	-	x		-
Porquipine	*Hystrix* spp.		x	-
Leopard	*Panthera pardus*		x	V
Mongoose	*Herpestes* spp.		x	-
Wild Boar	*Sus scrofa*		x	LC

Table 7. Total phosphorus, nitrate, and ammonia measurements for February (10 wetland locations, see Fig. 3e) and May (three wetland locations—outlet, inlet, and WQ3). Fields in this table represent a single data point; only one sample was taken in each location in each season.

Sample location	T. phosphorus (mg/l as P)		Nitrate (mg/l as NO_3)		Ammonia (mg/l as NH_3)	
	February	May	February	May	February	May
Outlet	<0.1	<0.1	<0.1	0.3	<0.1	<0.1
Inlet	<0.1	<0.1	2.3	2.9	<0.1	<0.1
WQ1	<0.1		0.7		<0.1	
WQ2	<0.1		0.4		<0.1	
WQ3	<0.1	<0.1	<0.1	0.4	<0.1	0.8
WQ4	<0.1		<0.1		<0.1	
V3	<0.1		0.1		<0.1	
V5	<0.1		<0.1		<0.1	
V6	<0.1		0.5		0.1	
V8	<0.1		<0.1		<0.1	
V9	<0.1		<0.1		<0.1	
V10	<0.1		0.1		<0.1	
V11	<0.1		0.7		<0.1	

Table 8. Temperature, dissolved oxygen, pH, and electrical conductivity in 18 sampling locations in the wetland. Averages and standard deviations (SD) from the three sample points (November 2016 and February and May 2017) are presented. Note: pH was measured only in February and May and conductivity was measured only in November and February.

Sample location	Temperature (°C)		Dissolved oxygen (mg/l)		pH		Conductivity (mg/l)	
	Average	SD	Average	SD	Average	SD	Average	SD
Outlet	13.0	2.6	7.4	0.6	7.0	0.5	30.0	0.0
Inlet	12.2	0.6	7.3	0.5	6.2	0.3	20.0	0.0
WQ1	15.5	4.3	5.8	1.2	6.8	0.4	30.0	0.0
WQ2	14.7	6.3	5.5	3.1	5.9	1.0	20.0	0.0
WQ3	20.9	4.1	9.0	6.5	7.0	1.1	20.0	10.0
WQ4	13.9	8.8	8.4	0.7	6.1	0.4	20.0	0.0
WT	15.8	3.5	2.1	1.2	4.5	0.3	10.0	0.0
V1	13.7	2.1	9.4	0.9	6.8	0.4	30.0	0.0
V2	18.0	0.7	10.8	5.8	6.4	1.1	20.0	0.0
V3	16.7	7.0	5.3	2.9	5.9	0.8	30.0	10.0
V4	12.7	4.0	5.5	1.7	5.7	1.1	20.0	0.0
V5	13.4	6.9	6.0	3.2	6.6	0.6	25.0	5.0
V6	14.2	7.7	7.9	1.1	6.4	0.4	20.0	0.0
V7	18.0	10.1	6.7	2.3	6.6	0.2	35.0	5.0
V8	14.0	8.6	7.6	1.2	6.8	0.2	30.0	0.0
V9	14.9	10.2	7.9	0.9	7.0	0.5	30.0	0.0
V10	12.6	9.0	5.6	1.6	5.7	0.5	5.0	5.0
V11	12.8	3.5	5.3	0.3	6.7	0.8	40.0	0.0
V12	10.0	6.6	5.4	1.8	5.9	0.9	20.0	0.0

application of organic fertilizer from farmyard manure or compost found that nitrogen can be immobilized in soils and enrich the soil nitrogen pool over a long term (Gutser et al. 2005). A similar phenomenon may happen near Parvati Kunda, leading to slow release of nitrogen from the soil rather than from the water. Furthermore, nutrient addition studies on invasive *Phragmites australis* in the United States found that nitrogen added to soil, rather than water, significantly increased the growth of the wetland plant *P. australis* compared to the native *Spartina pectinata* (Rickey & Anderson 2004). This indicates that nutrient enrichment of soil can indeed increase growth rates of wetland plants. Additionally, *A. calamus* itself might absorb much of the nitrogen entering the wetland. Zhang et al. (2018) investigated the nitrite stress tolerance of *A. calamus* during wastewater treatment and found that *A. calamus* could tolerate up to 30mg/l of nitrite (2018). Likewise, a study in northeastern China identified *A. calamus* as a tool for nitrogen removal in floating treatment wetlands, finding that *A. calamus* demonstrated an 84.2% removal efficiency for total nitrogen (Li & Guo 2017). Thus, ensuing studies on *A. calamus* in Parvati Kunda should focus on the nutrient content of both *A. calamus* and the soil to fully understand the nutrient dynamics in the wetland.

Nutrient pollution and microorganisms

The presence of excess nutrients in Parvati Kunda is also indicated by the 'poor' LWQC score, which suggests a 'heavy' degree of nutrient pollution (Tachamo-Shah et al. 2011). This rating is related to the diversity and types of macroinvertebrates present in the wetland. Several direct explanations for a poor rating may exist; for example, lack of substrate and lack of dissolved oxygen as a result of excess nutrient inputs and eutrophication processes can inhibit macroinvertebrate breeding, emergence, and overall survival (Boles 1981; Connolly et al. 2004). When abiotic conditions such as nutrient levels, substrate type, and dissolved oxygen are unfavourable, the rest of the ecosystem is affected. It should be noted, however, that although Parvati Kunda received a poor rating, the pipe method used for macroinvertebrate sampling was quantitative instead

Table 9. Results of tests conducted in February and May 2017 for presence (P) or absence (A) of microorganism per 100ml of water samples from 12 different locations near Parvati Kunda, Nepal. MPN Index stands for most probable number of colonies present in 100ml of sample, which is determined by comparing the pattern of positive results with statistical tables (Bartram & Pedley 1996). Key to sample locations (Fig. 4): 1 - PK Inlet, 2 - PK Outlet, 3 - PK WQ1, 4 - Chyange Spring, 5 - Chyange Tap, 6 - Sanglang Ghode Spring, 7 - Sanglang Ghode Tap, 8 - Shernemba Spring, 9 - Shernemba Pipe, 10 - Mill Tap, 11 - Old Parvati Kunda Tap, 12 - New Parvati Kunda Tap.

Sample location	Total coliform (MPN index/100ml)		*E. coli* (MPN index/100ml)		*Salmonella* spp. (P/A)*		*Shigella* spp. (P/A)*		Ova (P/A)*		Worms (P/A)*		*Giardia* (P/A)*		Amoeba (P/A)*		Cyst (P/A)*	
	Feb	May	Feb	May	Feb	May	Feb	May	Feb	May	Feb	May	Feb	May	Feb	May	Feb	May
1	9	150	4	9	-	A	-	A	-	A	-	A	-	A	-	A	-	A
2	0	1100	0	460	A	A	A	A	-	A	-	A	-	A	-	A	-	A
3	-	210	-	39	-	A	-	A	-	A	-	A	-	***P***	-	A	-	A
4	93	23	43	4	***P***	A	***P***	***P***	-	***P***	-	A	-	A	-	A	-	A
5	-	4	-	4	-	A	-	A	-	A	-	A	-	A	-	A	-	A
6	-	0	-	0	-	A	-	A	-	A	-	A	-	A	-	A	-	A
7	-	1100	-	43	-	A	-	***P***	-	A	-	A	-	A	-	***P***	-	A
8	-	240	-	43	-	A	-	A	-	A	-	A	-	A	-	A	-	A
9	-	240	-	43	-	A	-	A	-	A	-	A	-	A	-	A	-	***P***
10	460	1100	240	240	***P***	A	***P***	A	-	A	-	A	-	A	-	***P***	-	A
11	43	1100	15	460	-	A	-	A	-	A	-	A	-	A	-	A	-	A
12	23	240	3	93	-	A	-	A	-	A	-	A	-	A	-	A	-	A

of qualitative as the NLBI method suggests (Tachamo-Shah et al. 2011). Even if a more extensive qualitative survey were conducted, however, the LWCQ rating would most likely only move one step from poor to fair. This would still indicate a moderate level of nutrient pollution. Furthermore, wetlands in monsoonal systems such as in Nepal are known to change in water quality ranking depending on the season. For example, a study in the Rampur Ghol ecosystem in Chitwan, Nepal, used a similar macroinvertebrate indicator system to describe water quality and found that water quality and macroinvertebrate diversity was generally lower in the dry season than the rainy season (Gautam et al. 2014). Likewise, the LWCQ rating in Parvati Kunda probably fluctuates throughout the year and additional qualitative sampling would possibly identify either poor or fair ratings depending on the season.

Several of the macroinvertebrate taxa identified in this study indicate specific details about Parvati Kunda and its level of pollution. For example, many prior studies found that Chironomidae abundance generally increases with decreasing habitat quality (Fore et al. 1996; Botts 1997) and red Chironomidae are easily identifiable and are therefore a good indicator of this pattern (Tachamo-Shah et al. 2011). Red Chironomidae was very abundant at all sampling locations within Parvati Kunda, indicating high levels of pollution. Furthermore, various species in Insecta families of Heteroptera and Corixidae were used as indicators of eutrophication and pollution in Finland (Jansson 1977). As the Finland study found, species within the Corixidae family vary greatly in their tolerance to pollution, they were assigned a low rating (2) in the NLBI, and their presence in Parvati Kunda indicates high levels of nutrient pollution that may be specifically linked to eutrophication.

Threat of Eutrophication

Although wetland eutrophication is a natural process, it is unclear whether human activities within the Parvati Kunda watershed may also be influencing degradation of the wetland. Activities such as agriculture, livestock raising, and timber harvesting within the wetland watershed may lead to higher levels of nutrient inputs that could explain the patterns we observed in Parvati

Kunda (Foote et al. 1996; Prasad et al. 2002). Most of the Kunda watershed is encompassed by Bongjomane Community Forest, which is utilized by Gatlang community members for fodder and timber harvesting. Livestock also inhabits the area around the wetland. Future studies should use GIS and satellite imageries to assess landuse changes in the Parvati Kunda watershed over the last 30 years. Such a study could help indicate how and why nutrient pollution occurred in the wetland.

Public records available at the village health centre showed persistent infections of intestinal worms, Salmonellosis, and Shigellosis, and we indeed found associated contaminants in Parvati Kunda, three alternative water sources, and several village taps. Of all water sources and taps tested, only one water source at Sanglang Ghode spring was in compliance of Nepal national drinking water quality standards (Ministry of Physical Planning and Works 2005). Livestock waste is a probable source of contamination in the wetland itself, especially since livestock was frequently observed inside the wetland enclosure and was observed defecating near the water during bacterial sampling. Other reasons for contamination may include leaking pipes through which contaminants can seep between the water source and village taps. The extent and source of bacterial contamination in the wetland, other water sources, pipes, and taps should be further investigated, and methods for maintaining acceptable drinking water quality in the village should be pursued.

CONCLUSION

The present study is a baseline study in the Parvati Kunda Wetland and is believed to provide the first description of its wetland vegetation, riparian trees, aquatic macroinvertebrates, birds, physiochemical water parameters, and biological contaminants. Our data indicate a moderate to heavy degree of nutrient pollution, although the direct and indirect causes and consequences of pollution are not fully understood. Continued monitoring of the Parvati Kunda wetland will provide a valuable and interesting example of the process of wetland degradation and conservation in the Nepal Himalaya.

Future studies should focus on amphibian- and mammal-specific surveys. Additionally, watershed level studies that describe land-use changes and may indicate reasons for wetland degradation are necessary.

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