EFFECTS OF WATER HYACINTH INVASION TO AQUATIC BIODIVERSITY OF LAKES IN POKHARA VALLEY, NEPAL

A THESIS SUBMITTED TO THE

CENTRAL DEPARTMENT OF ZOOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE AWARD OF

DOCTOR OF PHILOSOPHY

IN ZOOLOGY

BY

RAJENDRA BASAULA

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DEAN'S OFFICE
Kirtipur, Kathmandu, Nepal

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EXTERNAL EXAMINERS

The Title of Ph.D. Thesis: " Effects of Water Hyacinth Invasion to Aquatic Biodiversity of Lakes in Pokhara Valley, Nepal "

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October 3, 2023

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Asst. Dean

DECLARATION

Thesis entitled **"Effects of Water Hyacinth Invasion to Aquatic Biodiversity of Lakes in Pokhara Valley, Nepal"** which is being submitted to the Central Department of Zoology, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Kumar Sapkota and Co-supervised by Assoc. Prof. Dr. Hari Prasad Sharma of Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

....

Rajendra Basaula

RECOMMENDATION

This is to recommend that **Rajendra Basaula** has carried out research entitled **"Effects of Water Hyacinth Invasion to Aquatic Biodiversity of Lakes in Pokhara Valley, Nepal"** for the award of Doctor of Philosophy (Ph.D.) in **Central Department of Zoology** under our supervision. To our knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

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LETTER OF APPROVAL

[Date: 11/06/2023]

On the recommendation of **Supervisor Prof. Dr. Kumar Sapkota** and **Co-supervisor Assoc. Prof. Dr. Hari Prasad Sharma**, this Ph.D. thesis submitted by **Rajendra Basaula** entitled **"Effects of Water Hyacinth Invasion to Aquatic Biodiversity of Lakes in Pokhara Valley, Nepal"** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U.

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Rajendra Basaula

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ABSTRACT

Proliferation of non-native invasive species poses a serious hazard on ecosystem structure and functioning, leading to decline in native species, habitat alteration, and nutrient cycling. Water hyacinth (*Pontederia crassipes*) is native in Brazil and now it is spreading globally in tropical and subtropical regions with detrimental impact in aquatic ecosystem. Despite the presence of water hyacinth in the Pokhara valley lakes for over two decades, its impact on water quality and aquatic biodiversity in the lakes of Pokhara Valley remains largely unexplored. To minimize the gap, the current study explored the effect of invasion of water hyacinth on physicochemical parameters, and abundance and diversity of macro-invertebrates, fishes and waterbirds in the lakes of Pokhara. The lakes were categorized into water hyacinth presence and absence habitat on the basis of occurrence of water hyacinth. Water hyacinth was present in Phewa, Begnas and Rupa throughout the study period, however, all area was not occupied by it. Therefore, each lake was categorized in water hyacinth presence and absence habitat, and the small lakes are without water hyacinth and are kept under water hyacinth absence habitat. The seasonal data on water quality, macro-invertebrates, fish and waterbird species were collected from 24 sampling plots from 2019 to 2020. The water sample, macro-invertebrates and fish were collected from each plot of 50×50 m^2 using plastic bottles, Peterson's Grab Sampler and gill net enclosures, respectively. Waterbirds data were collected from 50 m radius of each plot by direct observation. Water quality was found to be degraded in areas with water hyacinth. Free carbon dioxide and water temperature had the significant and positive association with water hyacinth coverage but opposite for same was recorded for other parameters including depth, transparency, pH, dissolved oxygen, total alkalinity and nitrate. Water hyacinth invasion has had the detrimental impact on the abundance and diversity some macro-invertebrates, fish and waterbirds in the lakes of Pokhara Valley. During the study period, 29 macro-invertebrate's species were collected including 26 genera from 21 families and 15 orders, with orders Diptera and Haplotaxida being less abundant in areas infestation with water hyacinth. The study also documented overall 20 species of fishes including 18 genera, eight families and six orders with Cyprinidae the most abundant family. The exotic species Nile tilapia (*Oreochromis niloticus*) was found to be the most abundant fish in both areas, with higher abundance in areas with water hyacinth ($p < 0.001$). Exotic fish species were positively correlated with water hyacinth coverage ($R = 0.066$; $p < 0.001$). A total of 32 waterbird species of 11 families were recorded in the lakes of Pokhara Valley. The study found the water hyacinth infested area had lower threatened waterbird abundance compared to areas without it ($p = 0.023$). The abundance and species richness of threatened waterbird declined in water hyacinth presence habitat during this study period but these were positively correlated with depth of and overall bird abundance. The current study provided the baseline data of invasion of water hyacinth and its effect on the physicochemical parameters and aquatic fauna of the lakes. Therefore, the lake managers and policymakers can use these data to develop policies for removing or managing water hyacinth in the lakes of Pokhara Valley to improve aquatic biodiversity conservation.

नेपालको पोखराका तालहरूको जैविक विविधतामा जलकुम्भीको फैलावटले पारेका असरहरू

सार

आयातित मिचाहा प्रजातिहरूको फैलावटले पारिस्थितिक प्रणालीको संरचना र कार्यप्रणालीमा गम्भीर खतरा निम्त्याउँछ, जसले स्थानीय प्रजातिहरूमा गिरावट, बासस्थान परिवर्तन र पोषक तत्व चक्र संचालनमा असर गर्दछ । जलकुम्भी (*Pontederia crassipes*) ब्राजिलको स्थानीय विरूवा हो र अहिले यो संसारभरका उष्ण र उपोष्ण क्षेत्रका सबै जसो मुलुकहरूमा मिचाहा प्रजातिको रूपमा फैलिरहेको छ र जलीय पारिस्थितिक प्रणालीमा नराम्रो असर पारिरहेको छ । पोखरा उपत्यकाका तालहरूमा दुई दशकभन्दा अगाडिदेखि जलकुम्भी भित्रिएको भए तापनि यसले तालको पानीको गुणस्तर र जलीय जैविक विविधतामा पारिरहेको प्रभावका बारेमा धेरै हदसम्म अस्पष्ट छ । यो अस्पष्टतालाई न्यूनीकरण गर्न, हालको अध्ययनले पानीको भौतिक तथा रासायनिक गुणहरू, आँखाले देख्न सकिने ढाडमा हाड नभएका जलीय जीवहरु, माछाहरू र जलपक्षीहरूको उपस्थिति र विविधतामा जलकुम्भीले पार्ने असरहरूका बारेमा अन्वेषण गरेको छ । यो अध्ययनको लागि सर्वप्रथम तालहरूलाई जलकुम्भी पाइने र नपाइने ठाउँको आधारमा वर्गीकरण गरियो । फेवाताल, वेगनासताल र रूपातालमा जलकुम्भी भएका र नभएका दुवै खालका अध्ययन क्षेत्रहरू निर्धारण गरियो भने साना तालहरूमा जलकुम्भी नभएको हुनाले प्रत्येक तालमा जलकुम्भी नभएको एउटा मात्र अध्ययन क्षेत्र निर्धारण गरिएको थियो । यसरी सन् २०१९ देखि २०२० सम्ममा जम्मा ५० मिटर × ५० मिटरका २४ वटा अध्ययन क्षेत्रहरूबाट पानी, ढाडमा हाड नभएका जीवहरू, र माछाहरूको मौसमी नमुनाहरू सङ्कलन गरिएको थियो । पानी, ढाडमा हाड नभएका जीवहरू र माछाहरूको नमुना सङ्कलन गर्न क्रमश प्लाष्टिकका बोतलहरू, पेटरसन ग्रयाब स्याम्पलर (Peterson's Grab Sampler) र गिल नेट प्रयोग गरियो । जलपक्षीहरूको नमुना भने प्रत्येक अध्ययन क्षेत्रमा ५० मिटर अर्धब्यासभित्र प्रत्यक्ष अवलोकन विधिद्वारा गरिएको थियो । जलकुम्भीको कारणले गर्दा तालको पानीको गुणस्तर खस्किएको पाइएको छ । जलकुम्भीले ओगटेको प्रतिशतसँग कार्वन डाइअक्साइड र पानीको तापक्रमको अत्यन्तै सकरात्मक सम्बन्ध रहेको पाइयो तर पानीको गहिराइ, पारदर्शिता, घुलनशील अक्सिजन, कुल क्षारीयता र नाइट्रेट लगायतका अन्य मापदण्डहरूमा यसको नकरात्मक असर पाइयो । पोखरा उपत्यकाका तालहरूमा मिचाह जलकुम्भीको फैलावटले गर्दा ढाडमा हाड नभएका जीवहरु, माछा र जलपक्षीहरूको उपलब्धता तथा विविधतामा नकरात्मक प्रभाव पारेको भेटियो । अध्ययन अवधिभरमा ढाडमा हाड नभएका जीवहरुका २१ परिवारहरू (Families) र १५ अर्डरहरूबाट जम्मा २६ जातिसहित २९ प्रजातिहरू सङ्कलन गरिएको थियो । अर्डरहरू डिप्टेरा (Diptera) र ह्याप्लोटाक्सिडा (Haplotaxida) समुहका जीवहरूको उपस्थिति जलकुम्भी भएको क्षेत्रमा धेरै कम पाइयो । त्यसैगरी माछाका आठ परिवारहरू (Families) र छ अर्डरहरूबाट १८ जातिसहित २० प्रजातिहरू अभिलेख गरिएको छ, जसमा साइप्रिनिडे (Cyprinidae) सबैभन्दा प्रचुर

मात्रामा पाइएको परिवार रह्यो। जलकुम्भी भएको र नभएको दुबै ठाउँहरूमा मिचाहा प्रजातिको माछा नाइल तिलापिया (*Oreochromis niloticus*) प्रचुर मात्रामा पाइएको थियो तर पनि तुलनात्मकरूपमा जलकुम्भी भएको क्षेत्रमा यसको उपस्थिति बढी थियो (p = ०.००१)। मिचाहा प्रजातिका माछाहरू र जलकुम्भीले ओगटेको क्षेत्रफल बिच असाध्यै सकारात्मक सम्बन्ध भएको पाइयो (p = ०.००१)। यो अध्ययन अवधिमा पोखरा उपत्यकाका तालहरूमा ११ परिवार (Families) भित्र ३२ प्रजातिका जलपक्षीहरू पाइएका थिए। जलकुम्भी नभएको क्षेत्रको तुलनामा जलकुम्भी भएको क्षेत्रमा धेरै रैथाने जलपक्षीहरू पाइएको थियो (p = ०.०२३)। संसारभरि नै खतरामा परेका जलपक्षीहरूको प्रचुरता र यिनीहरूको प्रजाति सङ्ख्या जलकुम्भी भएको बासस्थानमा अति नै कम पाइयो तर यी पक्षीहरूको सम्बन्ध पानीको गहिराइ र समग्र पक्षी उपलब्धतासँग सकारात्मक रहेको भेटियो। यस अध्ययनले तालको पानीको भौतिक तथा रासायनिक गुणहरूमा र जलीय जीवहरूमा जलकुम्भीको प्रभावका बारेमा आधारभूत तथ्याङ्क प्रदान गरेको छ। तसर्थ पोखरा उपत्यकाका तालहरूको जलीय जैविक विविधता संरक्षणमा सुधार गर्न, तालबाट जलकुम्भी हटाउन वा व्यवस्थापन गर्न र नीति निर्माण गर्न ताल व्यवस्थापकहरू र नीति निर्माताहरूको निमित्त यी तथ्याङ्कहरू उपलब्धिमूलक हुनेछन्, जसले गर्दा त्यहाँ रहेका जीवजन्तुहरूको संरक्षण गर्न मद्दत गर्ने छ।

LIST OF ACRONYMS AND ABBREVIATIONS

APHA	: American Public Health Association
CCA	: Canonical Correspondence Analysis
CDZ	: Central Department of Zoology
CDZMTU	: Central Department Zoology Museum of Tribhuvan University
CO_2	: Free Carbon dioxide
CR	: Critically endangered
DCA	: Detrended Correspondence Analysis
DO	: Dissolved Oxygen
GPS	: Geographic Positioning System
HA	: Water hyacinth absent
HP	: Water hyacinth presence
IAP	: Invasive Alien Plants
IAPS	: Invasive Alien Plant Species
IUCN	: International Union for Conservation of Nature
LC	: Least Concern
NT	: Near Threatened
NTU	: Nephelometric Turbidity Unit
RA	: Potential of Hydrogen ion
RA	: Relative Abundance
RDA	: Redundancy Analysis
TDS	: Total Dissolved Solids
USM	: Ultraviolet Spectrophotometric Methods
VU	: Vulnerable

LIST OF SYMBOLS

*	: Asterisk
H'	: Shannon Wiener diversity index
J	: Pielou’s evenness index
Σ	: Summation
^{o}C	: Degree Celsius
=	: Equal
$<$	: Lesser than
$>$	: Greater than
×	: Multiplication
α	: Alpha

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CHAPTER 1

1. INTRODUCTION

1.1 Background

Biological invasion is believed one of the prominent causes that threat the native species and biodiversity loss (Nentwig, 2008; Courchamp *et al.*, 2017; Mannino *et al.*, 2017; Gazoulis *et al.*, 2022). Invasive Alien Species (IAS) proliferating is the global phenomena, and it has been escalating gradually because of human movement all over the world (Seebens *et al.*, 2017). IAS is native to a particular region and may introduce to regions outside of the native range accidentally or intentionally by different media such as human trades in the form of decorative in the garden, pets, nursery stock, agriculture and forest products (Budha, 2015; Bhatta *et al.*, 2021). IAS has grown and invaded new habitats, threatening the biodiversity and functions of the whole ecosystem of that habitat (Budha, 2015; Shrestha, 2016; Bhatta *et al.*, 2021; Pathak *et al.*, 2021).

Both the aquatic and terrestrial ecosystem are vulnerable due to the invasion of IAS, it causes worldwide undesirable adverse effects on their structure and function (Havel *et al.*, 2015; Shrestha, 2016; Ongore *et al.*, 2018; Shah *et al.*, 2020). For example, in Chitwan National Park, the rapid proliferation of mikania (*Mikania micrantha)* in grassland possess severe problems of food deficiency to greater one-horned rhino (*Rhinoceros unicornis*) (Murphy *et al.*, 2013). Similarly, water hyacinth (*Pontederia crassipes*) is problematic in the aquatic ecosystem (Shrestha, 2016). Invasion of non-native species is considered a major factor for species extinction as well as the over-all change in the habitats which rises in efficient failures of natural ecosystem and enormous loss in biodiversity and economy (Mack *et al.*, 2000; Shrestha, 2021). Among the 100 worst invasive plant species in the world, the four species with significant threat to native species in Nepal are: *Chromolaena odorata*, *P. crassipes*, *Lantana camara* and *M. micrantha* (Lowe *et al.*, 2000; Bhatta *et al.*, 2021). The invasive floating plants harm aquatic ecosystems (Dersseh *et al.*, 2019); as they form dense mats that cause a significant change in the physicochemical parameters of water (Pinero *et al.*, 2021). Six aquatic invasive plant species recorded in Nepal are *Alternanthera philoxeroides*, *Myriophyllum aquaticum*, *P. crassipes*, *Ipomoea carnea fistulosa*, *Pistia stratiotes* and *Leersia hexandra* (Shrestha, 2016; Shah *et al.*, 2020; Pathak *et al.*, 2021). The invasive water hyacinth is considered one of 100 most aggressive and the top 10 worst aquatic weeds with significant harmful effects on aquatic biodiversity globally (Lowe *et al.*, 2000; Gichuki *et al.*, 2012; Ghoussein *et al.*, 2023).

Water hyacinth is perennial, freely floating vascular aquatic weed of the family Pontederiaceae. Lakes, streams, ponds and reservoirs in tropical and subtropical regions of five continents are invaded by this problematic and attractive water hyacinth throughout the Central America, Western and Central Africa, Southeast of United States, and Southeast of Asia (Bartodziej & Weymouth, 1995; Brendonck *et al.*, 2003; Mangas-Ramírez & Elías-Gutiérrez, 2004). Furthermore, it forms interlocking dense mats within a short period due to its complex root structure and massive reproductive output (Thakuri *et al.*, 2019). Proliferation and infestation of water hyacinth are fast in polluted rivers, lakes and ponds because of its capacity to absorb the heavy metals, pesticides and nutrients load from water bodies (Coetzee *et al.*, 2014). Therefore, the water hyacinth invasion is found higher in water bodies with high nutrient loads due to insufficient wastewater treatment, the high load of agricultural runoff and deforestation (Villamagna, 2009; Coetzee *et al.*, 2014; Thakuri *et al.*, 2019).

The nuisance water hyacinth is originated from Amazon basin of Brazil (Gopal, 1987). The first introduction from its native range was in Egypt sometime between 1879 and 1882 and officially reported from African country Ethiopia in 1956 (Friend, 1989; Firehun *et al.*, 2014; Degaga, 2018). It was first time recorded in Asia around 1888, in India around 1896 and in Japan about 1900 from Brazil due to human trade and other activities such as decorative in garden and ponds as its purple flowers are attractive and beautiful (Gopal, 1987). Moreover, the rapid spreading of water hyacinth could be attributed to its fast growth rate, greater capacity in seed production, environmental tolerance and massive reproductive output (Zhang *et al.*, 2010). The entrance of water hyacinth in Nepal might be from India around 1914 to 1947 due to the geographic proximity and easy movement of humans on the border, however, it was first reported in 1996 and highly sprayed in the wetlands of Tarai and mountains within the altitude of 75 - 1500 m from sea level (Tiwari *et al.*, 2005; Dahal, 2006; Maharjan & Ming, 2012; Thakuri *et al.*, 2019). The harmful aquatic weed; water hyacinth is distributed in more than 50 tropical and subtropical countries of the globe primarily because of transport of this plant as decorative and ornamental plant in the gardens and ponds. Three main ways for weeds to spread from one location to another were explained by Minakawa *et al.* (2008): (i) the weeds introduce from one water body to another through the connection chamber between them; (ii) wetland birds and other animals that forage in water hyacinth proliferation habitat carry the vegetative parts and seeds of weeds from one habitat to another habitat in their body parts and fecal matters over a long distance. For example, invasion of weeds in Lake Victoria from the excreta of diving birds; (iii) transport of weeds by the human activities and using as a decorative in garden and pond due to extremely attractive colourful flower. Now the water hyacinth is problematic and rapidly spreading invasive weed in the lakes, streams and reservoirs of Nepal that deteriorates the water quality and hampers the aquatic biodiversity (Maharjan & Ming, 2012; Thakuri *et al.*, 2019). It creates severe socioeconomic and environmental burdens for societies that depend on aquatic resources (Villamagna, 2009; Tifuh, 2012; Begam *et al.*, 2021).

Furthermore, the proliferation of water hyacinth in lakes and reservoirs may form thick mat and occupy the enormous surface of open water, decrease the intensity of light below the mat and obstruct the exchange of air between water and atmosphere and adversely affect the aquatic ecosystem (Fontanarrosa *et al.*, 2010). The fragmented and small mat may have the positive impacts on the aquatic ecosystem, for example, it absorbs pollutants and reduces the nutrient loads in the waterbodies, and it also provides the suitable habitat for the epiphytic organisms because of its well-developed root system (Wang *et al.*, 2012; Wang & Yan, 2017). Additionally, it also affects the aquatic ecosystem in terms of energy flow and nutrient cycling (Villamagna, 2009). As a result, a comprehensive perception about the effects of water hyacinth infestation on the relevant systems, including relationships, interdependence, and competition is necessary (Villamagna & Murphy 2010). The most commonly observed effect due to the dense mat of water hyacinth are lower dissolved oxygen concentration and lower phytoplankton productivity under the mats (Rommens *et al.*, 2003; Villamagna, 2009). Water hyacinth's dense mat prevents the pass of light and atmospheric oxygen from the water surface, it causes difficulty in photosynthesis to algae and other aquatic vegetation and the plants can not release the oxygen into the water (Villamagna & Murphy, 2010). The water hyacinth-infested area has a lower value of dissolved oxygen concentration (1.92±0.29 mg/L) than the non-infested area (5.89±0.85 mg/L) in Lake Naivasha, Kenya (Mironga *et al.*, 2012) and the water quality is degraded in the infested area (Asmath *et al.*, 2022). Moreover, when the concentration of dissolved oxygen in water reaches below 2 mg/L, then such condition will be hypoxic and the fish and other aquatic fauna cannot survive and causing the disruption of aquatic species diversity that results in unproductive ecological conditions (Yongo & Outa, 2015; Ongore *et al.*, 2018; Begam *et al.*, 2021).

In addition, the fragmented patch of water hyacinth is not harmful all the time, but it also supports the occurrences of macro-invertebrates including Ephemeroptera, Oligochaeta, Turbellaria, and Gastropoda (Marco *et al.*, 2001; Rocha-Ramírez *et al.*, 2007). Most of these macro-invertebrates depend on the dissolved oxygen available in and around the roots of water hyacinth, therefore, macro-invertebrates are abundantly found within aquatic macrophytes than in habitats without these plants (Masifwa *et al.*, 2001; Villamagna, 2009). In addition, the higher macro-invertebrate's diversity was recorded in higher water hyacinth coverage (>75%) and prolific coverage (50-75%), but highly diverged fish was recorded in the mat of frequent coverage (25-50%) in Koshi Tappu Wildlife Reserve (Pandey *et al*., 2020).

Furthermore, fish diversity, fish species richness, fish abundance and biomass were found higher in littoral zones having water hyacinth than without in St. Marks River in Florida (Brendonck *et al.*, 2003). The niche with water hyacinth is more favorable to insectivorous fish (Bartodziej & Weymouth, 1995; Bartodziej & Leslie, 1998). Therefore, sometimes it will be detrimental if we plan the complete removal of water hyacinth, for example, three fish species such as *Poecilia sphenops, Cyprinus carpio and Heterandria jonessi* completely lost from the Mexican Reservoir, probably due to the expansion of toxic nutrients (Mangas-Ramírez & Elías-Gutiérrez, 2004).

Waterbirds are one of the major constituents in wetland food webs and their distribution is obstructed by the floating thick mat of water hyacinth above the surface of water (Masifwa *et al.*, 2001; Villamagna, 2009). The close and compact structure floating weed; water hyacinth provides the space for a higher abundance of bird prey, such as insects, amphibians, reptiles and fish, which influence the bird's distribution (Masifwa *et al.*, 2001). Moreover, the aquatic vegetation like submerged macrophytes and floating mat of water hyacinth provide the habitat heterogeneity to protect from predator, a shelter and possible nursery territory for aquatic fauna like macro-invertebrates and fishes which are considered the prey base for the waterbirds (Svingen & Anderson, 1998; Brendonck *et al.*, 2003). For example, birds were feeding prey frequently in and around the mats of water hyacinth in River St. Mark's in Florida

(Bartodziej & Leslie, 1998). The water hyacinth with its disintegrated mat allows the possible foraging habitation specially for wading birds, such as the great-blue heron (*Ardea herodias*), tri-colored heron (*Egretta tricolor*), great egret (*Ardea alba*) and snowy egret (*Egretta thula*) (Villamagna, 2009). A dense mat of water hyacinth is harmful for aquatic biodiversity of lakes and rivers of the Ramsar sites in Nepal. It is equally harmful for the livelihood of the community around the catchment area of the lakes and rivers near the periphery of Begnas in Pokhara (Shrestha, 2016). Moreover, the daily life of people who depend on boating and fishing is adversely influenced because of the invasion of water hyacinth in the lakes. The excessive growth of invasive water hyacinth is a major threat in the lakes of Chitwan (Bhusal & Devkota, 2020).

In most cases, invasive species support other invasive species and facilitate to loss of native species. There is a close association among invasive species and they have a negative interaction with native species of that ecosystem, as discussed by Simberloff & Von Holle (1999) in invasional meltdown hypothesis. The larger lakes of Pokhara valley have been invaded by the nuisance invasive water hyacinth for more than two decades. The future climatic data also predicted the lakes Phewa, Begnas, Rupa and Khaste in Pokhara have the higher possibility of water hyacinth's potential invasion in 2030 and 2050 (Thakuri *et al.*, 2019). Therefore, it is crucial to prevent the biological invasion and their threats to the aquatic ecosystem of the lakes in Pokhara Valley. However, the invasion of water hyacinth and its effects on the water parameters and aquatic biodiversity of lakes in Pokhara Valley is not documented. Therefore, the current study was focused on the way to investigate the influence of water hyacinth's invasion on water quality and the aquatic biodiversity of the lakes in Pokhara Valley, Nepal.

1.2 Rationale

Pokhara metropolitan city encompasses the nine-lake cluster and large catchment area forming the large area of wetlands and it was established as the largest Ramsar site on 2 February 2016 (Ramsar Convention Secretariat, 2016; MoFE, 2018). The Pokhara Ramsar site has a key function in delivering ecosystem services and harbor a diverse array of biodiversity (MoFE, 2018; Pathak *et al.*, 2021). Moreover, the wetlands are rich in biological diversity and are considered the major foundations of water resource for various purposes such as drinking, fishing and irrigation. However, the larger lakes in Pokhara such as Phewa, Begnas and Rupa have been infested by invasive water hyacinth (Preliminary field visit), which can cause significant harm to the water quality, aquatic organisms and waterbird species in the area. The water hyacinth rapid growth rate and dense coverage can result in the loss of native flora and fauna, as well as migratory and threatened waterbirds (Cilliers *et al.*, 2003; Villamagna, 2009; Guo, 2017). The excessive expansion of the water hyacinth forms the dense mat and reduces the open water area for water-dwelling darters, ducks, gooses, cormorants and grebes. Additionally, its impact on the water quality and aquatic biodiversity in the lakes of Pokhara Valley remains under studied.

The diversity and species composition of macro-invertebrates are integral components of aquatic ecosystems have a pivotal role in their functioning. Moreover, they can serve as indicators of changing environmental changes. Macro- invertebrates play a significant role to transfer the energy in different trophic levels from detritus to consumers in ecological process. The understanding of the invasion of water hyacinth and its impacts on physicochemical parameters of water, and macro-invertebrate's abundance and diversity around the lakes in Pokhara remains limited, despite its undeniable significance.

The trend of increasing catches of exotic and invasive fish species and a decline of the same for native and local fish species in the lakes of Pokhara highlights the necessary for further investigation into the potential association between these species and other unknown invasive species. For example, the catch of non-native Nile tilapia (*Oreochromis niloticus*) is increasing significantly during recent years after the invasion of water hyacinth in the lakes of Pokhara. In most cases the invasive species support other invasive species and facilitate to loss of native species. The association between invasive water hyacinth and exotic Nile tilapia as well as other native species of fish in the lakes of Pokhara Valley is not documented scientifically. In the preliminary observation, it was noticed that the mat of water hyacinth provided the foraging grounds and hiding places for many residential wading birds like cattle egret (*Bubulcus ibis*), Indian pond heron (*Ardeola grayii*), common moorhen (*Gallinula chloropus*), purple swamphen (*Porphyrio porphyrio*), common coot (*Fulica atra*), etc. Aquatic food webs are critically impacted by the deteriorating water quality and restricted access to winter migratory and threatened waterbirds brought on by the invasion of water hyacinth. Furthermore, the winter migratory birds including many threatened species are declining continuously. Therefore, this study aimed to investigate the invasion effects of water hyacinth on the physicochemical parameters, as well as the abundance of macro-invertebrates, fishes, and waterbirds in the lakes of Pokhara.

The output of this research could be fruitful for the conservationists, policy makers and managers for developing policy to control and management of invasive aquatic plants and the conservation of aquatic biodiversity in the lakes of the Pokhara. The findings of this study could serve as the baseline data for further investigation about the invasion of aquatic weeds and their impacts on biological diversity.

1.3 Objectives

The general objective of this study was to examine the ecological effects of invasion of water hyacinth on abiotic and biotic components of lake ecosystems in the lakes of Pokhara. The specific objectives were:

1. To assess the impact of water hyacinth on physicochemical parameters of water and abundance of macro-invertebrates.
2. To illustrate the influence of invasive water hyacinth on the abundance and diversity of fish.
3. To identify the effects of invasion of water hyacinth on the abundance and diversity of waterbirds.

1.4 Organization of the thesis

The present study investigated the invasion of water hyacinth and its impacts on physicochemical parameters and aquatic biodiversity of the lakes in Pokhara Valley. In Chapter 1, the overarching elements of this thesis are succinctly outlined through subheadings including Background, Rationale and the main Objectives of the study. The Background section of this study provides an overall scenario of previous research and identified any research gaps in knowledge about the effect of invasive aquatic weeds on water quality, macro- invertebrates, fish and waterbirds. In Chapter 2, the comparison of physicochemical parameters in water hyacinth presence (HP) and absence (HA) habitats and the effect of infestation of water hyacinth on macro-invertebrates are documented. The overall abundance, and the abundance of dominant orders of macro-invertebrates in HP and HA habitats are also compared in this Chapter.

In Chapter 3, the influence of the water hyacinth's invasion on the diversity and abundance of both exotic and native fish species are evaluated. In Chapter 4, the study documents the invasion of water hyacinth and its impacts on the diversity and abundance of waterbirds in the lakes of the Pokhara. This Chapter also contains the factors affecting on the abundance and species richness of threatened waterbirds in these lakes. In Chapter 5, the overall Conclusions of this work and Recommendations on the basis of output of present study for future research in the same field are pointed out. The Summary of the thesis is presented in Chapter 6 which is the last chapter.

CHAPTER 2

THE EFFECT OF INVASION OF WATER HYACINTH ON WATER QUALITY AND ABUNDANCE OF MACRO-INVERTEBRATES IN THE LAKES OF POKHARA VALLEY, NEPAL

Abstract

Alien Invasive Plant Species appear an important threat to the wetland ecosystems and results in the loss of aquatic biodiversity. Freshwater environments like streams and lakes are especially vulnerable to loss of native species. The extensive distribution of water hyacinth (*Pontederia crassipes*) in the lakes of Pokhara Valley has jeopardized the survival of a number of aquatic species. The present study explored the association of water hyacinth coverage with water parameters and it also sought to identify the impact of water hyacinth infestation on water parameters and macro-invertebrate's abundance in the lakes. Samples of water and macro-invertebrates were collected in water hyacinth absence (HA) and presence (HP) habitats from 24 sampling plots seasonally from the lakes of Pokhara Valley, using bottles and Grab Sampler respectively. Temperature and turbidity of water were recorded comparable but rest of the parameters were significantly differed in HA and HP habitats. The depth, transparency, pH, and dissolved oxygen were shown to be negatively related with water hyacinth, while temperature and free carbon dioxide were found to be closely related. Furthermore, in total 21 families, 15 orders, 26 genera and 29 species of macro-invertebrates were recorded. The adverse effect of water hyacinth's invasion was found on the abundance of Diptera and Haplotaxida while other groups such as Odonata, Ephemeroptera, Coleoptera, Caenogastropoda and sphaeriida were significantly more abundant in the mat of water hyacinth. Macro-invertebrates were recorded less numerous and diversified in the HA habitat than in the HP habitat. The present study explored the adverse effect on some groups of macro-invertebrates due to the invasion of water hyacinth and it may cause changes in the faunal structure of lakes of Pokhara Valley. Therefore, developing a plan to manage water hyacinth of lakes in Pokhara Valley is advised.

2.1 INTRODUCTION

The phenomenon of wetland habitat deterioration caused by invasive alien plant species (IAPS) invasion is an urgent issue that needs the attention of ecologists and conservationists globally (Coetzee *et al.*, 2014; Shrestha, 2016; Pathak *et al.*, 2021; Lamelas-López *et al.*, 2021). The invasion of invasive species not only alters habitats of the ecosystems of invaded areas but also impacts the diversity and richness of native plant and animal species. The introduction of IAPS can lead to increased predation, hybridization, and competition ultimately leading to change the habitat and the structure of community (Blackburn *et al.*, 2011; Shrestha, 2016; Gentili *et al.*, 2021). Further, IAPS invasion can have a negative influence on all types of ecosystems, freshwater environments such as streams, rivers and lakes are more vulnerable to loss of species due to the invasion of IAPS and alteration of habitats (Havel *et al.*, 2015; Shrestha & Shrestha, 2021). In Nepal, a total of six aquatic invasive plants have been reported, out of them five species are found in the lakes of Pokhara (Shrestha, 2016; Pathak *et al.*, 2021). The invasive aquatic plants recorded in lakes of Pokhara Valley are: *Leersia hexandra*, *Pistia stratiotes*, *Alternanthera philoxeroides*, *Pontederia crassipes* and *Ipomoea carnea fistulosa* (Pathak *et al.*, 2021). The water hyacinth (*Pontederia crassipes*) is considered one of the world's 100 worst species among these five invasive species (Lowe *et al.*, 2000; Gichuki *et al.*, 2012).

The infestation of water hyacinth is recorded in the water bodies of approximately 50 countries in tropical and subtropical regions across the five continents (Bartodziej & Weymouth, 1995; Villamagna, 2009). The native home country of water hyacinth is Brazil in South America, now it is abundantly found in Southeast Asia, New Zealand, North America, Africa and Nigeria (Ndimele *et al.*, 2011). The species was first noticed in Nepal in 1966 in wetlands of Tarai in the western section, most likely brought from India (Tiwari *et al.*, 2005; Dahal, 2006; Maharjan & Ming, 2012; Khatri *et al.*, 2018). Human endeavors including gardening, decoration, trade and transportation helped in the expansion of invasive water hyacinth from native range to new countries in the word (Villamagna, 2009). Eutrophic lakes and average water temperatures of 30°C are typically advantageous for the growth and development of this invasive weed (Harun *et al.*, 2021). The growth is very fast in the water bodies of its contamination because of absence of natural enemies and its invasive nature (Khatri *et al.*, 2018). Since it has rapid multiplication rate, the population of water hyacinths could double within 10 to

12 days under suitable environmental condition and water temperature ranges from 25°C to 35°C (Gunnarsson & Petersen, 2007; Villamagna, 2009). Moreover, the freshwater ecosystem is vulnerables because of the invasion aquatic weeds like water hyacinth resulting the degradation of water quality, alteration of habitats and loss of native (Villamagna, 2009; Villamagna *et al.*, 2012; Hailu *et al.*, 2020). Additionally, water hyacinth generates significant ecological and economic problems, including the unpleasant samell in water, challenges with water-based enjoyment, such as boating, swimming and water transportation, as well as difficulties in fisheries and farming (Njiru *et al.*, 2002; Villamagna, 2009; Villamagna & Murphy, 2010; Yigermal & Assefa, 2019).

Under the thick mat of invasive water hyacinth, the slower rate of photosynthesis by phytoplankton results an increase in water temperature and free carbon dioxide and decrease in dissolved oxygen and (Villamagna, 2009; Mironga *et al.*, 2012). As a consequence, the concentrations of manganese, iron, ammonia and sulfide increase, leading the aquatic environment to enter a state of anoxia and altering the composition as well as the functioning of the entire aquatic ecosystem (Yongo & Outa, 2015). The mats reduce the amount of oxygen produced by other aquatic vegetations like algae (Villamagna & Murphy, 2010). It could lower the amounts of dissolved oxygen in water hyacinth-infested habitats (McVea & Boyd, 1975; Masifwa *et al.*, 2001; Mironga *et al.*, 2012). After the death of water hyacinth, the dead plant body sinks to the bottom, the amount of oxygen is decreased because the biomass of rotting plants consumes a lot of oxygen. As a result, the concentration of dissolved oxygen may fall to extremely low and the condition will be problematic to the native flora and fauna. Additionally, low dissolved oxygen levels enable the sediment to release phosphorus, accelerating eutrophication and possibly increasing in the water hyacinth growth and proliferation or algal blooms (Bicudo *et al.*, 2007).

The floating water hyacinth and other macrophytes serve a distinctive complex structure and the most favorable environments for larger macro-invertebrate colonization in fresh water (Masifwa *et al.*, 2001; Arora & Mehra, 2003). Occurrence of zooplanktons in aquatic system determines the abundance and diversity of macro-invertebrates because zooplankton directly generates energy to them. Micro-crustacea and copepods were more abundant in open water habitats, while epiphytic rotifers were more abundant in water hyacinth presence habitats (Arora & Mehra 2003; Mangas-Ramirez & Elias-Gutierrez 2004).

Water hyacinth forms the mats of interconnecting roots due to its structural complexity and varying length of the root (Gopal, 1987). This mat provides a unique habitat for development and colonization of many macro-invertebrates for example amphipods, arachnids and snails (Brendonck *et al.*, 2003; Rocha-Ramrez *et al.*, 2007; Villamagna, 2009). The water hyacinth edge has been found to have more macro-invertebrates than the rooted emergent vegetation (Bailey & Litterick, 1993; Masifwa *et al.*, 2001). Additionally, the expansion of invasive species into freshwater streams and lakes may alter the habitat complexity, resulting a shift of the macro-invertebrates and change in the structure of community (Wahl *et al.*, 2021).

Among the nine lakes of Pokhara, the larger size lakes Phewa, Begnas, and Rupa are highly proliferated with water hyacinth, lowering the diversity and abundance of winter migratory waterbirds (see in Chapter 4). Macro-invertebrates serve a crucial role in the biological process of energy transfer to consumers through detritus. However, little is known regarding the water hyacinth's impacts on the water parameters and abundance of macro-invertebrates in the lakes of Pokhara. The relationship between water parameters and coverage of this weed in these lakes is also not studied. Therefore, the present study attempted to investigate the effects of water hyacinth's invasion on the physicochemical parameters of water, and abundance and diversity of macro-invertebrates in the lakes of Pokhara Valley, which provided the baseline information on noxious aquatic weeds and biodiversity conservation.

2.2 MATERIALS AND METHODS

2.2.1 Study area

Pokhara is one of the rapidly urbanizing beautiful and historically important Metropolitan City in Nepal. It is the administrative headquarter of Gandaki Province and is located 200 kilometers west of the capital city Kathmandu. It is the largest metropolitan city of Nepal and it includes the nine ecologically significant lakes. Among these lakes, the Phewa and Kamal Pokhari are located within the city of Pokhara and other seven lakes Begnas, Rupa, Dipang, Gunde, Niureni, Khaste, and Maidi are located within Lekhanath in the Lake Cluster of Pokhara Valley (LCPV); the latest and largest Ramsar Site of Nepal (Figure 1).

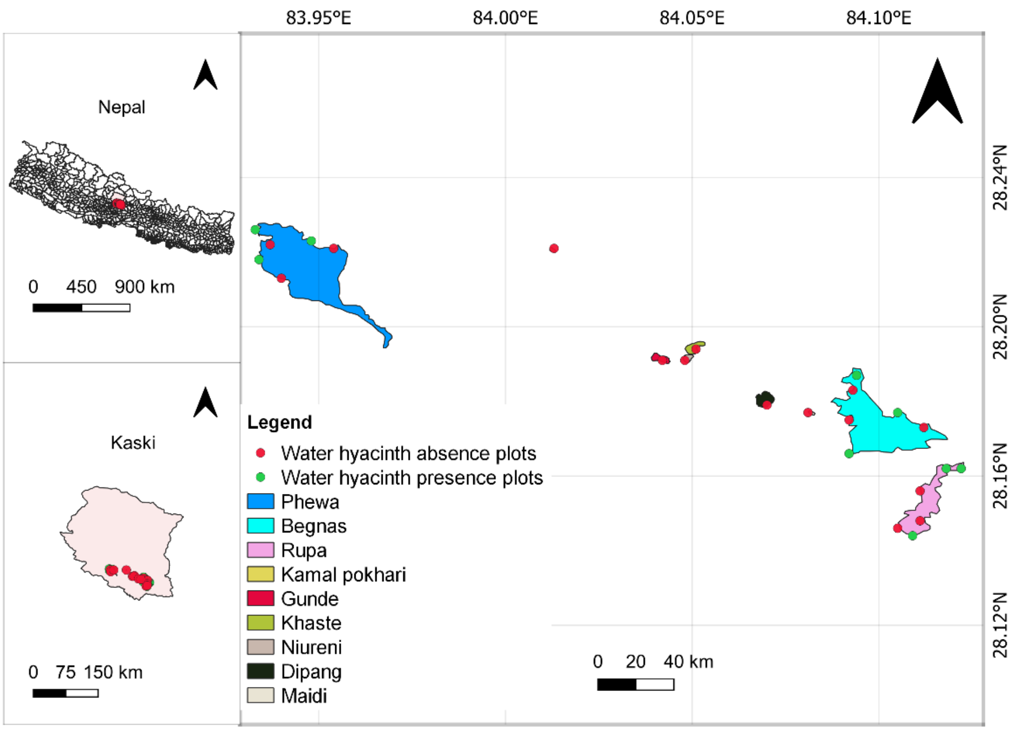


Figure 1: Study area showing the lakes of Pokhara Valley, Nepal

The LCPV extends from 27˚55'−28˚23' N latitude to 83˚48'−84˚11' E longitude with larger areas in Pokhara Metropolitan City and a small portion in Annapurna and Rupa Village Municipalities in central Nepal (Pathak *et al.*, 2021). Pokhara Metropolitan City, which is in a variety of climatic conditions within the Seti River basin, is one of the cities in Nepal that is urbanizing the fastest. The climatic variations in the northern half of the basin range from temperate to alpine with a high precipitation rate, about 3000 mm per year, while the middle part of the basin's climatic conditions range from sub-tropical to cool temperate (Tripathee *et al.*, 2016). The whole wetland area of LCPV was designated as the 10^{th} Ramsar site on February 2, 2016. The area of the LCPV is 262 km^2 and the total area of water bodies is 9 km^2 within this cluster, and it is the largest Ramsar site of Nepal. Phewa is the largest lake, it fluctuates between eutrophic and mesotrophic. Similarly, Begnas is the second largest lake, it fluctuates between mesotrophic and oligotrophic while the remaining Rupa and other smaller lakes fluctuate as eutrophic (MoFE, 2018).

The wetland of LCPV is considered as a hotspot in terms of biodiversity; its southern section is covered by tropical to the sub-tropical broad-leaved Sal Forest (*Shorea robusta*) and the western and northern parts are covered with Chilaune-katus forest (*Schima-Castanopsis*). The cluster supports over 360 plant species, 32 mammal species, 140 wetland bird species, 24 reptilian species, 11amphibian species and 27 fish species (MoFE, 2018). The LCPV is the natural habitat of varieties of plant species such as yellow grass orchid (*Apostasia wallichii*), champak (*Michelia champaca*), satawari or asparagus (*Asparagus racemosus*), bulbophyllum (*Bulbophyllum plyrhiza*), white cheese-wood (*Alstonia scholaris*); cymbidium (*Cymbidium iridioides*), pineapple orchid (*Dendrobium densiflorum*), fringe-lipped dendrobium (*D. fimbriatum*), tree fern (*Cyathea spinulosa*), elephant’s foot (*Dioscorea deltoidea*), oberonia or Nepal’s orchid (*Oberonia nepalensis*), Sikkim’s orchid (*O. iridifolia*), water chestnut (*Trapa natans*), Indian trumpet flower (*Oroxylum indicum*), terete vanda (*Papilionanthe teres*), malabar gulbel (*Tinospora sinensis*), common hornwort (*Ceratophyllum demersum*), and lesser bulrush (*Typha angustifolia*) (MoFE, 2018). The LCPV is rich in avian fauna for example, Nepal wren babbler (*Pnoepyga immaculate*), comb duck (*Sarkidiornis melanotos*), spiny babbler (*Turdoides nepalensis*) (MoFE, 2018), and numerous other wetland birds are found in this periphery.

LCPV also supports fauna of globally threatened categories such as leopard (*Panthera pardus*), clouded leopard (*Neofelis nebulosa*), yellow-breasted bunting (*Emberiza aureola*), common pochard (*Aythya ferina*), woolly necked stork (*Ciconia episcopus*), baer’s pochard (*Aythya baeri*), and ferruginous pochard (*Aythya nyroca*) (MoFE, 2018). Further, the presence of invasive species, including giant African land snail (*Achatina fulica*), African cuttlefish (*Clarias gairiepinus*), Nile tilapia (*Oreochromis nilotica*), mikania (*Mikania micrantha*), water hyacinth, cut grass (*Leersia hexandra*), parthenium (*Parthenium hysterophorus*), alligator weed (*Alternanthera philoxeroides*), water lettuce (*Pistia stratiotes*) and bush morning glory (*Ipomoea carnea fistulosa*) has also been documented (MoFE, 2018; Adhikari *et al*., 2020; Pathak *et al*., 2021).

2.2.2 Methods

Based on the size of lakes and occurrence of water hyacinth in the lakes, over all 24 sampling plots each of 50 × 50 m^2 were demarcated and seasonal data were collected from these plots in the lakes of Pokhara Valley. In larger lakes Phewa, Begnas and Rupa, three water hyacinth presence (HP) and three water hyacinth absence (HA) plots were demarcated. While each of the smaller lake contained a single HA plot because of absence of water hyacinth in those lakes. The minimum distance from one HP plot to another HA plot was 500 m in all large lakes. The water hyacinth coverage in HP plots was not less than 90% throughout the study period, while this weed was totally lacked in HA plots over the previous one decade and the confirmation was taken by the consultation with Jalari community and management committees of each lake. The coverage was estimated visually and as well as from photographs. Each plot is further divided into 1 × 1 m^2 sub plots. In each HP plots the water hyacinth coverage was measured using a measuring tape. GPS (Garmin eTrex Touch 35) was used to record the coordinates of each plot. Seasonal data were collected from each lake during September to November 2019, December to February 2020, March to May 2020 and June to August 2020.

2.2.2.1 Physicochemical Parameters of water

The physicochemical parameters of water including temperature, transparency, pH, depth, turbidity, total dissolved solids (TDS), dissolved oxygen (DO), free carbon dioxide (CO_2), nitrate (NO_3) and total alkalinity (TA) were measured at each plot. The water samples were collected from five subplots, one from the center each plot and four along each plot edge and all five samples were mixed equally to make a single sample of water. The physicochemical parameters were investigated following the methods Golterman *et al.* (1978); Trivedy and Goel (1984); Adoni *et al.* (1985) and APHA (1998). The water parameters such as depth, temperature and transparency were measured in field, DO, CO_2 and TA were determined in laboratory of Department of Zoology, Prithvi Narayan Campus. The remaining parameters pH, turbidity, TDS and

NO_3 were analysed in the laboratory of Federal Water Supply and Waste Management Project, Pokhara.

2.2.2.1.1 Depth of water

A weight, rope, and measuring tape were used to determine the depth of the water (m). A rope was linked to the weight and progressively lowered into the water until the weight struck the bottom, at which point the rope was marked. The weight was removed and the rope's markings were measured with a measuring tape. The reading was taken three times, with the mean value used in the analysis.

2.2.2.1.2 Transparency (m)

A Secchi disc of diameter 20 cm was used to measure the transparency (m) of water. The disc was fallen downward below from surface of water until it completely disappeared, the point of depth from surface was recorded. Similarly, the Secchi disc was then pulled up until it reappeared, and again the point of depth was recorded. The procedure was repeated three times, with the mean used in the analysis.

$$D = \frac{a+b}{2}$$

Where,

D = Visibility depth,

a= Depth disappearance and

b= Depth reappearance

2.2.2.1.3 Water surface temperature

Standard mercury thermometer calibrated to $0^{o}C$ -$50^{o}C$ and with a precision of $0.1^{o}C$ was used to measure the temperature of the water surface. It was repeated three times, with the mean used in the analysis.

2.2.2.1.4 Potential of Hydrogen ion (pH)

The pH of water was measured using a Hanna water test pH-meter (4500-B, APHA) with a calibration range of 1.0-14.0 and an accuracy of 0.1. The mean value of three repeated measures was used in the analysis.

2.2.2.1.5 Dissolved Oxygen (DO)

A popular Winkler's method of titration was used to determine the amount of dissolved oxygen. A BOD bottle (300 ml.) was filled with water sample and a stopper was placed on the bottle carefully to prevent air trapping within it. After removing the stopper from the BOD bottle, 2 ml. of manganese sulphate ($MnSO_4$) and 2 ml. of alkaline potassium iodide (KI) were added at a few second intervals using a separate pipette. Again, the stopper was carefully inserted to avoid air bubbles, and the solution was well mixed by inverting the bottle for a few minutes. After the settled of precipitation at the bottom sufficiently, 2 ml. of concentrated H_2SO_4 was added to it. The stopper was gently reinstated, and the bottle was shaken numerous times with figure 8 until the precipitate was totally dissolved. A 50 ml. of well mixed sample was dropped in burette from the BOD bottle using a pipette and titrated against sodium thiosulphate solution $Na_2S_2O_3$ (0.025 N) in a conical flask until a light coffee brown hue developed. At the moment a drop of starch solution was added as an indicator and again titrated against sodium thiosulphate until blue colour disappeared. The volume of titrant used in getting the end point was noted. The amount of DO in water was calculated using the formula below. The mean value of three repeated measure was used for analysis.

$$\text{Dissolved oxygen (mg/L)} = \frac{\text{ml.} \times \text{N of titrant} \times 8 \times 1000}{\frac{\text{V2} \times \text{V1} - \text{V}}{\text{V1}}}$$

Where,

N = Normality

V = Volume of $MnSO_4$ and Potassium iodide used

V1 = Volume of the bottle after placing the stopper

V2 = Volume of the part of the contents titrated

2.2.2.1.6 Free Carbon dioxide (CO_2)

The water sample of 50 ml. was taken in a conical flask and a few drops of Phenolphthalein indicator was added to it for simple titrimetric process. If the sample did not become pink and remained colourless it indicated the presence of CO_2 in the water sample. As soon as a faint pink colour developed at the endpoint, the sample was titrated against a sodium hydroxide (NaOH) solution (0.05 N). The total volume of NaOH consumed is the amount of free CO_2. Three reading of burette were noted and the amount of free CO2 was calculated by using the formula given below. The mean value of three repeated measure was used for analysis.

$$\text{Free Carbon dioxide (mg/L)} = \frac{\text{ml.} \times \text{N of NaOH} \times 1000 \times 44}{\text{ml.of sample}}$$

Where,

N = Normality

2.2.2.1.7 Total Alkalinity (TA)

The water sample of 50 ml. was kept in conical flask and a few drops of the phenolphthalein indicator was added to measure the total alkalinity. The sample changed to pink, showing that phenolphthalein alkalinity was present. The titration was conducted with hydrochloric acid (HCL - 0.1 N) until the pink colour disappeared. The same sample was then treated with two or three drops of methyl orange indicator then it was titrated with hydrochloric acid of the same normality (0.1 N) until its colour turned pink. It was noted the amount of total hydrochloric acid used. Total alkalinity contained in sample as Calcium carbonate was calculated three times using the formula given below and mean was used for analysis.

$$\text{TA as } CaCO_3 \text{ (mg/L)} = \frac{\text{A} \times \text{N of titrant} \times 1000 \times 50}{\text{ml.of sample}}$$

Where,

A = ml of HCl used with only phenolphthalein indicator

TA = total alkalinity

2.2.2.1.8 Total dissolved solids (TDS)

TDS (mg/L) were determined using an instrument refractometer following the destructive method of TDS measurement.

2.2.2.1.9 Turbidity- Nephelometric Turbidity Unit (NTU)

Turbidity was calculated instrumentally using APHA methods, 3130 B.

2.2.2.1.10 Nitrate (NO_3^-)

Nitrate (NO_3^-) was determined using Ultraviolet Spectrophotometric Methods (USM-4500 NO_3^-), ranged 0.2 – 11 mg nitrate-Nitrogen.

2.2.2.2 Macro-invertebrates survey

Samples of macro-invertebrates were collected from the same plots and subplots used to collect the water samples using Peterson's Grab Sampler of size 0.0289 m (Sly, 1969) in each subplot. The content of each Grab samples was collected in well labeled polythene bags. The contents of the bags were screened by using a standard sieve of 40 mesh size/inch. The water hyacinth plants from each subplot were taken out and the epiphytic macro-invertebrates found in stem and leaf were manually picked up using entomological forceps and brush, but the root was combed in a bucket containing 70% ethanol (Figure 2). The sieved samples and manually picked samples were transferred to plastic bottles containing 5% formalin and kept the details of the sampling site and date following (Toft *et al.*, 2003). The samples of macro-invertebrates were taken to the laboratory and poured into a white enamel tray. All the macro-invertebrate taxa were identified up to possible taxonomic level (species) following the taxonomic manuals (Pennak, 1953; Edmondson, 1959; Macan, 1977; Subramanian & Sivaramakrishnan, 2007). All the individuals were counted from each sub-plot and its average was calculated for analysis. The abundance of macro-invertebrate taxa was expressed as individuals per square meter. The samples of macro-invertebrate specimens were kept in the Museum at Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Figure 2: Sampling of macro-invertebrates using Grab Sampler (a), combing of roots of water hyacinth (b), screening of grab samples (c) and identification of macro-invertebrates (d)

2.2.2.3 Data analysis

The following diversity indices of macro-invertebrates were calculated in both HP and HA habitats of the lakes using given formula.

a) Shannon–Wiener diversity (H′) (Shannon & Weaver, 1949)
b) Species evenness (J) (Pielou, 1966) and
c) Species richness (S) (Margalef, 1968)

Shannon Wiener's diversity index (H') = $-\sum \left(\frac{ni}{N}\right) \ln(\frac{ni}{N})$

Where,

ni = Importance values for each species is sum of individuals in each species, the abundance of each species.

N = Total importance value, is the sum of the number of individuals observed.

The Pielou's species evenness (J) was determined by using the formula given below.

Pielou's species evenness (J) = $\frac{H'}{\ln S}$

Where,

H'= Shannon Wiener's diversity index

S = Species richness is the number of species and is simply a count of the number of different species in a given area.

The Shapiro-wilk test was performed to check the normality of data, it indicated that the data were non-normally distributed. Therefore, the Mann-Whitney test was used to compare the variation of physicochemical parameters of water and macro-invertebrate abundance in HP and HA habitat. Correlation matrix was performed to show the relation of water hyacinth coverage and physicochemical parameters. The association of abundance macro-invertebrates with the water hyacinth coverage and water depth was performed by Spearman's correlation and Simple linear regression.

Multivariate ordination analysis was conducted to identify the association of macro-invertebrates' taxa with physicochemical parameters of water and water hyacinth coverage.

Detrended Correspondence Analysis (DCA) was applied using the function decorana following Yang *et al.* (2020) to find whether the data supported Canonical

Correspondence Analysis (CCA) or Redundancy Analysis (RDA). The RDA was supported by the data because the lengths of all four axes were less than two in DCA. RDA was used to show the relation of the macro-invertebrates with the environmental variables using the function rda. Moreover, the Monte-Carlo permutation test under the reduced model of 999 permutations at ($\alpha = 0.05$) was used for the significance test following method described by Powell (2019). The DCA and the RDA were conducted in the vegan package of R statistical software. The data analysis was carried out using program R (R Core Team, 2020).

2.3 RESULTS

2.3.1 Physicochemical parameters of water

The water parameters such as transparency, depth, pH, total dissolved solids, dissolved oxygen, free carbon dioxide, nitrate and total alkalinity were significantly varied in HA and HP habitats ($p < 0.001$; Table 1). While the water temperature and turbidity were similar in both areas ($p > 0.05$). The parameters of lake water such as depth, transparency, pH, dissolved oxygen, total alkalinity and nitrate were recorded higher in HA areas, but the amount of free carbon dioxide and total dissolved solid were higher in HP areas.

Table 1: Physicochemical parameters of water in water hyacinth presence (HP) and absence (HA) habitats in lakes of Pokhara Valley, Nepal from 2019 to 2020. Range of reported values are in parentheses

Water parameters	**Habitat (HP)** **Median**	**Habitat (HA)** **Median**	**Mann–Whitney U test**
Transparency(m)	0.77 (0.5-1)	1.4 (0.7-2.6)	U = 2057; p < **0.001**
Temperature (°C)	25 (15.6-30.6)	24.5 (15-29.6)	U = 850.5; p = 0.082
Depth (m)	3 (2-4)	4(1.9-11)	U = 1817; p < **0.001**
TDS (mg/L)	23.5 (19-34)	19(15-17)	U = 602; p < **0.001**
Turbidity (NTU)	3.05 (2.1-6.7)	3.05 (0.9-11.1)	U = 949; p = 0.323
Total alkalinity (mg/L)	126.5 (109-196)	149.5 (121-298)	U = 1677; p < **0.001**
DO (mg/L)	4 (2.84-4.5)	6.6 (2.4-8.6)	U = 1997; p < **0.001**
CO_2 (mg/L)	11.85 (10.12-15)	6.8 (3.8-12)	U = 96.5; p < **0.001**
NO_3 (mg/L)	1.25 (0.6-2.3)	2.05 (0.76-3.2)	U = 1766; p < **0.001**

The range of depth of water was from 2 m to 4 m in HP habitats and 1.9 m to 11 m in HA habitats. Similarly, the amount of dissolved oxygen was from 2.84 mg/L to 4.5

mg/L in HP area and 2.4 mg/L to 8.6 mg/L in HP habitats. Likewise, free carbon dioxide was 10.12 mg/L to 15 mg/L in HP habitats and 3.8 mg/L to 12 mg/L in HA habitats (Table 1).

2.3.2 Correlation of water hyacinth coverage with physicochemical parameters

There was positive correlation between water hyacinth coverage and free carbon dioxide (r = 0.84) and water temperature (r = 0.86) but it had negative correlation with transparency (r = -0.73), pH (r = - 0.64), depth (r = -0.51), dissolved oxygen (r = -0.77) and nitrate (r = -0.63) (Table 2).

Table 2: Correlation among physicochemical parameters and water hyacinth coverage in lakes of Pokhara Valley, Nepal from 2019 to 2020

Parameters	Depth	Temp.	Transp.	pH	DO	Free CO_2	TA	Nitrate	WHCOV
Depth	1								
Temp.	-0.620	1							
Transp.	**0.**822	-0.761	1						
pH	0.676	-0.655	0.744	1					
DO	0.693	**-0.798**	0.788	0.633	1				
Free CO_2	-0.785	**0.819**	**-0.930**	**-0.799**	-0.763	1			
TA	0.326	-0.302	0.341	0.548	0.265	-0.449	1		
Nitrate	0.435	-0.674	0.581	0.550	0.677	-0.586	0.551	1	
WHCOV	**-0.512**	**0.861**	**-0.739**	**-0.641**	**-0.768**	**0.858**	-0.463	**-0.631**	1

Abbreviations: Temp. = Water temperature, Transp. = Transparency, TA = Total Alkalinity and WHCOV = Water hyacinth coverage.

2.3.3 Abundance and diversity of macro-invertebrates

A total of 29 Species of macro-invertebrates belonging to 26 genera representing 21 Families under 15 Orders of the three phyla Annelida, Arthropoda and Mollusca were identified during the study period in the lakes Pokhara Valley (Table 4). The highest relative abundance of macro-invertebrates 16.47% (n = 4644) was found in the HP habitat in the autumn of 2019 and 16.25% (n = 4583) in the HA habitat in the spring of 2020. Similarly, the lowest relative abundance of 9.85% (n = 2776) was found in the HP habitat and 10.57% (n=2981) in the HA habitat during the winter of 2020 (Table 3). The highest mean abundance of macro-invertebrates (102.35±7.04) was recorded in bottom sediments, followed by (50.38±3.67) in the root of water hyacinth and the lowest abundance (7.47±0.33) was in the stem of water hyacinth and sand (Figure 3).

All the macro-invertebrates with their mean abundance in all the seasons in both HP and HA habitat was listed in Appendix IV.

Table 3: Seasonal abundance and relative abundance (RA) of macro-Invertebrates in lakes of Pokhara Valley, Nepal from 2019 to 2020

Seasons	HP	RA%	HA	RA%
Autumn	4644	16.47	3889	13.79
Winter	2776	9.85	2981	10.57
Spring	3860	13.69	4583	16.25
Summer	3006	10.66	2457	8.71

The highest mean abundance of macro-invertebrates (102.35±7.04) was recorded in bottom sediments, followed by (50.38±3.67) in the root of water hyacinth and the lowest abundance (7.47±0.33) was in the stem of water hyacinth and sand (Figure 3). All the macro-invertebrates with their mean abundance in all the seasons in both HP and HA habitat was listed in Appendix IV.

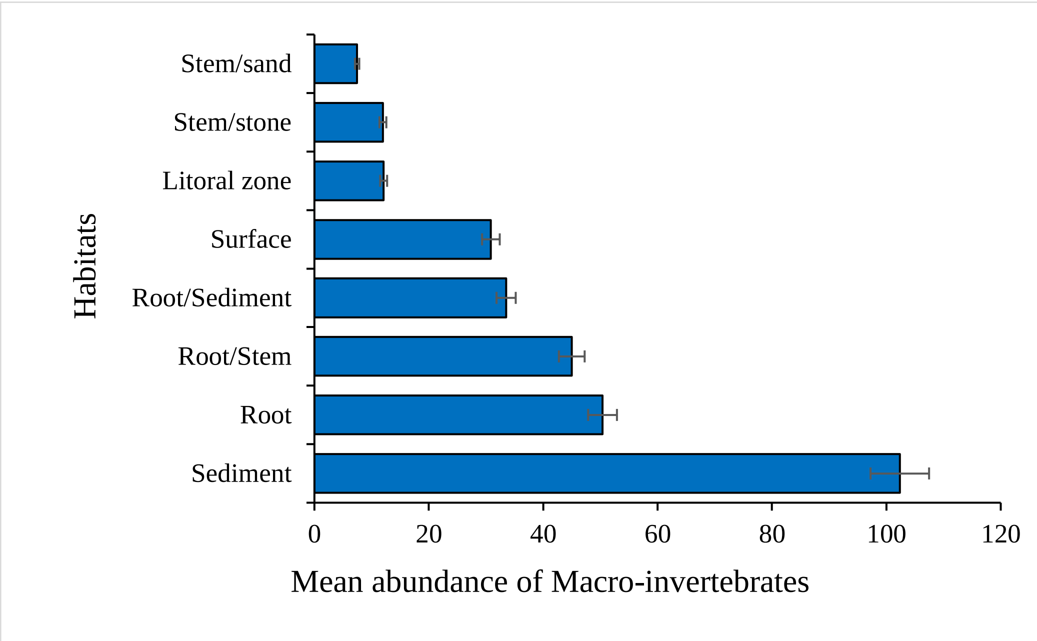


Figure 3: Abundance of macro-invertebrates in lakes of Pokhara Valley, Nepal

The highest mean abundance for *Chironomus* sp. (37.52±4.07) was recorded in the study and was followed by *Aquarius remiges* (30.30±1.92) in the HA habitat whereas the lowest abundance was found for *Dolomedes tenebrosus* (0.25±0.10). Similarly, the highest mean abundance for *Thermonectus* sp. (72.69±4.79) was recorded in the HP habitat, followed by *Sphaerium* sp. (64.06±3.53) whereas the lowest abundance was found for *Nepa cenerea* (0.08±0.08) (Table 4).

Table 4: Abundance of the macro-invertebrates in water hyacinth presence (HP) and absence (HA) habitats in lakes of Pokhara Valley, Nepal from 2019 to 2020

SN	Order	Family	Phylum	Scientific name	Mean±SE (HP)	Mean±SE (HA)
1	Haplotaxida	Tubificidae	Annelida	*Limnodrilus hoffmeisteri* Claparède, 1862	11.42±1.15	20.93±2.41
2	Haplotaxida	Tubificidae	Annelida	*Branchiura sowerbi* Beddard, 1892	13.25±1.47	21.27±2.14
3	Haplotaxida	Naididae	Annelida	*Tubifex tubifex* Mueller, 1774	5.61±0.94	16.95±1.75
4	Haplotaxida	Naididae	Annelida	*Stylaria lacustris* Linnaeus, 1767	5.83±0.55	13.47±1.72
5	Hirudinida	Hirudinidae	Annelida	*Hirudinaria granulosa* Lucknow, 1941	2.64±0.4	2.75±0.59
6	Hirudinida	Hirudinidae	Annelida	*Hirudo medicinalis* Linnaeus, 1758	5.33±0.67	3.55±0.45
7	Rhynchobdellida	Glossiphonidae	Annelida	*Helobdella stagnalis* Linnaeus, 1758	6.81±0.97	2.95±0.47
8	Rhynchobdellida	Glossiphonidae	Annelida	*Placobdella parsitica* Say, 1824	5.36±0.77	2.18±0.4
9	Diptera	Chironomidae	Arthropoda	*Chironomus* sp. Meigen, 1803	21.69±4.44	37.52±4.07
10	Ephemeroptera	Leptophlebiidae	Arthropoda	*Leptophlebia marginata* Linnaeus, 1767	39.92±2.95	11.75±0.92
11	Odonata	Libellulidae	Arthropoda	*Diplacodes* sp. Rambur, 1842	13.67±0.98	4.85±0.83
12	Odonata	Libellulidae	Arthropoda	*Brachythemis contaminate* Fabricius, 1793	15.69±0.98	4.23±0.71
13	Odonata	Gomphidae	Arthropoda	*Gomphidia* sp. Selys, 1854	10.31±0.8	4.03±0.68
14	Odonata	Hemiphlebiidae	Arthropoda	*Schnura heterosticta* Burmeister, 1842	15.75±1.09	8.77±0.69
15	Hemiptera	Gerridae	Arthropoda	*Aquarius remiges* Say, 1824	31.81±2.19	30.3±1.92

16	Hemiptera	Nepidae	Arthropoda	*Nepa cinerea* Linnaeus, 1758	0.08±0.08	1.67±0.48
17	Coleoptera	Dytiscidae	Arthropoda	*Thermonectus* sp. Dejean,1833	72.69±4.79	16.67±1.66
18	Araneae	Pisauridae	Arthropoda	*Dolomedes tenebrosus* Hentz, 1844	2.08±1.07	0.25±0.1
19	Decapoda	Paleomonidae	Arthropoda	*Paleomon* sp. Weber, 1795	1.89±0.56	0.53±0.23
20	Sphaeriida	Sphaeriidae	Mollusca	*Sphaerium* sp. Scopoli, 1777	64.06±3.53	15.2±1.99
21	Acroloxoidea	Acroloxidae	Mollusca	*Acroloxus lacustris* Linnaeus, 1767	12.64±1.03	2.03±0.49
22	Unionoida	Unionidae	Mollusca	*Lamellidens* sp. Simpson, 1900	3.08±0.76	2.3±0.46
23	Unionoida	Veneridae	Mollusca	*Radiatula* sp. Simpson, 1900	0.75±0.37	1.17±0.35
24	Basommatophora	Planorbidae	Mollusca	*Helisoma* sp. Brown, 1967	1.64±0.91	
25	Basommatophora	Planorbidae	Mollusca	*Segmentina* sp. Fleming, 1817	0.97±0.35	0.28±0.12
26	Caenogastropoda	Thiaridae	Mollusca	*Thiara requeti* Grateloup, 1840	1.47±0.86	
27	Caenogastropoda	Thiaridae	Mollusca	*Thiara tuberculata* Mueller, 1774	10.97±1.52	2.05±0.35
28	Caenogastropoda	Thiaridae	Mollusca	*Thiara granifera* Lamarck, 1822	6±1.51	1.25±0.41
29	Caenogastropoda	Viviparoidae	Mollusca	*Bellamya bengalensis* Lamarck, 1822	13.42±1.39	2.93±0.42

Mcro-invertebrate's abundance varied between HA and HP habitats (U = 358; p <0.001) (Table 5). Orders Odonata, Coleoptera, Ephemeroptera, Caenogastropoda and sphaerrida had significantly higher abundance in the HP habitats than they did in the HA habitat, however, two orders Diptera and Haplotaxida had significantly lower abundance in HP habitats. Abundance of *Limnodrilus hoffmeisteri*, *Branchiura sowerbi*, *Tubifex tubifex*, *Stylaria lacustris* (Haplotaxida) and *Chironomus* sp. (Diptera) was recorded higher in HA habitats in all the seasons (Table 5, Appendix IV).

Table 5: Abundance of macro-invertebrates in water hyacinth presence (HP) and absence (HA) habitats in lakes of Pokhara Valley, Nepal from 2019 to 2020

Variables	Habitat (HP) Median	Habitat (HA) Median	Mann Whitney U test
Total Abundance	392	231	U = 358; p < 0.001
Orders (Abundance)			
Diptera	10.5	26.5	U = 1499.5; p = 0.001
Haplotaxida	27.5	66	U = 1390; p = 0.01
Ephemeroptera	37	11	U = 92; p < 0.001
Odonata	52.5	13	U = 263.5; p < 0.001
Coleoptera	68.5	18	U = 65.5; p < 0.001
Sphaeriida	60.5	14	U = 64.5; p < 0.001
Caenogastropoda	23.5	5	U = 110; p < 0.001

HP habitat had more macro-invertebrate diversity but the evenness was somewhat lower (H' = 2.77; J = 0.82) than HA habitat (H' = 2.74; J = 0.83). Both habitats were comparable in species richness (HP = 29, HA= 27). *Helisoma* sp. and *Thiara requeti* were not recorded in HA habitat (Figure 4, Table 4)

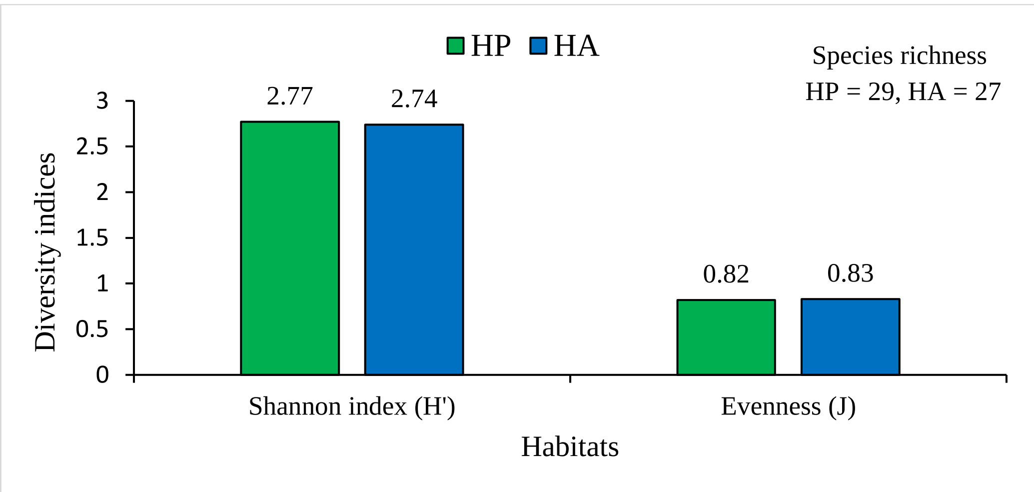


Figure 4: Diversity indices of macro-invertebrates in water hyacinth presence (HP) and absence (HA) habitats in lakes of Pokhara Valley, Nepal

2.3.4 Association of macro-invertebrates with environmental variables

The abundance of orders Diptera and Haplotaxida decreased significantly with the increase of water hyacinth coverage ($p < 0.001$) but it was opposite for the depth of water ($p = 0.09$; Figure 5).

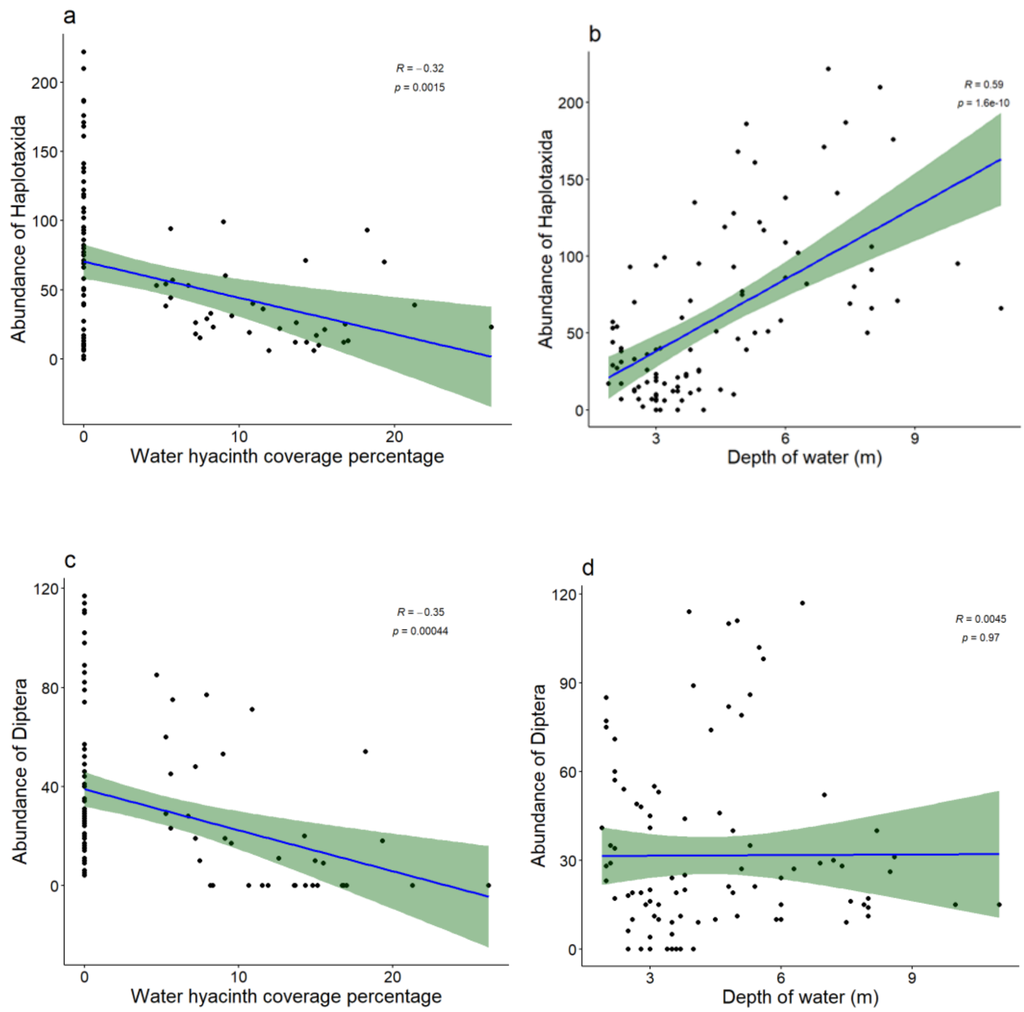


Figure 5: Relation of abundance of Haplotaxida and Diptera with Water hyacinth Coverage and Depth of Water in lakes of Pokhara Valley, Nepal

The DCA analysis showed the first and second axis length of 1.21 and 1.41 respectively, and similarly, the Eigenvalue of 22.12% (Table 6). Therefore, the RDA ordination was accepted for analysis.

Table 6: Detrended correspondence analysis of macro-invertebrates and environmental variables in lakes of Pokhara Valley, Nepal

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.2212	0.10915	0.07952	0.083977
Decorana values	0.2222	0.07494	0.01275	0.007482
Axis lengths	1.2111	1.41122	1.38577	1.385018

The canonical axes of RDA-biplot ordination analysis explained the variance in the interaction between macro-invertebrates and environmental variables, with the first axis accounting for 69.55% and the second axis for 33.98%. The result of ANOVA (F = 5.83, p = 0.001) showed all the canonical axes of RDA were significant. The water hyacinth coverage was closely associated with free carbon-dioxide and temperature whereas oppsite of the same for other parameters of water (Figure 6). Similarly, the macro-invertebrates such as *Gomphidia* sp., *Schnura heterosticta*, *Leptophlebia marginata*, *Thermonectus* sp. and *Sphaerium* sp. were closely associated with the water hyacinth coverage, but *Stylaria lacustris*, *Chironomus* sp. *Limnodrilus hoffmeisteri*, *Branchiura sowerbi* and *Tubifex tubifex* were negatively associated with the same (Figure 6). All the acronyms used for macro-invertebrates are listed in Appendix III.

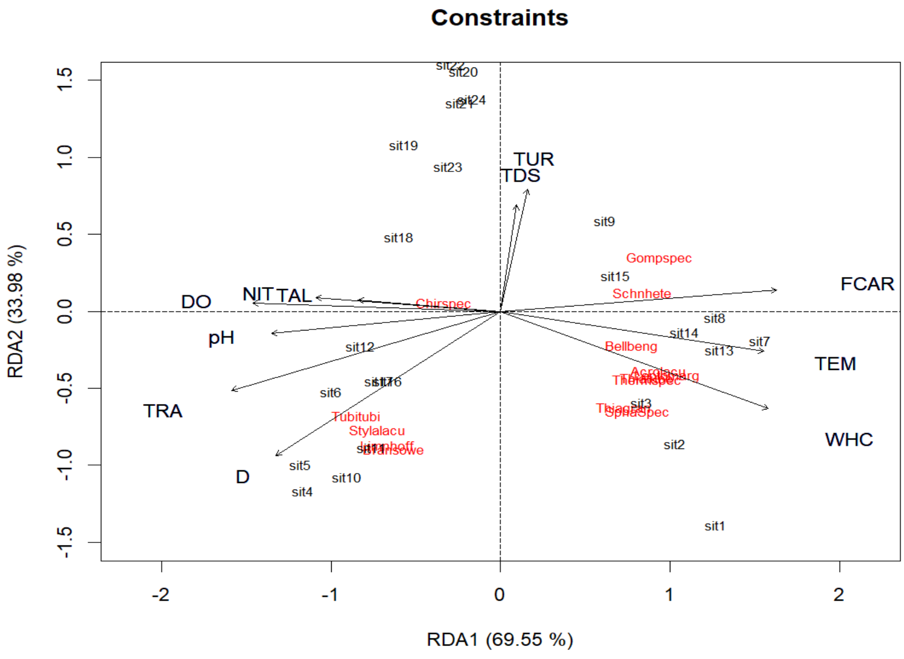


Figure 6: RDA – biplot ordination showing the relationship between the macro-invertebrates and environmental variables in lakes of Pokhara Valley, Nepal

Symbols used for environmental variables:

D = Depth, DO = Dissolve oxygen, FCAR = Free carbon dioxide, NIT = Nitrate, pH = Potential of hydrogen ion, TAL = Total alkalinity, TDS = Total dissolved solids, TEM = Temperature, TRA = Transparency, TUR = Turbidity, WHC = Water hyacinth coverage

2.4 DISCUSSION

The findings of the current study showed the sign of deterioration of water quality of the lake water in Pokhara because of the invasion. The analysis of physicochemical parameters confirmed that all parameters except water temperature and turbidity varied significantly in the HA and HP habitats; however, the temperature of water was somewhat higher in the HP habitats. This nominal increase of temperature in the plots with water hyacinth in the lakes could be attributed to water hyacinth mats blocking sunlight penetration. Therefore, the photosynthesis rate of phytoplankton and other aquatic plants would be reduced, it might be due to the increase in free carbon dioxide and decrease in dissolved oxygen in HP areas (Mangas-Ramrez & Elas-Gutiérrez, 2004; Villamagna, 2009; Mironga *et al.,* 2012). The increase in free carbon dioxide might cause the higher acidic level in water, it could be the consequences for the lower value of pH in HP habitats in the lakes of Pokhara Valley (Mironga *et al.,* 2012; Dersseh *et al.,* 2019). In all seasons, the higher value of pH of the water was recorded in the HA habitats, indicating that the mat of water hyacinth moderately acidifies the water in HP habitats (Mironga *et al*., 2012; Dersseh *et al.*, 2019) it could be possibly due increase in production of free carbon dioxide in the HP habitats than in HA habitats, it could form carbonic acid when dissolve in lake water.

The decomposition of dead bodies of water hyacinth and sources of secondary pollution may increase the lake's eutrophic level, potentially leading to major issues with the lake ecosystem (Chen *et al.*, 2021). Ammonia, iron, manganese, and sulfide concentrations rise as a result, causing the aquatic system to enter anoxic conditions and it could be attributed in changing the overall structure and function of the entire ecosystem of aquatic habitats (Yongo & Outa, 2015, Mao *et al.*, 2023). Since water hyacinths cannot grow in water deeper than six meters, the HA habitat had a greater depth of water than the HP habitat (Dersseh *et al.,* 2019). The water hyacinth has the capacity to absorb the load of nutrients such as nitrates and ammonia contained in water bodies (Masifwa *et* *al.*, 2001; Villamagna *et al.*, 2010), and it could be attributed for the lower nitrate content in water hyacinth presence habitat than in the absence habitat in the lakes of Pokhara. The low dissolved oxygen value in the HP habitat with high free carbon dioxide could be attributed to the epiphytic organism's metabolic activities (Masifwa *et al.*, 2001; Villamagna *et al.*, 2010). According to the correlation analysis, water temperature, free carbon dioxide and total dissolved solids increased with the increase in water hyacinth coverage, but opposite for the same was noticed for transparency, depth, pH, dissolved oxygen, and nitrate. This scenario of water parameters in lakes of Pokhara Valley could be attributed to the deteriorating quality of water.

The present study primarily focused to explore how the water hyacinth invasion affected the abundance and diversity of macro-invertebrates in the lakes of Pokhara Valley. In comparison to habitats without water hyacinth, the water hyacinth and other floating macrophytes presence habitats specially the littoral zone comprised more macro-invertebrates' colonization and had a greater abundance (Salmon *et al.*, 2022). The presence of substrate structures of fragmented mat of water hyacinth such as roots, stems, and leaves providing suitable habitats may explain the more diversified macro-invertebrates in the habitat with mat of water hyacinth (O'Hara, 1967; Hansen *et al.*, 1971; Villamagna & Murphy, 2010; da Silva & Henry, 2020). The variation in abundance of macro-invertebrates could be attributed to the availability of nutrients such as nitrogen, ammonium concentration and habitat structures like substrates, sediments and presence of aquatic macrophytes (Mao *et al.*, 2023).

Low water temperature during the winter months might be the cause of lower occurrence of macro-invertebrate in the lakes of Pokhara Valley because it is unsuitable for seasonal invertebrates, especially mayflies and other Ephemeroptera, and the summer days are taken as the suitable for the development and metamorphosis of these insect larvae (Watanabe *et al.*, 1999). Development of aquatic insects is influenced by water temperature, the high temperature of water decreases the period of development of the larvae of Diptera (Campobasso *et al.*, 2001). The higher rate of rainwater runoff could add the nutrients required for the development of macro-invertebrates during summer monsoon (Lal & Lal, 2023) it could be attributed for the greater abundance of macro-invertebrates during summer season. Water hyacinth and other wetlands' vegetation, which starts their proliferation in the summer and become dense in autumn, offer appropriate habitat for macro-invertebrates. As a result, the abundance of macro-invertebrates and the size of the water hyacinth mats were positively associated in the study area throughout the autumn. Not only in the present study area but also in a reservoir in the western part of Ecuador, the water hyacinth had a positive relationship with macro- invertebrates' density (Thi Nguyen *et al.*, 2015). The flat area of the water hyacinth mat may provide the shelter for many species such as dragonfly, mayfly and diving beetle larvae (Villamagna, 2009), it could be attributed to a greater abundance of macro-invertebrates during summer months.

Macro-invertebrate abundance is influenced by the morphological features and structural complexity of vegetation (Villamagna, 2009; da Silva & Henry, 2020). For macro-invertebrates like snails, arachnids, and amphipods, the floating water hyacinth's root and leaves offer an exhaustive habitat for their survival in aquatic environment (Brendonck *et al.*, 2003; Villamagna, 2009). However, macro-invertebrates were more numerous in the HA habitats than in the HP of artificially created environments by Coetzee *et al.* (2014). It could be due to variation in substrates and age of habitat. A greater abundance of macro-invertebrates in HP habitat of lakes in Pokhara also attributed for the greater abundance of insectivore and omnivore waterbirds in the HP habitat of these lakes (see in Chapter 4). However, macro-invertebrates from the two orders Diptera (*Chironomous* sp.) and Haplotaxida (*Branchiura sowerbi*, *Limnodrilus hoffmeisteri*, *Stylaria lacustris*, *Tubifex tubifex*) had significantly lower abundance in the HP habitats than in the HA habitats.

The abundance of Diptera and Haplotaxida decreased with the increase in water hyacinth coverage, it indicated that water hyacinth has adverse impact on macro-invertebrates from these groups. These taxa have a high tolerance for toxic substances (Mandaville, 2002). Higher abundances of Diptera and Haplotaxida were discovered in the water hyacinth absence habitat in the lakes of Pokhara at greater depths in bottom sediments where decomposed organic matters were obtained. The deposition of the water hyacinth's pieces may not always occur in the same area of the lake because it is buoyant and floats from one site to another. Also prevalent in the HA habitats were oligochaetes and chironomid larvae, which were high-tolerance taxa and had a greater rate of organic material decomposition (Shah *et al.*, 2011). Further, the HP habitat in the lakes of Pokhara Valley was more diverse than the HA habitat. As a result, the diversity indices were found regularly greater in these habitats (Brendonck *et al.*, 2003). The multivariate RDA ordination also revealed that the taxa from two orders Diptera and Haplotaxida were abundant groups in HA habitats and other groups in HP habitats, it could be the reason for the greater abundance of residential birds of feeding guilds insectivore and omnivore waterbirds in HP habitats of the lakes in Pokhara Valley (see in Chapter 4).

2.5 CONCLUSION

The findings of current study indicated the sign of degrading water quality. The variance in macro-invertebrate abundance in the lakes of Pokhara Valley between the HP and HA habitats is predominantly caused by alterations of the structural composition and degrading quality of water. The increased free carbon dioxide and water temperature, and decreased dissolved oxygen in the areas with areas covered water hyacinth indicates the possibility of variation in macro-invertebrate abundance in two habitats of the lakes in LCPV. The occurrence of some species of macro-invertebrates was supported by water hyacinth while the negative effect was noticed on other species of the orders Haplotaxida and Diptera. In this regard, in order to protect the aquatic biodiversity of the lakes in Pokhara, it is advised to implement the management of invasive water hyacinth with integrated methods of control of weeds. Multidisciplinary research approaches are also for other invasive plants and their impact on aquatic biodiversity in the lakes of Pokhara.

CHAPTER 3

INFLUENCE OF INVASIVE WATER HYACINTH ON FISH ABUNDANCE AND DIVERSITY IN THE LAKES OF POKHARA VALLEY, NEPAL

Abstract

Invasion of aquatic weeds like water hyacinth is a global problem that threatens aquatic lives. Similar to the global scenario, the lakes of Pokhara Valley, which is designated the largest Ramsar sites, are also experiencing the adverse effects due to water hyacinth's invasion. Though, the effect of invasion on aquatic ecosystem is little known in the lakes of Pokhara Valley. Therefore, the current study explored the impacts of water hyacinth invasion on the abundance and diversity of fishes in the lakes of Pokhara Valley. The present study assessed the seasonal survey of fishes from a total 24 sampling plots in two habitats with water hyacinth presence (HP) and absence (HA) in the lakes between 2019 and 2020. The present study identified all together 20 fish species from the lakes of Pokhara included in 18 genera, eight families and six orders; the Cyprinidae was the most abundant family. Fish abundance was recorded greater during the winter season ($p < 0.001$) and it was found more in the HP habitat ($p < 0.001$). The invasive *Oreochromis niloticus* was the most abundant fish in both habitats, however, the abundance of this species was significantly greater in HP habitat. The exotic species *Ctenopharyngodon idella* had a greater abundance in the HP habitat but the commercially growing exotic fish *Ciprinus carpio* had a greater abundance in the water hyacinth absence habitat. The abundance of the exotic fish species was positively associated with water hyacinth coverage ($R = 0.066$; $p < 0.001$). The results revealed that the invasion of water hyacinth and introduced *O. niloticus* have had negative impacts on native fish species, as a result, the decline in native fish catch in lakes of Pokhara Valley was reported. The study recommended the regular monitoring programs of restoring stocks for native fish species. It also suggested for integrated control methods of invasive weeds with their sustainable management to support the conservation of native fishes and the livelihood of the fishery-dependent community.

3.1 INTRODUCTION

The biological parameters of all types of ecosystems depend on the abundance and species diversity of plants and animals (Jewel *et al.*, 2018). Among the amimals, fishes are believed as the most important constituents of the aquatic environment. More than 35,100 fish species in the world was reported in fishBase by Froese & Pauly (2023). Nepal is rich in freshwater resources with the variation in altitudes from lowland to high mountain, bearing 252 fish species (Rajbanshi, 2012; Shrestha, 2019). The fish species abundance and occurrence are affected by the invasion of aquatic weeds, habitat connectivity and physicochemical parameters of water (Jin *et al.*, 2019; Mamilov *et al.*, 2021; Virgilio *et al.*, 2022). Additionally, the invasion of aquatic invasive weeds shows the severe adverse effects in the aquatic ecosystem (Villamagna, 2009; Ongore *et al.*, 2018) such as increasing rate of evapotranspiration, degradation of quality of water, flooding, siltation and loss of native species (Shrestha, 2016; Ongore *et al.*, 2018). Among 27 invasive plants in Nepal, the water hyacinth has been reported to pose a threat to the ecology and human livelihoods by diminishing fish catches, hindering transportation, and degrading quality of water (Kateregga & Sterner, 2009; Asmare, 2017).

The Lake Cluster of Pokhara Valley (LCPV) is largest Ramsar site of Nepal, which contains nine ecologically significant lakes (Paudel *et al.*, 2018). People nearby the lakes get benefited from these lakes in terms of fisheries, irrigation, tourism, recreation, washing and bathing. About 200 families of fisher communities are fully dependent on fisheries like capture, cage culture and pen culture (Husen *et al.*, 2019). A total of 34 species of fish species, including seven exotic and 27 native species of fish were described by Timilsina *et al.* (2019) from five lakes of Pokhara and Lekhanath. Among these species the frequently occuring species in the lakes are common bhitti (*Danio devario*), least concern rewa (*Chagunius chagunio*), endangered flame sahar (*Tor tor*), endangered sahar (*Tor putitora*) and near threatened katlae (*Neolissochilus hexagonolepi*) (Husen & Sherpa, 2017).

The lakes in Pokhara Valley are invaded with five species of aquatic invasive plants, among them the water hyacinth is the abundant invasive plant (Pathak *et al.*, 2021). The invasion of this has had an adverse influence on the occurrence and species richness of wintering and threatened waterbirds in the lakes of Pokhara (see in Chapter 4). During the rainy season, near about 25 to 60% of the surface of lakes remain covered by water hyacinth and it is considered as the major contributor for the loss of fish habitats in the lakes of Pokhara Valley (Husen *et al.*, 2019). Because of the rapid expansion of water hyacinth, two fish species *Neolissochilus hexagonolepis* and *Puntius sarana* in Begnas Lake, were not reported. It was also noted that the catches of *Tor putitora* and *N. hexagonolepis* were diminishing in Phewa and Rupa lakes of the Pokhara (Husen *et al.*, 2016). Nevertheless, the impact of invasion due to water hyacinth on the abundance and diversity of fishes in the lakes of Pokhara remains unexplored. Collection of information about fish assemblage in lakes and reservoirs is crucial for the formulation of effectual management plan and conservation of the Ramsar sites like the lakes of Pokhara Valley. Therefore, the current study investigated the influence of the water hyacinth on the abundance and diversity of fishes in the lakes of Pokhara Valley. The results provided baseline data for future research and conservation of fish in lakes of Pokhara Valley.

3.2 MATERIALS AND METHODS

3.2.1 Methods (It is explained in Chapter-2)

3.2.1.1 Physicochemical parameters of water (It is explained in Chapter-2)

3.2.1.2 Fish survey

The survey was carried out seasonally during autumn 2019 (September to November), winter 2020 (December to February), spring 2020 (March to May) and summer 2020 (June to August) with the help of experienced and skillful fishermen of the catchment area of the lakes using a rectangular gill-net of 50 × 1 m^2 of mesh size 20 mm. The net was fitted overnight and the fish samples were collected next morning in a bucket containing water (Figure 7). All the fish were identified with the help of local fisherman and fish expert following the taxonomic monographs (Shrestha, 1981, 1994, 2002; Shrestha, 2019). All the fish were released in the respective lakes. The species and number of each fish species per catch in each plot and in each season were noted for the analysis.

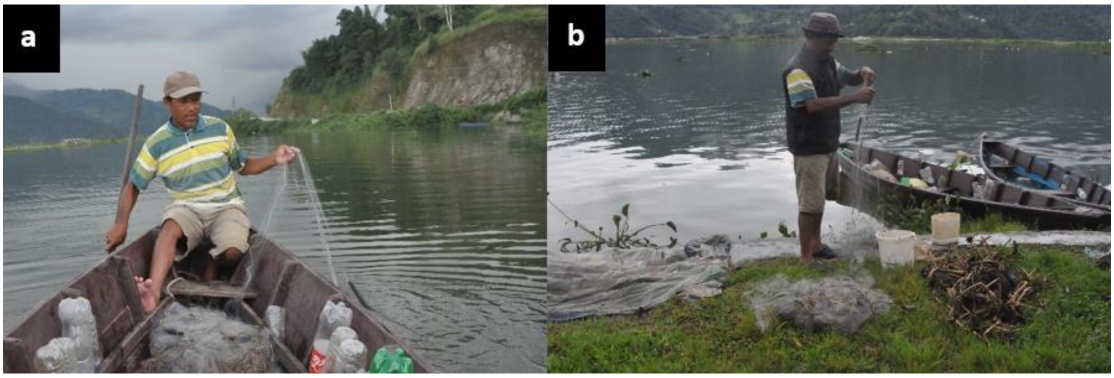


Figure 7: Rectangular gill net enclosure fixing in Phewa lake to collect the samples of fish (a) and fish samples collecting in Phewa from gill net enclosure (b)

3.2.1.3 Data analysis

Diversity indices of the fish were calculated by the same method used for macro-invertebrates in Chapter 2.

Normality of the data was evaluated using the Shapiro-wilk test, it indicated that the data were not distributed normally. Therefore, non-parametric Kruskal-Wallis test was applied to compare the seasonal variation in fish abundance. Similarly, Mann-Whitney test was used to compare the variation of fish abundance in HP and HA habitat. The association of abundance and diversity indices of fish with the water hyacinth coverage was performed by Spearman's correlation and Simple linear regression.

Multivariate ordination analysis was conducted to identify the association of fish with physicochemical parameters of water and coverage of water hyacinth. The analytical methods of multivariate ordination are explained in Chapter 2.

The factors affecting the native fish abundance in the lakes was determined using Generalized linear model. The model parameters used were transparency (%), temperature, water hyacinth coverage (%), depth (m), and hyacinth presence and absence habitats. Negative binomial distribution was used to escape over dispersion on the model. R Core Team (2020) was used for all statistical analysis in R program.

3.3 RESULTS

3.3.1 Abundance and diversity of fish

Out of total 20 fish species included in 18 genera, eight families and six orders explored during the present study from the lakes in Pokhara Valley, 17 species were native and three species were exotic fishes (Table 7). The outcomes of the present study showed that the family Cyprinidae was the most abundant family (60%, n = 12), it was followed by Clariidae (10%, n =2) and (5%, n =1) each from the families Bagridae, Belonidae, Mastacembelidae, Channidae, Cichlidae, and Ambassidae (Figure 8).

Table 7: Fish species in lakes of Pokhara Valley, Nepal from 2019 to 2020

Orders of fish	Family	Scientific name	Local name	IUCN Status
Cypriniformes	Cyprinidae	*Puntius chola* Hamilton, 1822	Bhurluk	LC
Cypriniformes	Cyprinidae	*Puntius sophore* Hamilton, 1822	Bhitta	LC
Cypriniformes	Cyprinidae	*Danio devario* Hamilton, 1822	Sera	LC
Cypriniformes	Cyprinidae	*Tor putitora* Hamilton, 1822	Sahar	**EN**
Cypriniformes	Cyprinidae	*Naziritor chelynoides* McClelland, 1839	Karange	LC
Cypriniformes	Cyprinidae	*Labio rohita* Hamilton, 1822	Rahu	LC
Cypriniformes	Cyprinidae	*Labio angra* Hamilton, 1822	Gardi	LC
Cypriniformes	Cyprinidae	*Cirrhinus mrigala* Hamilton, 1822	Naini	LC
Cypriniformes	Cyprinidae	*Chagunius chagunio* Hamilton, 1822	Rewa	LC
Cypriniformes	Cyprinidae	*Catla catla* Hamilton, 1822	Bhakur	LC
Cypriniformes	Cyprinidae	*Ctenopharyngodon idella* Valenciennes, 1844	grass carp	LC*
Cypriniformes	Cyprinidae	*Cyprinus carpio* Linnaeus, 1758	Common Carp	LC*
Siluriformes	Bagridae	*Mystus bleekeri* Day, 1877	Junge	LC
Siluriformes	Clariidae	*Heteropneustes fossilis* Bloch, 1794	Balim	LC
Siluriformes	Clariidae	*Clarias batrachus* Linnaeus, 1758	Magur	LC
Beloniformes	Belonidae	*Xenentodon cancila* Hamilton, 1822	Chuche Bam	LC
Channiformess	Channidae	*Channa punctatus* Bloch, 1794	Bhoti	LC
Perciformes	Cichlidae	*Oreochromis niloticus* Linnaeus, 1758	Tilapia	LC*
Synbranchiformes	Mastacembelidae	*Mastacembelus armatus* Lacepede, 1800	Dhunge Bam	LC
Perciformes	Ambassidae	*Chanda nam*a Hamilton, 1822	Glass fish	LC

(EN = Endangered, LC = Least Concern, * = Exotic species)

The seasonal survey of fish indicated that, the the winter season was recorded as the most abundant season in both habitats (HA = 19.48% and HP = 12.79%), followed by spring (HA = 16.36% and HP = 11.8%) and the summer season was recorded as the least abundant season in both the habitats (HA = 9.54% and HP = 7.71%). In terms of overall abundance, the fish catch was found to be lesser in the HP habitat (42.4%) than in the HA habitat (57.6%) (Table 8).

Table 8: Abundance and Relative abundance (RA) of fish in lakes of Pokhara Valley from 2019 to 2020

Seasons	Abundance (HP)	RA%(HP)	Abundance (HA)	RA%(HA)	Total
Autumn	761	10.10	920	12.21	1681
Winter	964	12.79	1468	19.48	2432
Spring	889	11.80	1233	16.36	2122
Summer	581	7.71	719	9.54	1300
Grand Total	3195	42.40	4340	57.60	7535

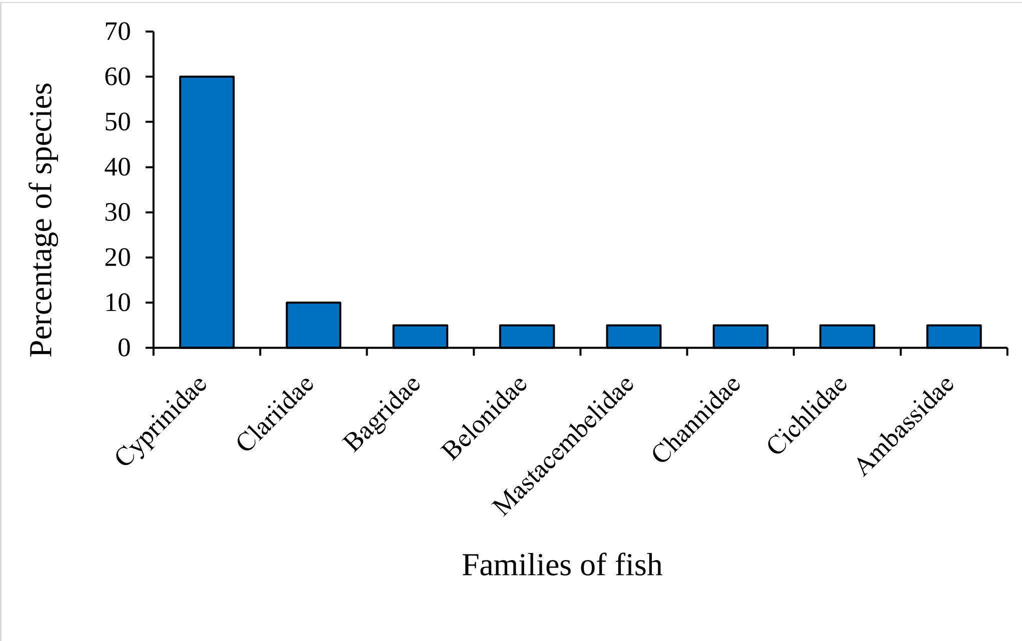


Figure 8: Families of fish with species percentage in lakes Pokhara Valley

The fish species *Orechromis niloticus* was recorded as the abundant fish species in all the seasons and in both habitats of lakes in Pokhara Valley. It was followed by *Puntius sophore* and *Puntius chola* in all the seasons but *Chanda nama* in HP habitat (Table 9). Both fish abundance and relative abundance in the lakes of Pokhara are given in Table 9.

Table 9: The abundance and relative abundance (RA) of each species of fish in lakes of Pokhara Valley, Nepal between 2019 and 2020

	Abundance and Relative abundance (RA) of fish											
Taxa	**Autumn**	**RA%**	**Winter**	**RA%**	**Spring**	**RA%**	**Summer**	**RA%**	**HP**	**RA%**	**HA**	**RA%**
Puntius chola	228	13.56	346	14.23	281	13.24	169	13.00	293	9.17	731	16.84
Puntius sophore	148	8.80	289	11.88	263	12.39	120	9.23	241	7.54	579	13.34
Danio devario	130	7.73	216	8.88	192	9.05	90	6.92	194	6.07	434	10.00
Tor putitora	4	0.24	7	0.29	13	0.61	2	0.15	3	0.09	23	0.53
Naziritor chelynoides	27	1.61	29	1.19	20	0.94	21	1.62	7	0.22	90	2.07
Labio rohita	16	0.95	14	0.58	14	0.66	10	0.77	0	0.00	54	1.24
Labio angra	21	1.25	22	0.90	15	0.71	14	1.08	0	0.00	72	1.66
Cirrhinus mrigala	7	0.42	5	0.21	12	0.57	5	0.38	4	0.13	25	0.58
Chagunius chagunio	14	0.83	20	0.82	17	0.80	18	1.38	3	0.09	66	1.52
Catla catla	37	2.20	29	1.19	23	1.08	26	2.00	102	3.19	13	0.30
Ctenopharyngodon idella	26	1.55	19	0.78	12	0.57	23	1.77	45	1.41	35	0.81
Cyprinus carpio	3	0.18	34	1.40	27	1.27	9	0.69	0	0.00	73	1.68
Mystus bleekeri	23	1.37	19	0.78	19	0.90	21	1.62	17	0.53	65	1.50
Heteropneustes fossilis	88	5.23	75	3.08	56	2.64	63	4.85	5	0.16	277	6.38
Clarias batrachus	35	2.08	187	7.69	147	6.93	32	2.46	96	3.00	305	7.03
Xenentodon cancila	71	4.22	73	3.00	67	3.16	53	4.08	76	2.38	188	4.33
Mastacembelus armatus	54	3.21	67	2.75	50	2.36	38	2.92	117	3.66	92	2.12
Channa punctatus	14	0.83	29	1.19	25	1.18	8	0.62	7	0.22	69	1.59
Orechromis niloticus	603	35.87	737	30.30	636	29.97	418	32.15	1514	47.39	880	20.28
*Chanda nam*a	132	7.85	215	8.84	233	10.98	160	12.31	471	14.74	269	6.20
Total	**1681**		**2432**		**2122**		**1300**		**3195**		**4340**	

The invasive *Oreochromis niloticus* was the most abundant fish in the lakes of Pokhara in all seasons throughout the study period, other abundant species were *Chanda nama, P. sophore*, *Puntius chola.* The winter season was recorded as the most abundant period for the fish, abundance of native and exotic fish species (H = 30.06, $p < 0.001$; H =19.5, $p < 0.001$ and H = 7.51, p = 0.05 respectively; Table 10). The species, *P. sophore* and *P. chola* were found to be abundant only during the winter season (H = 23.98, $p < 0.001$ and H = 22.87, $p < 0.001$ respectively) among the top most abundant eight fish species in the lakes (Table 10).

Table 10: Seasonal comparison of abundance and diversity of fishes in lakes of Pokhara Valley, Nepal from 2019 to 2020

Variables	**Autumn Median**	**Winter Median**	**spring Median**	**Summer Median**	**Kruskal Wallis test**
Total abundance	68.5	95	86	53	H = 30.06, p < **0.001**
Native species	38	58	55.5	31	H = 19.5, p **< 0.001**
Exotic species	21	29	23.5	15	H = 7.51, p = **0.05**
Abundant species					
Puntius chola	8	14	11	6	H = 22.87, p < **0.001**
Puntius sophore	6	11.5	10.5	4.5	H = 23.98, p < **0.001**
Chagunius chagunio	0	0	0	0	H = 0.67, p = 0.8
Heteropneustes fossilis	0	0.5	1	0	H = 2.59, p = 0.4
Chanda nama	0	0	0	2	H = 1.94, p = 0.5
Oreochromis niloticus	21	28.5	22	14.5	H = 5.98, p = 0.1
Ctenopharyngodon idella	0.5	0	0	0	H = 5.82, p = 0.12
Cyprinus carpio	0	0	0	0	H = 1.09, p = 0.77

The overall abundance of fish differed in HP and HA habitats of the lakes in Pokhara Valley (U = 721.5, p = 0.006; Table 11). The abundance of native fishes was found similar in both habitats, but greater abundance was found for exotic fishes in the HP habitat (U = 182, $p < 0.001$). Out of the most abundant eight fish species, three native species *C. chagunio*, *P. chola* and *H. fossilis* had greater abundance in the HA habitat (U = 1637.5, $p < 0.001$; U = 1441, p = 0.002 and U =1666, $p < 0.001$ respectively). However, one native species *C. nama* and two exotic species *C. Idella* and *O. niloticus* had greater abundance in the HP habitat (U = 844, p = 0.03; U = 737.5, p = 0.002 and U = 172.5, $p < 0.001$ respectively) and one commercially grown exotic species *Cyprinus carpio* had greater abundance in HA habitat (U= 1224, $p < 0.02$; Table 11).

Table 11: The abundance and diversity of fishes in HP and HA areas in lakes of Pokhara Valley, Nepal from 2019 to 2020. Range of reported values are in parentheses

Variables	**Habitat (HP)**	**Habitat (HA)**	**Mann Whitney U test**
	Median	**Median**	
Total abundance	84.5 (44-155)	66 (11-200)	U = 721.5, p = **0.006**
Native species	39 (16-120)	49 (11-168)	U = 1294, p = 0.1
Exotic species	40 (13-102)	15 (0-43)	U = 182, p < **0.001**
Abundant species			
Puntius chola	8.5 (2-15)	11 (0-37)	U = 1481, p = **0.002**
Puntius sophore	6.5 (1-15)	8.5 (0-31)	U = 1292, p = 0.1
Chagunius chagunio	0 (0-2)	1(0-5)	U = 1637.5, p < **0.001**
Heteropneustes fossilis	0 (0-2)	2(0-30)	U = 1666, p < **0.001**
Chanda nama	0 (0-61)	0 (0-32)	U = 844, p = **0.03**
Oreochromis niloticus	39.5 (13-102)	15 (0-39)	U = 172.5, p < **0.001**
Ctenopharyngodon idella	1 (0-5)	0 (0-6)	U = 736.5, p = **0.002**
Cyprinus carpio	0 (0-0)	0 (0-17)	U = 1224, p = **0.02**

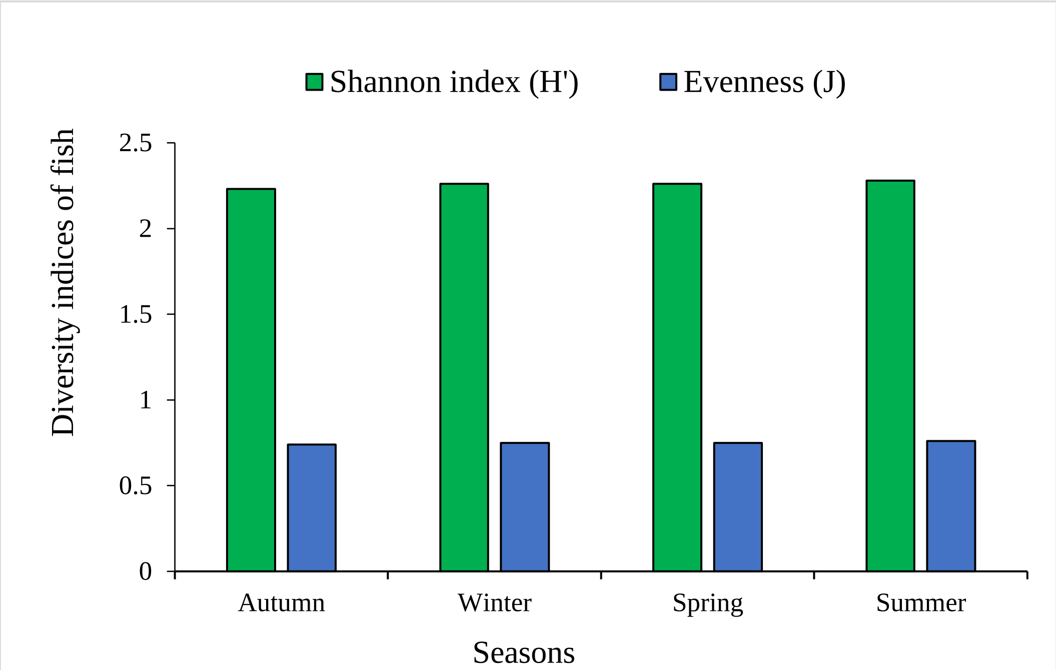


Figure 9: Seasonal fish diversity indices of fish in Lakes of Pokhara Valley, Nepal

The fish diversity in the lakes were comparable in all the seasons (Figure 9). Further, the water hyacinth absence habitats had a slightly higher fish diversity (species richness S = 20 and H' = 2.45; J = 0.81) compared to the presence habitats (S = 17, H' = 1.79; J = 0.63; Figure 10, Table 9).

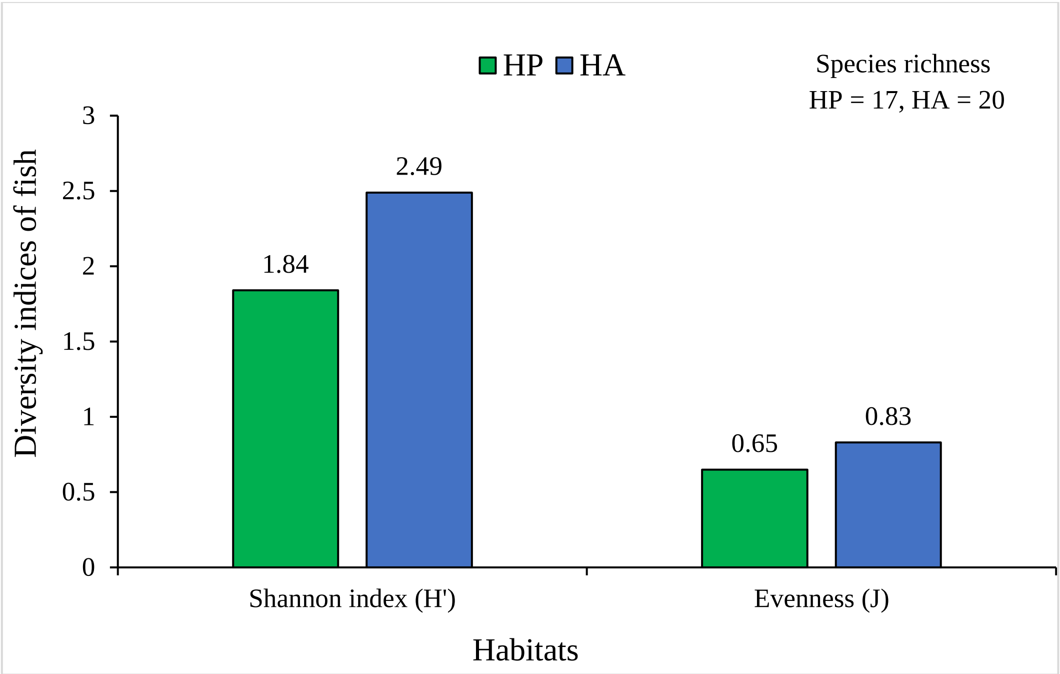
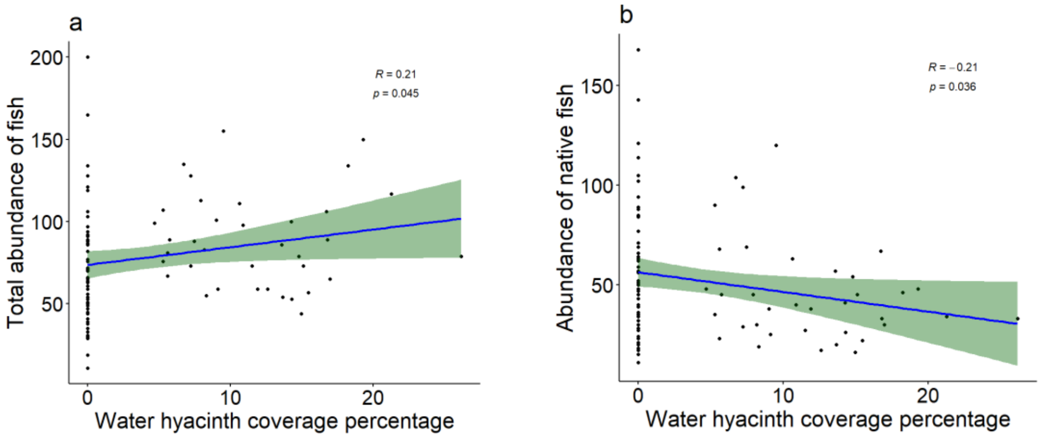


Figure 10: Diversity indices of fish in water hyacinth presence (HP) and absence (HA) habitats in lakes of Pokhara Valley, Nepal

3.3.2 Factors affecting the abundance and diversity of fish

There was a significant positive correlation of the coverage of water hyacinth with overall abundance and the abundance of exotic fishes ($R = 0.21$, $p = 0.04$ and $R = 0.67$, $p < 0.001$ respectively) but a significant negative association with the abundance of native fishes was noticed ($R = -0.36$, $p < 0.001$). Likewise, a significant negative association with Shannon Wiener diversity index ($R = -0.42$, $p < 0.001$) and species evenness index was observed ($R = -0.51$, $p < 0.001$; Figure 11).



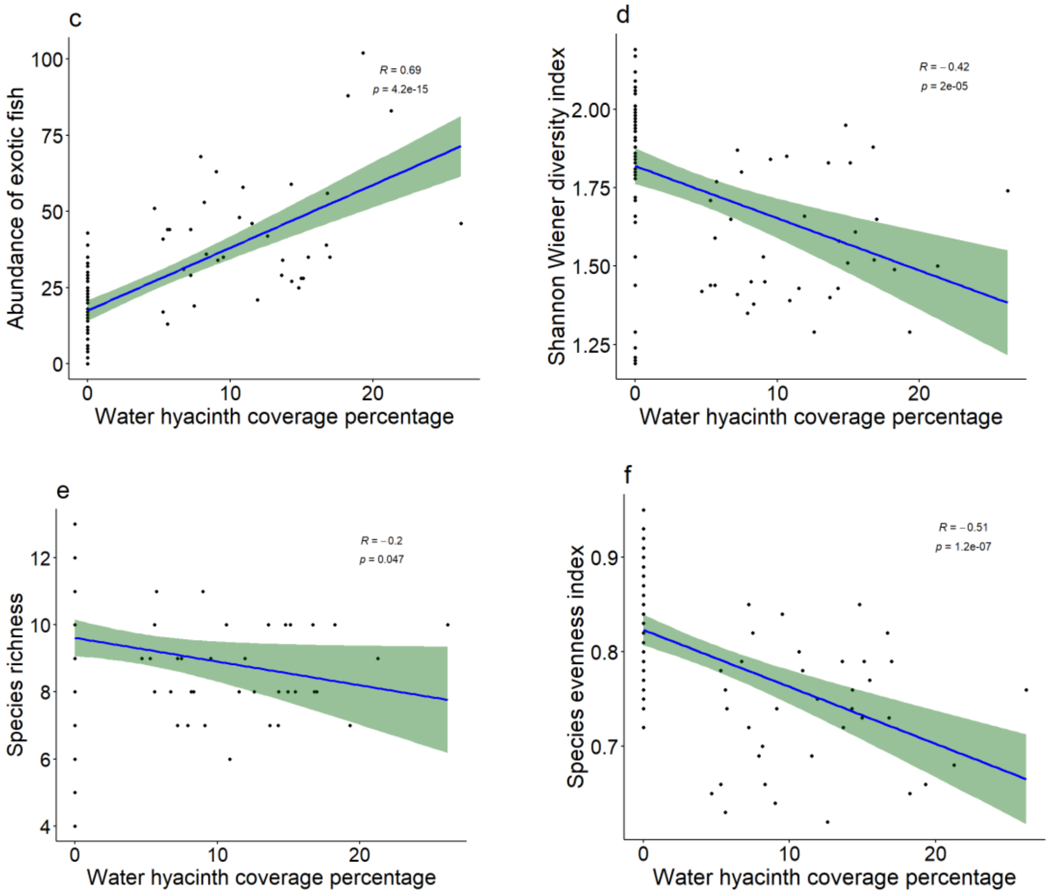


Figure 11: Influence of water hyacinth cover on abundance and diversity of fish in lakes of Pokhara Valley, Nepal

The DCA analysis showed the 39% Eigenvalue, the length of first and second axis 2.1 and 1.49 respectively (Table 12). Therefore, the RDA ordination was accepted for analysis.

Table 12: Detrended correspondence analysis of fish species and environmental variables in lakes of Pokhara Valley, Nepal

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.3951	0.2048	0.05582	0.09876
Decorana values	0.407	0.1983	0.03508	0.01415
Axis lengths	2.104	1.4979	0.83515	1.36829

In HP habitat, the abundance of native fish species declined. Furthermore, the abundance of native fish species declined with decreasing water depth and transparency of lake water, whereas the abundance of native fish species increased with rising abundance of exotic fish species (Table 13).

Table 13: GLM showing the factors affecting the abundance of native fish species in lakes of Pokhara Valley, Nepal from 2019 to 2020

Parameters	Estimate	SE	z	p
(Intercept)	4.3848	0.2092	20.96	**< 0.001**
Water hyacinth presence	-0.9117	0.1946	-4.686	**0.001**
Exotic fish abundance	0.0136	0.0032	4.288	**0.000**
Transparency (%)	-0.2661	0.1828	-1.456	0.145
Depth (m)	-0.1033	0.0357	-2.893	**0.004**

The canonical axes of RDA analysis explained the variance in the interaction between fish species and environmental variables, with the first axis accounting for 54.57% and the second axis for 43.14%. The ANOVA showed all the canonical axes of RDA were significant ($F = 3.08$, $p < 0.001$; Figure 12). The RDA ordination revealed that, the coverage of water hyacinth, free carbon dioxide, water temperature and the fish species such as *O. niloticus*, *C. nama* and *C. idella* were closely associated each other. Whereas, the depth, pH, transparency, dissolved oxygen, nitrate and the fish species such as *C. chagunio, H. fossilis, Naziritor chelynoides, Danio devario, T. putitora, P. chola, P. sophore, C. carpio* were distantly or negatively associated with the water hyacinth coverage.

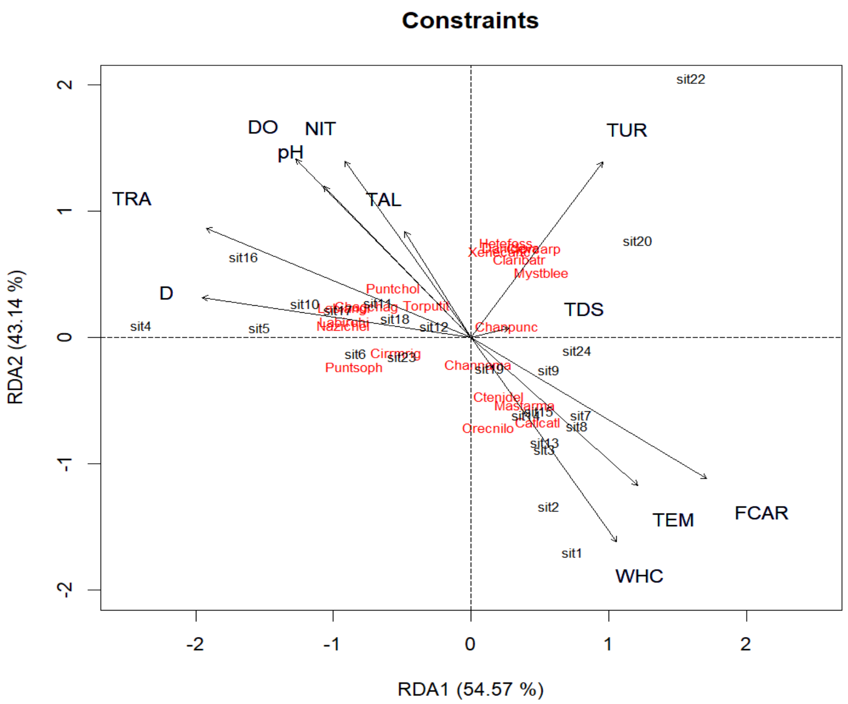


Figure 12: RDA – biplot ordination showing the relationship between the fish and environmental variables in lakes of Pokhara Valley, Nepal

Symbols used for environmental variables:

D = Depth, DO = Dissolve oxygen, FCAR = Free carbon dioxide, NIT = Nitrate, pH = Potential of hydrogen ion, TAL = Total alkalinity, TDS = Total dissolved solids, TEM = Temperature, TRA = Transparency, TUR = Turbidity, WHC = Water hyacinth coverage

3.4 DISCUSSION

The outcomes of the present study stated that, the invasion of water hyacinth has adverse effects on the occurrence of native fish species but supports the occurrence of exotic species in LCPV. Among the families recorded from study area the family Cyprinidae was the most abundant one. It might be due to the suitable water temperature of lakes for this widespread fish family (Swar, 2002). The large scaled cyprinids can withstand a wide range of temperature because they inhibit in the fresh water lakes and rivers of mid-hill and mountain regions with the average temperature ranges from 10°C to 20°C (Swar, 2002).

The fish species *H. fossilis* and ornamental fish *C. nama* have expanded rapidly in the lakes of Pokhara, with *C. nama* exclusively found in Phewa and Begnas. These species were first time reported by Husen *et al*. (2019) from Begnas lake. How were these fish species introduced in Begnas lake is still unknown, but the accidental release of fries in the lakes might be the ways of expansion of these species. The facultative *C. nama* feeds on hatchlings and scales of the fishes (Chakraborty *et al.,* 2018), therefore, it might kill the native carp species in the lakes. For example, 33.33% and 66.66% of native carp mortality were recorded in two cages of different mesh size due to falling of scales in cultivated carps in Kulekhani reservoir (Dahal *et al.,* 2012). The catch of *C. nama* is increasing in Phewa and Begnas but the catch of native fish is declining, it imight be due to the adverse effect of *C. nama* in these lakes.

The higher fish abundance during winter in lakes of Pokhara Valley could have attributed the sparse coverage of the water hyacinth mat, which might afford a suitable habitat for nursery as well as the greater chances of food availability for the planktivorous and omnivorous fish and greater abundance of macro-invertebrates (Toft *et al.,* 2003; Troutman *et al.,* 2007; Dos Santos *et al.*, 2020). It might be the cause for a significantly greater fish abundance in HP habitats of the lakes in Pokhara Valley. Nevertheless, the comparable abundance of *T. putitora* the endangered species in both

HP and HA habitats, it could be due to its foraging habits as it feeds on algae, other aquatic plants and insects (Bhatt & Pandit, 2016). The commercially cultivated exotic fish *C. idella* preferred the HP habitat as it is herbivorous fish, which could be attributed the use of this species to control the aquatic harmful invasive plants (Pipalova, 2006) and another exotic commonly growing species *C. carpio* denied the HP habitat as it is benthivores in feeding habit with bottom-up effect of sediments (Rahman, 2015). Moreover, this study revealed that the fish species *P. chola* and *P. sophore, Naziritor chelynoides*, *C. chagunio* and *H. fossilis* preferred the HA habitat and that *O. niloticus*, *C. idella* and *C. nama* preferred the HP habitat. The comparable species diversity of fish was observed in all the seasons in the lakes of Pokhara, however, HP habitat was found less diverse in comparison to the HA habitat, it could be attributed for the abundant native fishes in water hyacinth absence habitat. The higher overall abundance of fish in the water hyacinth presence habitat could be due to the structural complexity and food accessibility because the sparse mat of water hyacinth offers a unique environment for macro-invertebrates (Njiru *et al.*, 2002, 2004; Troutman *et al.*, 2007; Villamagna, 2009). Recent studies showed that, the catch of invasive species *O. niloticus* has been increasing significantly than that of native fish species in present studied lakes of Pokhara (Husen, 2014; Husen *et al.*, 2016; Husen *et al.*, 2019). Infestation of water hyacinth in lakes with fragmented mat may provide a suitable habitat for *O. niloticus* as it feeds on zooplankton, phytoplankton and aquatic insects, its feeding habit changes from omnivores to herbivores with the increase in the size of the body (Tesfahun & Temesgen, 2018). Similarly, Njiru *et al.* (2004) recorded the shifting of Nile perch (*Lates niloticus*) towards the water hyacinth mats that supported the invertebrate diets for this particular species in Lake Victoria, Uganda.

The temperature of water remains cool during the winter, while free carbon dioxide and pH levels rise in contrast to other seasons. As a result, fish abundance dropped with increasing water temperature and increased with increasing pH and free carbon dioxide. The abundance exotic fish was found to be higher in the water hyacinth presence habitat than in the HA habitat, which could have attributed the fragmented patch of water hyacinth mat support for feeding, nursery and fish breeding grounds (Toft *et al.*, 2003; Villamagna *et al.*, 2010). Further, it could be harmful in the case of dense mat because it may change the habitat to hypoxic due to a significant decrease in dissolved oxygen. For example, in lake victoria, the fish catch was significantly decreased and only two hypoxic species the catfish and lungfish were recorded in Lake Victoria (Ongore *et al.*, 2018). The native fish abundance increased with the increase in nitrate. Water hyacinth absorbs nutrients load from water bodies (Gichuki *et al.*, 2012), therefore, the HA habitat was recorded with more nitrate concentration in water and greater abundance of native fish species. More native species were recorded in the HA habitat than that in the HP habitat, it could be attributed to the lower species richness of fish in the HP habitat. The Shannon Wiener diversity index, evenness and species richness were negatively influenced by water hyacinth coverage, it could be the reason for the lower diversity indices of fish in the HP habitat of the lakes in Pokhra Valley.

The RDA ordination showed that the coverage of water hyacinth was closely associated with temperature and free carbon dioxide as their distance is nearer to each other, while the rest of all parameters of water were distantly associated with that. The concentration of dissolve oxygen below the mat of water hyacinth could be lowered because it blocks the pass of sunlight below the mat and the rate of photosynthesis in phytoplankton, algae and other aquatic plants gets lowered (McVea & Boyd, 1975; Mangas-Ramírez & Elías-Gutiérrez, 2004; Villamagna & Murphy, 2010). Furthermore, decline in the amount dissolved oxygen concentration in water enhances the release of phosphorus at the bottom, increases the temperature of water, free carbon dioxide and lowers the pH because of the breakdown of dead body of water hyacinth, all of these phenomena might initiate for the deterioration of quality of water and eutrophication of lakes (Ndimele *et al.*, 2011; Dersseh *et al.*, 2019). In the case of a compact mat of water hyacinth, there is a higher mass of dead bodies of plants that settle in the bottom and expends a large amount of dissolved oxygen during decomposition and the condition will be hypoxic for fish and other aquatic fauna (Bicudo *et al.*, 2007). The mat of water hyacinth supported the exotic fish species *C. Idella* and *O. niloticus*, and the introduced species *C. nama*, which might explain the inclining capture of these fish in the lakes of Pokhara. The noticeable decline in capture of native fishes has been observed after the invasion of water hyacinth and *O. niloticus* in these lakes (Husen, 2014; Husen *et al.*, 2016). The captured biomass of exotic *O. niloticus* raised, for example, its biomass was 0.6 metric tons during 2006, but it was increased to 58.1 metric tons during 2011 (Husen, 2014). The invasive *O. niloticus* showed adverse effect in mud carp species for their growth and development in a manipulative experiment (Gu *et al.*, 2015). The results of RDA ordination also suggested a negative association of the native fishes *C. chagunio, H.*

fossilis, Danio devario, Naziritor chelynoides, *T. putitora, P. sophore* and *P. chola* with the increasing coverage of water hyacinth but positive association of the exotic fishes *O. niloticus*, *C. idella* and a native (introduced for lakes of Pokhara) *C. nama* with the same.

3.5 CONCLUSION

The invasion of water hyacinth in the large lakes of Pokhara appeared as the detrimental factor in the abundance and diversity of fishes. Though, it was shown that the fish abundance was higher in winter, the diversity of fish was comparable during the study period. The study revealed that, the fragmented mat of water hyacinth provided supporting environment for the speedily spread of fish *C. nama*, the invasive fish *O. niloticus* and the recently introduced exotic fish *C. idella*. In contrast, the native fish species abundance was adversely affected by the occurrence of the floating mat of water hyacinth. The close association of the percentage coverage of water hyacinth with amount of free carbon dioxide and temperature of water while that of the negative association with pH, dissolved oxygen and transparency notified the sign of degrading of water quality in the lakes of Pokhara. The declining trend in the capture of native and local fishes in the lakes of Pokhara could be due to the degrading water quality, the introduction of *O. niloticus*; the invasive fish, and *C. nama*; the scale-feeding fish and the invasion of water hyacinth. Regular monitoring of native and exotic fish catches as well as other invasive weeds in lakes of Pokhara Valley is essential to realize the influence of invasive species on the lakes. The present study suggested for the regular stock of native fish and management of invasive weeds to conserve the native fish in the lakes of Pokhara Valley.

CHAPTER 4

INVASION OF WATER HYACINTH LIMITS THE ABUNDANCE AND DIVERSITY OF GLOBALLY THREATENED WATERBIRDS IN THE LAKES OF POKHARA VALLEY, NEPAL

Abstract

The invasion of alien species is the principal cause for the change in the structure and operation, of any type of ecosystem, for example, alteration of habitats, adverse effects on native species, energy flow and nutrient cycling. Out of 27 invasive alien plant species of Nepal, the water hyacinth (*Pontederia crassipes*) infestation is quickly expanding in the lakes of Pokhara in the last two decades. This study explored the effects of water hyacinth's invasion on waterbird diversity and abundance in the lakes of Pokhara Valley. Overall, 24 sampling plots were demarcated on the basis of size of lakes and occurrence of water hyacinth. The seasonal bird observation was conducted using the point count method within the 50 m radius of water hyacinth presence (HP) and absence (HA) plots. Feeding guilds of the birds were separated according to their foraging behaviors. The results showed the HP habitats had lowered abundance of threatened waterbirds in comparison to HA habitats ($p = 0.023$). The piscivorous bird abundance was higher in the HA plots compared to the HP, whereas the opposite was observed for insectivorous and omnivorous birds. The abundance and richness of globally threatened waterbirds were declined in HP areas and increased with the increase in overall waterbird abundance and depth of water. The findings of this study provided the baseline data in the field of biological invasion and can be used by the researcher for further research in future. It may also direct the lake managers and policymakers to formulate the strategies to remove or manage water hyacinth in the lakes of Pokhara Valley to improve water quality as well as waterbird conservation.

4.1 INTRODUCTION

Globally, biological invasion has a detrimental effect on the structure and function of the ecosystems in both aquatic and terrestrial environment (Havel *et al.*, 2015; Shrestha, 2016; Shah *et al.*, 2020; Pinero *et al.*, 2021). The primary effects of invading alien species comprise the loss of biodiversity including native species, modification of habitats, and declines in nutrient cycling and ecosystem productivity (Charles & Dukes, 2008; Keller *et al.*, 2018; Rai & Singh, 2020; Pathak *et al.*, 2021). For example, mikania (*Mikania micrantha*) invasion covers about 44% of the grassland habitat of the greater one-horn rhino (*Rhinoceros unicornis*) in Chitwan National Park, Nepal and it avoids the outgrowth of ground vegetaions, primary grasses and more tree species, reducing the amount of feed available to this animal (Murphy *et al.*, 2013; Shrestha, 2016; Shah *et al.*, 2020). The invasive plants with significant impact on native species in Nepal with detrimental impacts on ecosystems of various habitats are blue billygoat weed (*Ageratum houstonianum*) in agro-ecosystems, parthenium (*Parthenium hysterophorus*) in grasslands and residential areas, and water hyacinth (*Pontederia crassipes*) in wetlands (Shrestha, 2016; Pathak *et al.*, 2021). The detrimental impact of invasive species on native species of flora and fauna including common species are growing in Nepal (Rana *et al.*, 2018; Shrestha *et al.*, 2018; Shrestha & Shrestha, 2021).

Out of 182 naturalized alien flowering plants in Nepal, 27 species are considered invasive (Shrestha & Shrestha, 2021). Moreover, the main four species from them, lantana (*Lantana camara*), siam weed (*Chromolaena odorata*), mikania, and water hyacinth, are convinced among the World's 100 invasive species with the greatest unpleasant impacts (Lowe *et al.*, 2000; Villamagna, 2009). The perennial invasive water hyacinth native to Brazil is a mat-forming and rooted macrophyte that can proliferate rapidly in polluted water bodies, resulting in the reduced growth of native species (Villamagna, 2009). It is spreading in tropical and subtropical regions of the world, including Southeast Asia, New Zealand, Africa, North America, and Nigeria (Ndimele *et al.*, 2011), and human trade as a decorative plant has expedited the spread of this plant outside of its native range (Villamagna, 2009). The invasive water hyacinth was certainly introduced in Nepal from India (Maharjan & Ming, 2012). It was first noted in Nepal in 1966 in wetlands of Tarai in the western section (Dahal, 2006; Khatri *et al.*, 2018)). Water hyacinth has a massive rate of multiplication and the population could be double within a week (Gunnarsson & Petersen, 2007). The proliferation of water hyacinth in aquatic environment can deteriorate the quality of water, harm aquatic ecosystems and displace native species (Villamagna, 2009; Villamagna *et al.*, 2012; Hailu *et al.*, 2020). The dense mat of floating water hyacinth can impede the exchange of oxygen from the atmosphere to the water surface or curtail the production of oxygen by other plants and algae, owing to lower rate of photosynthesis (Villamagna & Murphy, 2010). Therefore, the reduced rate of photosynthesis of aquatic plants may result increasing water temperature, carbon dioxide and decreasing dissolve oxygen in the habitats of thick mat of water hyacinth (Mironga *et al.*, 2012; Hailu *et al.*, 2020), ensuing the aquatic environment in anoxic conditions with a substantial decrease in dissolved oxygen and high strengths of nutrient loads and pollutants like ammonia, iron, manganese, and sulphide (Yongo & Outa, 2015).

Interestingly, waterbirds are top level consumers in wetland ecosystems, the wetlands provide suitable habitats for numerous local residential and winter migratory birds for foraging (Kumar *et al.*, 2016), nesting, and reproduction (Firdausy *et al.*, 2021). Additionally, the ecosystems of wetland are more susceptible, with the unfavorable loss of wetlands occurring in Asia because of invasion of non-native species, that results in the alteration and destruction of habitats (Villamagna *et al.*, 2012; Inskipp *et al.*, 2016; Firdausy *et al.*, 2021). Moreover, the rapidly spreading water hyacinth's mat can lower water quality and restrict some threatened and migratory waterbird species' access to open water (Villamagna *et al.*, 2012). As a result, many winter migratory waterbird populations are reducing (Inskipp *et al.*, 2016). For instance, migratory waterbirds species declined when the water surface of Lake Chapala was covered by thick mat of water hyacinth in Mexico (Villamagna *et al.*, 2012).

Out of 892 bird species (BCN and DNPWC, 2022), more than 200 species are categorized as waterbirds in Nepal (Inskipp *et al.*, 2016). The waterbird population of Nepal is in a declining trend in present scenario, and nearby 25% of waterbird species are about extreme possibility of disappearance (Inskipp *et al.*, 2016). Degrading quality of water and limited access to threatened waterbirds because of the floating mat of the invasive water hyacinth (Villamagna *et al.*, 2012) are serious and harmful to aquatic food webs (Masifwa *et al.*, 2001; Toft *et al.*, 2003; Villamagna *et al.*, 2010). Nevertheless, the knowledge of water hyacinth's invasion and its impact on abundance and diversity of waterbirds is limited, which is critical for progressing wetland's invasive species management plans for the lakes in LCPV. As a result, the current study enumerated the effects of invasion of the water hyacinth on waterbird abundance and diversity in the lakes of Pokhara, and focused on winter migratory waterbirds.

4.2 MATERIALS AND METHODS

4.2.1 Methods (It is explained in Chapter-2)

4.2.1.1 Physicochemical parameters of water (It is explained in Chapter 2)

The sampling stations in large lakes Phewa, Begnas and Rupa in Pokhara Valley are shown in Figure 13 below.

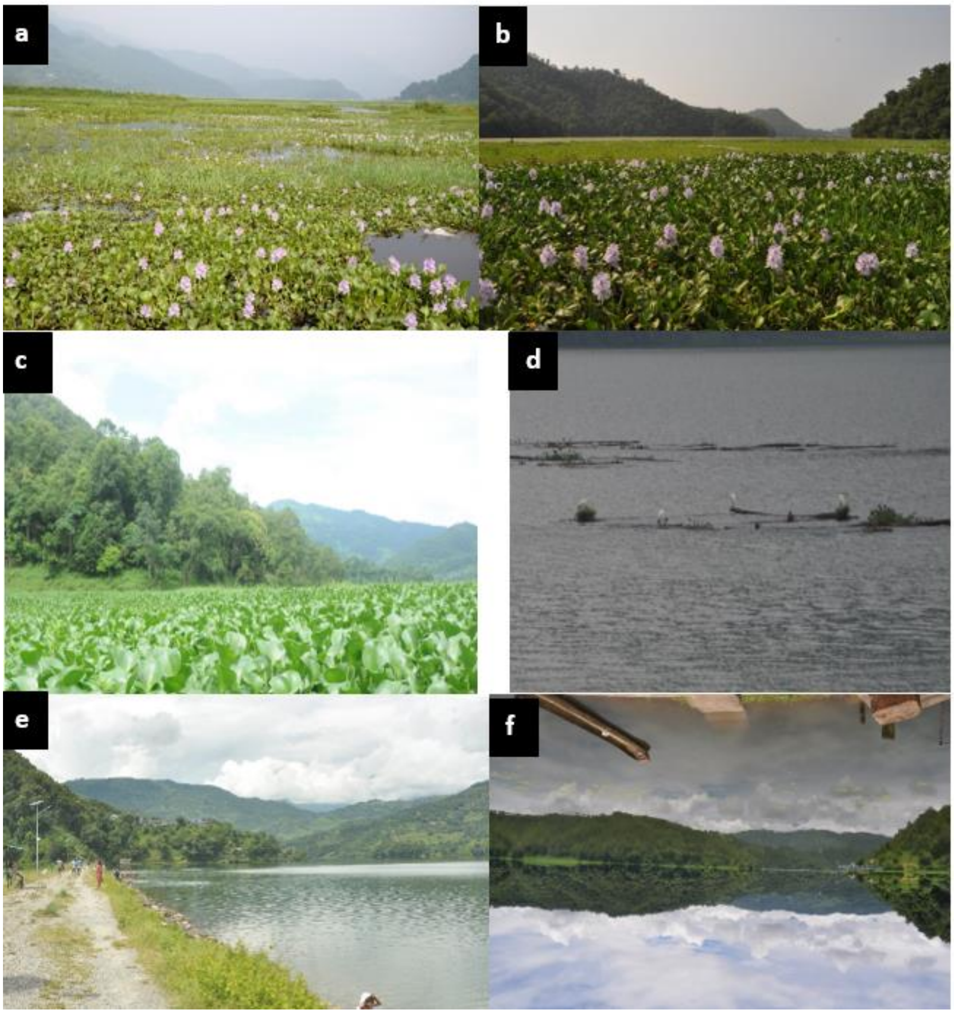


Figure 13: Sampling stations in largr lakes Phewa, Begnas and Rupa: a-c; water hyacinth presence habitats, d-f; water hyacinth absence habitats

4.2.1.2 Waterbird survey

The waterbird survey was conducted during morning hours from 7 AM to 11AM. Birds were observed for 30 minutes in each plot at 5 minutes interval to record the waterbird abundance and composition followed by Bibby *et al.* (2000). Waterbirds were counted in both HP and HA plots after 5 minutes of arrival at each plot to reduce potential disturbance effects to the birds due to the observers (Figure 14). Birds were observed on four occasions during summer (June and July 2019, July and August 2020) and winter (December 2019, January and February 2020). The maximum number of waterbird species with their total number of individuals were recorded in each plot in all the studied seasons and used in statistical analyses. For the identification of waterbirds, Grimmett *et al.* (2016); the field guide of birds was used and consultation with experts was taken in case of uncertain species for the confirmation.

Figure 14: The scholar recording the coordinates of Gunde lake using GPS (a) and observing the waterbirds with binoculars in Phewa lake, Khapaudi (b)

4.2.1.3 Data analysis

Diversity indices of the waterbirds were calculated by the same method used for macro-invertebrates in Chapter 2.

Waterbird feeding guilds were divided into four categories: piscivorous, insectivorous, omnivorous, and herbivorous on the basis of foraging behavior (Inskipp *et al.*, 2016). Normality of the data was evaluated using the Shapiro-wilk test, it indicated that the data were not distributed normally ($p < 0.05$). Therefore, the total bird abundance, threatened bird abundance and the bird abundance in each feeding guilds were compared between water hyacinth presence and absence habitats using the Mann-Whitney test (Mann & Whitney, 1947).

To determine the influencing environmental factors on threatened waterbird abundance and species richness, generalized linear mixed models with Poisson distribution and using the lake as a random factor was operated in the lme4 package (Bates *et al.*, 2018) in Program R (R Core Team, 2020). The model parameters in which the predictor variables were seasons (winter and summer), water hyacinth presence and absence, transparency of water (m), depth of water (m), temperature (°C), distance to lake edge (m) and distance to settlement (m) and overall bird abundance, similarly, the response variables were the abundance of threatened waterbirds, and richness of threatened waterbirds. The competing models with Akaike Information Criteria scores that were adjusted for small samples < 2, expected 95% confidence intervals for each variable and accepted statistical significance at 0.05 were used to do the model averaging.

4.3 RESULTS

4.3.1 Abundance and diversity of waterbirds

The current study observed overall 4114 entities of waterbirds belonging 11 families, 23 genera and 32 species (Figure 15, Table 13). The greatest number of waterbird species were counted from Anatidae family (N = 11, 35%), it was followed by Ardeidae (N = 5, 16%) and Rallidae (N = 3, 10%); the least number of species was recorded from three families Jacanidae, Dacelonidae and Podicipedade (N = 1, 5%) from each (Figure 15).

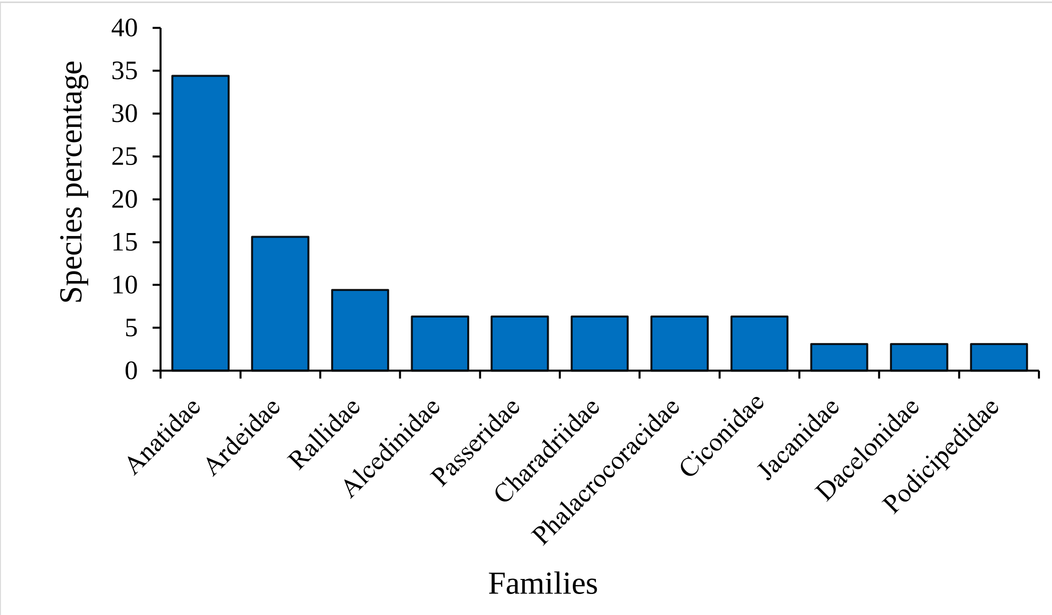


Figure 15: Waterbird families by species percentage in lakes of Pokhara Valley, Nepal

There were four globally threatened waterbirds recorded in lakes of Pokhara, which were: woolly-necked stork (*Ciconia episcopus*) and common pochard (*Aythya ferina*); vulnerable, Baer's pochard (*Aythya baeri*); critically endangered and ferruginous pochard (*Aythya nyroca*); near-threatened and the remaining species of birds were in the least concern category (Table 14).

Table 14: List of waterbirds recorded in lakes of Pokhara Valley, Nepal from 2019 to 2020

S.N.	Scientific Name	Common Name	Family	IUCN Status	Feeding Guild
1	*Phalacrocorax carbo* Linnaeus, 1758	Great Cormorant	Phalacrocoracidae	LC	Piscivore
2	*Aythya baeri* Güldenstädt, 1770	Baer's Pochard	Anatidae	CR	Omnivore
3	*Aythya ferina* Linnaeus, 1758	Common Pochard	Anatidae	VU	Omnivore
4	*Anser indicus* Latham, 1990	Bar-headed Goose	Anatidae	LC	Herbivore
5	*Mareca penelope* Linnaeus, 1758	Eurasian Wigeon	Anatidae	LC	Herbivore
6	*Aythya nyroca* Güldenstädt, 1770	Ferruginous Pochard	Anatidae	NT	Omnivore
7	*Anas platyrhynchos* Linnaeus, 1758	Mallard	Anatidae	LC	Omnivore
8	*Anas acuta* Linnaeus, 1758	Northern Pintail	Anatidae	LC	Omnivore
9	*Spatula clypeata* Linnaeus, 1758	Northern Shoveler	Anatidae	LC	Omnivore
10	*Tadorna ferruginea* Pallas, 1764	Ruddy Shelduck	Anatidae	LC	Omnivore
11	*Aythya fuligula* Linnaeus, 1758	Tufted Duck	Anatidae	LC	Omnivore
12	*Ciconia nigra* Linnaeus, 1758	Black Stork	Ciconiidae	LC	Piscivore
13	*Ciconia episcopus* Boddaert, 1783	Woolly Necked Stork	Ciconiidae	VU	Piscivore
14	*Ardea cinerea* Linnaeus, 1758	Grey Heron	Ardeidae	LC	Piscivore
15	*Dendrocygna javanica* Horsfield, 1821	Lesser Whistling duck	Anatidae	LC	Omnivore
16	*Tachybaptus ruficollis* Pallas, 1764	Little Grebe	Podicipedidae	LC	Insectivore
16	*Phalacrocorax niger* Gmelin, 1789	Little Cormorant	Phalacrocoracidae	LC	Piscivore
18	*Bubulcus ibis* Linnaeus, 1766	Cattle Egret	Ardeidae	LC	Insectivore
19	*Ardeola grayii* Sykes, 1832	Indian pond Heron	Ardeidae	LC	Insectivore
20	*Ardea intermedia* Wagler, 1829	Intermediate Egret	Ardeidae	LC	Insectivore
21	*Egretta garzetta* Linnaeus, 1766	Little Egret	Ardeidae	LC	Insectivore

22	*Fulica atra* Linnaeus, 1758	Common Coot	Ardeidae	LC	Omnivore
23	*Gallinula chloropus* Linnaeus, 1758	Common Moorhen	Rallidae	LC	Omnivore
24	*Porphyrio porphyrio* Linnaeus, 1758	Purple Swamphen	Rallidae	LC	Omnivore
25	*Metopidius indicus* Latham, 1790	Bronze-winged Jacana	Jacanidae	LC	Omnivore
26	*Alcedo atthis* Linnaeus, 1758	Common Kingfisher	Alcedinidae	LC	Piscivore
27	*Alcedo meninting* Horsfield, 1821	Blue-eared Kingfisher	Alcedinidae	LC	Piscivore
28	*Halcyon smyrnensis* Linnaeus, 1758	White Throated Kingfisher	Dacelonidae	LC	Piscivore
29	*Motacilla maderaspatensis* Gmelin, 1789	White-browed Wagtail	Passeridae	LC	Insectivore
30	*Motacilla alba* Linnaeus, 1758	White Wagtail	Passeridae	LC	Insectivore
31	*Charadrius dubius* Scopoli, 1786	Little Ringed Plover	Charadriidae	LC	Insectivore
32	*Vanellus indicus* Boddaert, 1783	Red Wattled Lapwing	Charadriidae	LC	Insectivore

*Status of IUCN: Vulnerable (VU), Near Threatened (NT), Least Concerned (LC), Critically Endangered (CR), Endangered (EN)

HP habitats had lower bird diversity and evenness (H' = 2.68; J = 0.84) than HA areas (H' = 3.01; J = 0.86). In comparison to HP habitats (21 species), HA habitats had a higher species richness (29 species; figure 16).

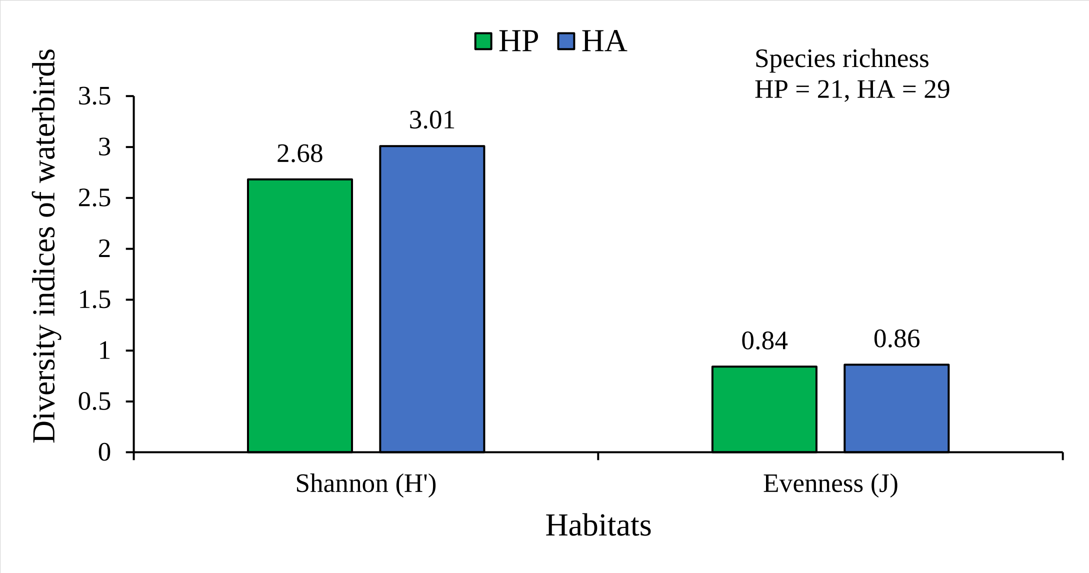


Figure 16: Diversity indices of waterbirds in areas with water hyacinth presence (HP) and absence (HA) in lakes of Pokhara Valley, Nepal

The waterbird abundance was varied in between HP and HA habitats (p = 0.04; Table 15). More residential waterbirds were found in HP habitats (p = 0.004; Table 15). The residential waterbirds such as cattle egret, Indian pond heron, purple swamphen, bronze-winged jacana and common moorhen were more dominant in HP habitats, and winter migratory waterbirds such as ruddy shelduck, tufted duck, great cormorant, common pochard, and lesser whistling duck were most frequent in HA habitats.

Birds that used foraging niches were omnivores in 41% of cases (n =13), insectivores in 28% (n =9), carnivores in 25% (n =8) and herbivores in 6% (n =2) (Figure 17). Piscivorous bird abundance was comparable in between water hyacinth presence and absence habitats (p = 0.90), although more insectivorous (p = 0.02) and omnivorous (p = 0.04) waterbirds were observed in HP habitats (Table 15).

Table 15: Abundance of waterbirds between water hyacinth presence (HP) and absence (HA) habitats in lakes of Pokhara Valley, Nepal from 2019 to 2020. Range of reported values are in parentheses

Parameters (Abundance)	**Habitat (HP) Median**	**Habitat (HA) Median**	**Mann–Whitney U test**
Total Abundance	33.5 (9–160)	25.5 (3–205)	U = 805.5; p = **0.04**
Resident birds	33.5 (9–131)	23 (3–116)	U = 700.5; p = **0.004**
Insectivore birds	20 (7–58)	16 (2–58)	U = 772.5; p = **0.02**
Piscivore birds	4 (1–21)	4.5 (1–41)	U = 1064; p = 0.91
Omnivore birds	15 (0–82)	6 (0–112)	U = 817; p = **0.04**
Threatened birds	0 (0–7)	0 (0–14)	U = 1275; p = **0.02**

Overall abundance of waterbirds and abundance of residential birds was greater in HP habitats (U = 805.5, p = 0.04 and U = 700.5, p = 0.004; respectively). However, the abundance of threatened waterbirds was found greater in HA habitats (U = 1275, p = 0.02) but the abundance of omnivore and insectivore birds were greater in HP habitats (U = 817, p = 0.04 and U = 772.5, p = 0.02; respectively. Whereas the abundance of piscivore birds was comparable in both the habitats (Table 15).

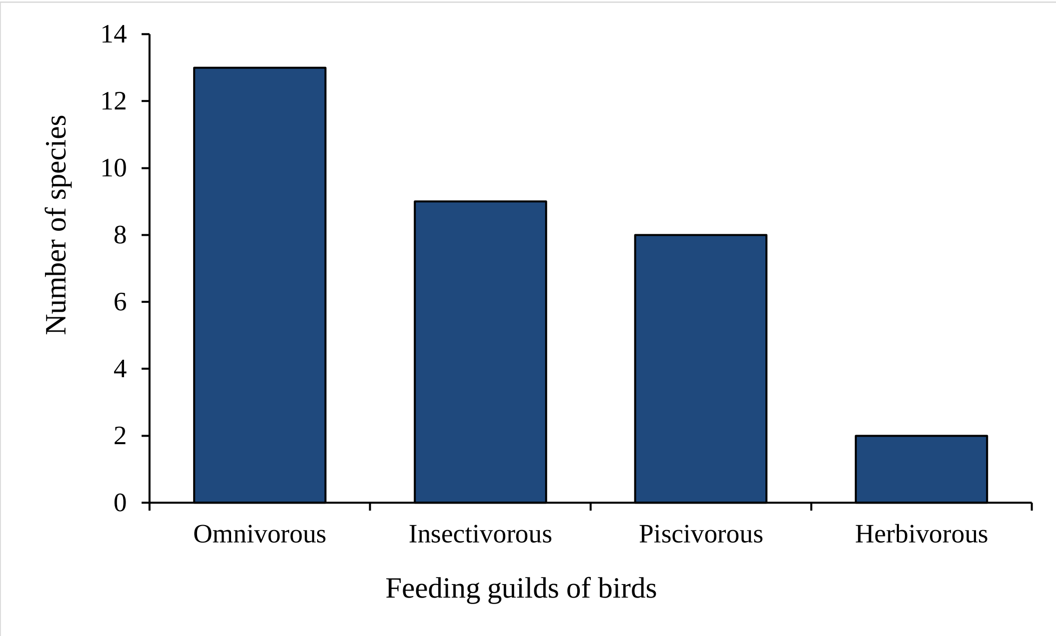


Figure 17: The waterbirds species number on the basis of feeding guild in lakes of Pokhara Valley, Nepal

4.3.2 Factors affecting the threatened waterbirds

The threatened waterbird abundance was higher in HA habitats but declined with increasing distance from settlements (Table 16). The abundance of threatened as well as overall bird abundance increased during the winter. The species richness of waterbirds declined with the decrease in water temperature. The abundance of threatened waterbirds increased with the increase in total abundance and depth of water and decreased in HP areas. Similarly, the richness of threatened waterbirds also increased with the increase in overall abundance of waterbirds and depth of water (Table 16).

Table 16: Generalize linear mixed model showing the influencing environmental factors on the abundance and species richness of threatened waterbirds in lakes of Pokhara Valley from 2019 to 2020

	Parameters	Estimate	Lower CL	Upper CL	Z	P
Threatened waterbird abundance	(Intercept)	-1.96×10^{1}	-5.27×10^{3}	5.23×10^{3}	−0.006	0.995
	Depth	4.47×10^{-1}	3.46×10^{-2}	6.92×10^{-1}	2.610	**0.009**
	Distance to settlement	2.70×10^{-5}	-2.23×10^{-3}	8.32×10^{-4}	0.022	0.982
	Abundance	1.23×10^{-2}	4.19×10^{-3}	2.18×10^{-2}	3.560	**0.000**
	Distance to edge	-1.01×10^{-3}	-2.23×10^{-3}	8.32×10^{-4}	−0.774	0.439
	Winter season	2.04×10^{1}	-5.23×10^{3}	5.27×10^{3}	0.006	0.995
	Water hyacinth	-1.45×10^{0}	-3.17×10^{0}	1.85×10^{-1}	−2.126	**0.033**
	Transparency	-1.38×10^{0}	-2.58×10^{0}	3.04×10^{-1}	−1.893	0.058
	Temperature	-6.25×10^{-2}	-2.16×10^{-1}	2.80×10^{-2}	−1.113	0.266
Threatened waterbird richness	(Intercept)	-2.22×10^{1}	-8.23×10^{3}	8.19×10^{3}	0.005	0.996
	Abundance	9.89×10^{-3}	2.19×10^{-3}	1.76×10^{-2}	2.517	**0.012**
	Depth	3.54×10^{-1}	$9.06\times10\text{-}^{2}$	6.18×10^{-1}	2.633	**0.008**
	Winter season	2.04×10^{1}	-8.20×10^{3}	8.24×10^{3}	0.005	0.996
	Temperature	-9.80×10^{-2}	-2.67×10^{-1}	7.11×10^{-2}	1.136	0.256
	Water hyacinth	-6.31×10^{-1}	-2.13×10^{0}	8.70×10^{-1}	0.824	0.410
	Transparency	4.29×10^{-2}	-1.64×10^{0}	1.73×10^{0}	0.050	0.960
	Distance to settlement	1.96×10^{-4}	-1.67×10^{-3}	2.06×10^{-3}	0.205	0.837
	Distance to edge	2.70×10^{-4}	-1.67×10^{-3}	2.21×10^{-3}	0.273	0.785

4.4 DISCUSSION

The proliferation of water hyacinth has detrimental effects on the abundance and diversity of waterbirds. Nevertheless, the lakes within the Pokhara Valley sustain a rich assortment of waterbird species. Wetlands that provide habitat and feeding possibilities may be the reason for the great species diversity (Villamagna *et al.*, 2012; Thapa & Saund, 2012; Adhikari *et al.*, 2018; Adhikari *et al.*, 2019). During winter, the waterbirds thrived abundantly, possibly due to the arrival of migratory waterbird species in the lakes. In HA, the waterbird community was dominated by the Anatidae family in winter, while in HP habitats of the lakes in the Pokhara Valley, resident birds like purple swamphen, egrets, and herons were prevalent during both winter and summer seasons. A similar observation was made in Pulau Rambut Wildlife Sanctuary in Jakarta, Indonesia, where herons, cormorants, and egrets were the dominant species (Firdausy *et al.*, 2021). The presence of Anatidae species in the lakes during winter is likely due to their migratory patterns and utilization of the lakes of Pokhara Valley as wintering grounds. Similar to this study, Lake Chapala in Mexico also reported Anatidae birds as the most abundant species (Villamagna *et al.*, 2012) and Beeshhazari Lake, Nepal (Adhikari *et al.*, 2018).

The Anatidae, Ardeidae and Jacanidae families of waterbirds were the most abundant families in water hyacinth presence habitats in the lakes of Pokhara Valley, it might be due to their migratory patterns, insectivorous foraging, and resident status. Additionally, these families were also recorded at Phewa Lake Pokhara (Gautam & Kafle, 2008; Giri & Chalise, 2008), Beeshhazari Lake (Adhikari *et al.*, 2018) and Khaste Lake Complex (Dhakal *et al.*, 2020).

Threatened waterbird populations were less numerous in HP habitats during the current study, which might be due to the changes in the structural composition of vegetation, heterogeneity in habitats, availability of the food to the waterbirds, and their foraging nature (Villamagna, 2009; Khan, 2010; Villamagna *et al.*, 2012). The increased presence of threatened waterbirds in HA regions may be attributed to the extensive open water areas, which provide many opportunities for easy foraging and swimming. Wintering waterbirds, such as cormorant, ducks and geese species, generally avoid dense floating and emergent vegetation because it restricts movement and reduces foraging efficiency (Villamagna *et al.*, 2010; Kumar *et al.*, 2016; Kaur *et al.*, 2018).

The negative impact of invaded sites and bush areas on the abundance and diversity of birds was reported by Grzędzicka (2023). The higher occurrence of resident birds in the HP habitat, such as common coots, common moorhens, purple swamphens, herons, and egrets, can be attributed to their omnivorous feeding behavior. This is likely due to the availability of abundant macro-invertebrates and vegetation in the HP habitat, which serve as potential food sources (Villamagna *et al.*, 2012; Kaur *et al.*, 2018). The majority of resident waterbirds were insectivores and omnivores, and the roots and leaves of water hyacinth offer a diverse array of macro-invertebrates, including insects, mollusks, and worms (Bartodziej & Leslie, 1998; Masifwa *et al.,* 2001). Additionally, invertebrate prey utilizes the dense mats of water hyacinths, which might promote the occurrence of more omnivorous and insectivorous bird species. Some species may also find refuge and breeding places in mat of water hyacinth (Villamagna *et al.,* 2012; Rajpar & Zakaria, 2015; Martins *et al.,* 2021). For instance, purple swamphen's population is increasing in the lakes of Pokhara than it did previously, most likely because of safe nesting and breeding grounds in and around water hyacinth habitats (Doss *et al.,* 2009; Mouslim *et al.*, 2014; Yang *et al.,* 2021). In recent years, the number of migratory waterbirds in the lakes of Pokhara has decreased (Inskipp *et al.,* 2016), could be possibly due to the increasing proliferation rate of this invasive weed; water hyacinth in the lakes of Pokhara that has resulted in the alteration of habitats. Similar kind of correlation of proliferation of water hyacinth with migratory birds was observed in Lake Chapala, Mexico (Villamagna *et al.,* 2012).

Overall waterbirds abundance in the present research site had a positive impact on the richness and diversity of threatened waterbirds, probably because of increased access to food and security offered by other birds. According to the more-individuals hypothesis, the presence of individual's species in a community increases with the increase of energy availability, which can increase overall species richness (Storch *et al.,* 2018). The degraded water quality detected during the current investigation in HP habitats may have had an impact on the abundance and diversity of globally threatened waterbird and wintering waterbirds. The higher overall abundance and richness of waterbirds in water hyacinth absence habitats could be due the greater depth, lesser detritus and sufficient open water access. Furthermore, in the present study area, there was a positive correlation observed between water depth and the overall presence of waterbirds. The water hyacinth absence areas in the lakes are shallow open water regions where the winter migratory waterbirds easily dive and catch the fish easily (Zhou *et al.,* 2020), this could potentially explain the greater abundance of these birds in the HA habitats within the lakes of Pokhara. Moreover, waterbird abundance and diversity were higher in habitats with less coverage of water hyacinth (Dahal, 2006), which could be attributed to provide more open water for foraging and easier movement for waterbirds. Detritus growth due to decaying of dead water hyacinth may result in lower water transparency, dissolved oxygen, pH, short light penetration and greater turbidity and free carbon dioxide (Rommens *et al.,* 2003; Mangas-Ramrez & Elas-Gutiérrez, 2004; Dereje, 2015; Hailu *et al.,* 2020). When the pH of water level reaches greater than nine and less than five it will be harmful to aquatic organisms (Thapa & Saund, 2012). According to Thapa and Saund (2012), the pH and dissolved oxygen support the waterbird abundance; higher pH and dissolved oxygen levels, as well as reduced free carbon dioxide, may contribute in the lakes of Pokhara for higher abundance of waterbirds in water hyacinth absence habitats. Due to the fact that threatened waterbirds are migratory and are more frequent in winter season, their abundance and richness declined as temperatures increased.

4.5 CONCLUSION

The outcomes of present study indicated the invasive water hyacinth has had a demonstrable adverse effect on the abundance and diversity of winter migratory waterbirds in lakes of Pokharra Valley. More waterbird species are expected in the lakes of Pokhara with better water quality, if the lakes are managed to concentrate on habitat reestablishment, particularly through the elimination of problematic water hyacinth. This is becoming more crucial to managers, especially in aquatic systems, as there are frequently more invasive species being introduced in the lakes of Pokhara Valley. The current study was focused only on the effects of water hyacinth but overall consequences of all non- native species on lake system are important to understand the invader's effect and their management. To advance the knowledge of invasion of weeds like water hyacinth and other invasive plant species and their effects on waterbird populations, it is recommended that the multidisciplinary research and continuous monitoring of waterbirds with the habitat restoration for the record of new and returning winter migratory bird species.

CHAPTER 5

SUMMARY AND CONCLUSION

5.1 Summary

The occurrence of invasive alien species can significantly alter both terrestrial and aquatic ecosystems, resulting in the decline and loss of native species, habitat modification, and changes in nutrient cycling. This phenomenon plays a substantial role in the global loss of biodiversity. The introduced non-native species negatively affects the structure and functioning of natural ecosystems. The invasion of non-native alien species poses a significant threat to biological diversity and ecological functions in habitats outside their native range, which can occur through intentional or accidental means of transportation. Invasion by non-native species is widely recognized as a leading cause of species extinction, habitat alteration, ecosystem disruptions, and significant losses in biodiversity and economic viability. Nepal has reported a total of 27 invasive plant species, among which water hyacinth (*Pontederia crassipes*) is rapidly spreading in the country's lakes, streams, and reservoirs, leading to deterioration of water health and disturbance of aquatic biodiversity. The infestation of invasive water hyacinth has posed a severe threat to various components of the lakes in the Pokhara Valley for over two decades. However, the effects of this invasive weed on water quality and aquatic organisms in these lakes remain unstudied. This study aimed to evaluate the ecological impacts of water hyacinth invasion on the physicochemical parameters of water, abundance and diversity of macro-invertebrates, fish, and waterbirds in the lakes of the Pokhara Valley.

The lakes in the Pokhara Valley were divided into two habitats based on the presence or absence of water hyacinth (HP and HA, respectively). In the larger lakes (Phewa, Begnas, and Rupa), three HP and three HA plots were established, while each of the smaller lakes had a single HA plot. In total, 24 sampling plots measuring 50 m × 50 m were established in both HP and HA habitats, with each plot designated for seasonal data collection. Physicochemical parameters of the water, such as transparency, temperature, pH, depth, total dissolved solids (TDS), turbidity, dissolved oxygen (DO), free carbon dioxide (CO_2), total alkalinity, and nitrate (NO_3), were measured at each plot. Macro-invertebrates were collected from the same plots using Peterson's Grab Sampler, and rectangular gill-net enclosures were set up overnight on a seasonal basis to sample fish populations. Bird surveys were conducted within a 50 m radius of each plot using the point count method between 7:00 AM and 11:00 AM.

The study revealed significant differences in physicochemical parameters between the HP and HA areas, including depth, pH, total dissolved solids, transparency, total alkalinity, dissolved oxygen, nitrate, and free carbon dioxide ($p < 0.001$). The coverage of water hyacinth was positively correlated with water free carbon dioxide and temperature but negatively correlated with other parameters, indicating degraded water quality in the HP areas.

A total of 29 macro-invertebrate species and 26 genera were identified, with Diptera and Haplotaxida being the least abundant in HP habitats. However, overall abundance and diversity were higher in the HP habitats compared to the HA habitats. Among the fish species identified (20 species under 18 genera belonging to eight families representing six orders), the Cyprinidae family had the highest number of species. The invasive species *Oreochromis niloticus* was the abundant fish in both study sites, but its abundance was significantly higher in HP habitats ($p < 0.001$), and there was a positive association between exotic fish species and water hyacinth coverage.

Throughout the survey period, a total of 4,114 individual waterbirds belonging to 32 species and 11 families were recorded. However, the abundance of threatened species within the Anatidae family was lower in areas with water hyacinth compared to areas without it ($p = 0.023$). The feeding guilds of waterbirds also varied between the presence and absence of this weed (water hyacinth). In HP habitats, insectivorous and omnivorous birds were more abundant, while piscivorous birds dominated the HA habitats. The absence of water hyacinth was associated with higher diversity of waterbirds ($H' = 3.01$; $J = 0.86$) compared to its presence ($H' = 2.68$; $J = 0.84$). The abundance and richness of threatened waterbirds increased with increasing water depth and overall waterbird abundance but decreased in HP habitats.

These findings provide valuable baseline data for policymakers and lake managers to formulate strategies for the removal or management of aquatic weeds, specifically water hyacinth, in the lakes of the Pokhara Valley to aid in the conservation of aquatic biodiversity.

5.2 Conclusions

The invasion of water hyacinth showed the remarkable degradation of water quality in the lakes of Pokhara Valley. The variances in the abundance and diversity of macro-invertebrates in water hyacinth presence and absence habitats in the lakes of Pokhara are primarily attributed to alterations in the structural composition and physicochemical characteristics of the water. Although water hyacinth provides a conducive environment for certain species, leading to higher abundance, the invasion of this weed has a negative impact on others, such as Diptera and Haplotaxida.

The declining catch of the native fishes in the lakes of the pokhara is primarily attributed to the invasion of water hyacinth, the degrading quality of water, and the introduction of *C. nama* and *O. niloticus*.

The proliferation of invasive water hyacinth has had a detrimental impact on the abundance and diversity of waterbirds especially winter migratory and threatened waterbirds in the lakes of the LCPV. The decline in winter migratory waterbird species is attributed in the decreasing open water areas due to rapid proliferation of the mat of water hyacinth. With the elimination of water hyacinth, we can expect the improved water quality and arrival of more species of winter migratory waterbird in the lakes of Pokhara.

5.3 Recommendations for Further work

Present study was specially focused on the habitat with presence and absence of water hyacinth in natural condition only. Here are some recommendations;

The study revealed that, the percentage cover of water hyacinth has the negative impacts on aquatic faunal abundance. Therefore, the removal of water hyacinth from the lakes is suggested. The removal of water hyacinth manually could be harmful as this process removes the macro-invertebrates attached in root, stem and leaf of water hyacinth. Therefore, the current study suggests for the biological control of the water hyacinth.

The study also suggests the comprehensive research on biodiversity of the whole wetland area of the lakes of Pokhara Valley. Such type of study will provide the complete check list of flora and fauna of this wetland, it will be helpful to formulate the policy for the conservation of wetland and biological diversity. The study also recommended to establish a regular program for the stocking of native fish species. So that, more faunal species can be expected from the lakes of Pokhara Valley.

This study suggests the habitat restoration efforts combined by the implementation of an integrated management strategy to control the invasive water hyacinth and other weeds aiming at conserving aquatic biodiversity in the lakes of Pokhara Valley. The study also suggests for management of the invasive plants and promotion of the native plant species such as waterthyme (*Hydrilla verticillate*), banana plant (*Nymphoides indica*), floating crystalwort (*Riccia fluitans*), water-nymph (*Najas graminea*), hornwort (*Ceratophyllum demersum*), lotus (*Nelumbo nucifera*), Narkat (*Arundo* spp.). The increase of native plants would be the suitable habitat for water hyacinth dependent waterbirds in the lakes. Additionally, some of these plants such as *Arundo donax* (Bonanno, 2012), are equally important for the purification of water.

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PHOTO PLATES

Photo plate 1: Macro-invertebrates of lakes of Pokhara Valley, Nepal

Photo 1: *Limnodrilus hoffmeisteri*

Photo 2: *Branchiura sowerbi*

Photo 3: *Tubifex tubifex*.

Photo 4: *Stylaria lacustris*

Photo 5: *Helobdella* sp.

Photo 6: *Placobdella* sp.

Photo 7: *Chironomus* sp.

Photo 8: *Leptophlebia marginata*

Photo 9: *Diplacodes* spp.

Photo 10: *Brachythemis contaminata*

Photo 11: *Gomphidia* spp.

Photo 12: *Aquarius remigis*

Photo 13: *Nepa cinerea*

Photo 14 *Thermonectus* sp.

Photo 15: *Dolomedes tenebrosus*

Photo 16: *Paleomon* sp.

Photo 17: *Acroloxus lacustris*

Photo 18: *Lamellidens* spp.

Photo 19: *Radiatula* spp.

Photo 20: *Thiara requeti*

Photo 21: *Thiara tuberculata*

Photo 22: *Bellamya* sp.

Photo Plate 2: Some fishes recorded in the Lakes of Pokhara Valley, Nepal

Photo 1: Bhitta (*Puntius chola*) in Kamal Pokhari

Photo 2: Sil Bhitta (*Danio devario*) in Phewa

Photo 3: Bhitta (*Puntius sophore*) in Kamal Pokhari

Photo 4: Grass carp (*Ctenopharyngodon idella*) in Gunde

Photo 5: Common carp (*Cyprinus carpio*) in Gunde

Photo 6: Balim (Chille) (*Heteropneustes fossilis*) in Niureni

Photo 7: Dhunge Bam (*Mastacembelus armatus*) in Phewa

Photo 8: Chuche Bam (*Xenentodon cancila*) in Phewa

Photo 9: Nile tilapia **(***Orechromis niloticus*) in Begnas

Photo 10: Glass fish **(***Chanda nama*) in Begnas

Photo Plate 3: Waterbirds observed in the Lakes of Pokhara Valley, Nepal

Photo 1: Black stork (*Ciconia nigra*) in Khapaudi, Phewa lake

Photo 2: Woolly necked stork (*Ciconia episcopus*) in Pame Phanta of Phewan lake

Photo 3: Mallard (*Anas platyrhynchos*) and other birds in Khapaudi, Phewa lake

Photo 4: Tufted duck (*Aythya fuligula*) in Khapaudi, Phewa lake

Photo 5: Common coot (*Fulica atra*) in Khaste lake

Photo 6: White-browed wagtail (*Motacilla maderaspatensis*) in Khapaudi, Phewa lake

Photo 7: Grey heron (*Ardea cinerea*) in Khapaudi, Phewa lake

Photo 8: Indian pond heron (*Ardeola grayii*) near the paddy field in Kamal Pokhari

Photo 9: Great cormorant (*Phalacrocorax carbo*) ready to take flight in Phewa

Photo 10: Purple swamphens (*Porphyrio porphyrio*) above the mat of water hyacinth in Rupa lake

Photo 11: Bronze-winged jacana (*Metopidius indicus*) foraging in mud in Kamal Pokhari

Photo 12: Blue-eared kingfisher (*Alcedo meninting*) with prey in Khapaudi, Phewa lake

Photo 13: Common kingfisher (*Alcedo atthis*) in Khapaudi, Phewa lake

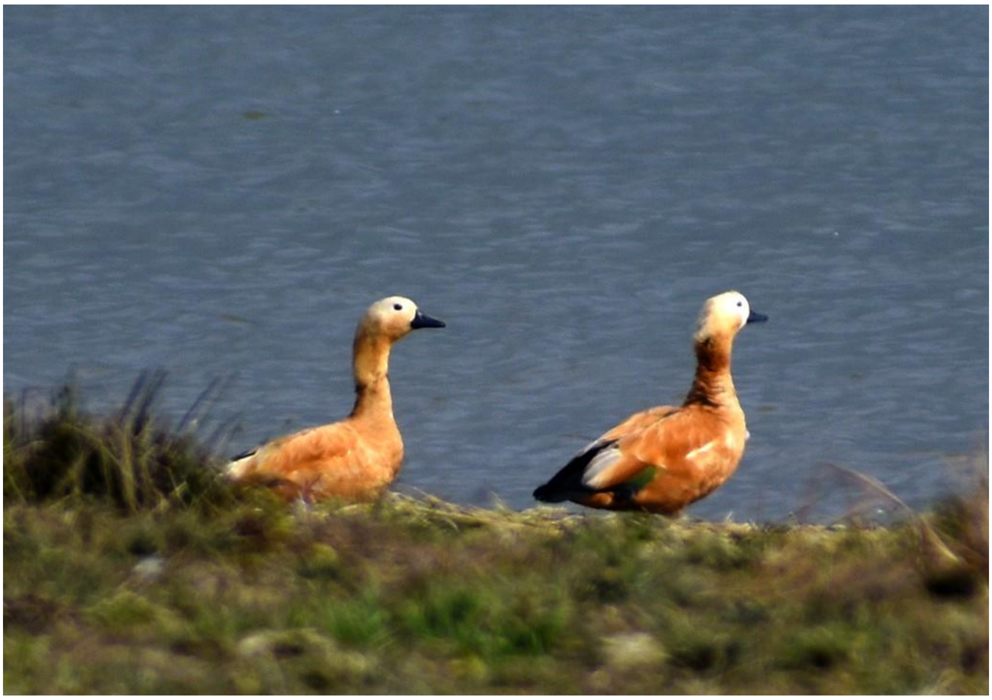

Photo 14: A pair of Ruddy shield ducks (*Tadorna ferruginea*) at the edge of Phewa lake in Khapaudi

Photo 15: White-throated Kingfisher (*Halcyon smyrnensis*) searching the prey in Rupa Lake

Photo 16: Little egret (*Egretta garzetta*) foraging in muddy water in Phewa lake

Photo 17: Bar headed goose (*Anser indicus*) in Phewa lake

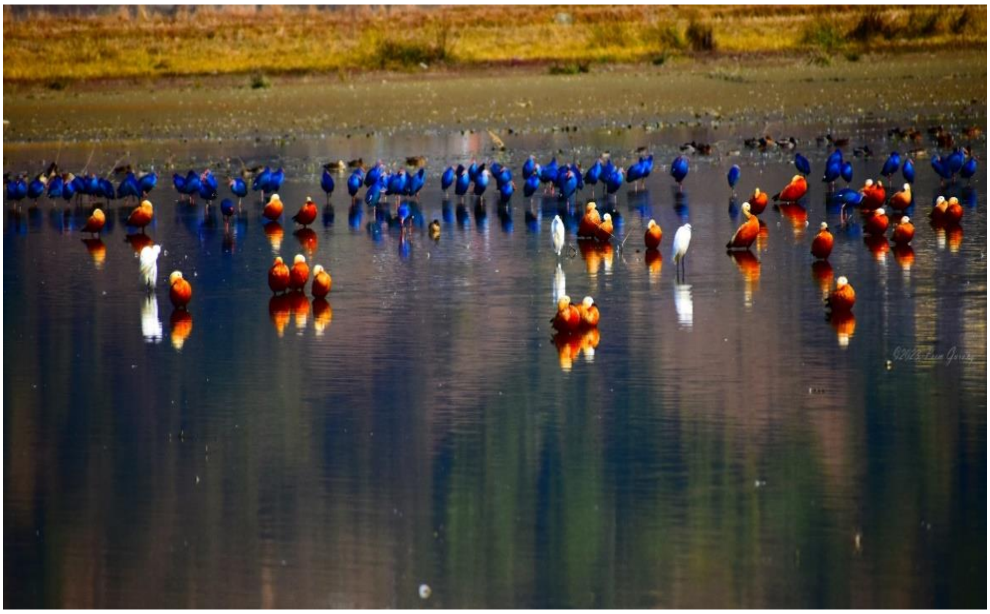

Photo 18: Flocks of winter migratory waterbirds in Khapaudi, Phewa lake. Ruddy shield ducks, purple swamphens and little egrets are seen in front line

Photo 19: Common moorhen (*Gallinula chloropus*) in Kamal Pokhari

Photo 20: Intermediate egret (*Ardea intermedia*) in Rupa

APPENDICES

Appendix I: Published research articles in national and international Journals

1.

sustainability

MDPI

Article

Invasive Water Hyacinth Limits Globally Threatened Waterbird Abundance and Diversity at Lake Cluster of Pokhara Valley, Nepal

Rajendra Basaula [1,2], Hari Prasad Sharma [1,3,*], Jerrold L. Belant [4] and Kumar Sapkota [1]

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ORIGINAL RESEARCH ARTICLE

The Invasion of Water Hyacinth and Its Impact on Diversity of Macro-Invertebrates in the Lake Cluster of Pokhara Valley, Nepal

3.

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ELSEVIER

Effects of invasive water hyacinth on fish diversity and abundance in the Lake Cluster of Pokhara Valley, Nepal

sustainability

Article

Invasive Water Hyacinth Limits Globally Threatened Waterbird Abundance and Diversity at Lake Cluster of Pokhara Valley, Nepal

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Abstract: Invasive species alter ecosystem structure and functioning, including impacts on native species, habitat alteration, and nutrient cycling. Among the 27 invasive plant species in Nepal, water hyacinth (*Eichhornia crassipes*) distribution is rapidly increasing in Lake Cluster of Pokhara Valley (LCPV) in the last several decades. We studied the effects of water hyacinth on threatened waterbird abundance, diversity, and physico-chemical parameters of water in the LCPV. We found areas with water hyacinth present (HP) had reduced threatened water bird abundance relative to areas where water hyacinth was absent (HA; $p = 0.023$). The occurrence of birds according to feeding guilds also varied between water hyacinth presence and absence habitats. Piscivorous birds were more abundant in HA areas than HP areas whereas insectivorous and omnivorous birds had greater abundance in HP areas than in HA areas. Threatened waterbird abundance and richness were greater in areas with greater water depth and overall bird abundance but declined in HP areas. Degraded water quality was also identified in HP areas. Our findings can be used as a baseline by lake managers and policy makers to develop strategies to remove or manage water hyacinth in LCPV to improve waterbird conservation.

Keywords: abundance; physicochemical; threatened; waterbirds; wetland

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1. Introduction

Biological invasions have adverse effects on ecosystem structure and functioning in terrestrial and aquatic ecosystems [1–5]. Major impacts of alien invasions include the loss of native species, habitat alteration, and deterioration of ecosystem productivity and nutrient cycling [6–9]. For example, mikania (*Mikania micrantha*) covers about 44% of the habitat of Greater one-horn rhino (*Rhinoceros unicornis*) in Chitwan National Park, Nepal, and has reduced the growth of primary grass and tree species resulting in limited forage for this species [4,5,10]. Other invasive plant species in Nepal alter ecosystems, including parthenium (*Parthenium hysterophorus*) in grasslands and residential areas, blue billygoat weed (*Ageratum houstonianum*) in agro-ecosystems, and water hyacinth (*Eichhornia crassipes*) in wetlands [5,8]. The effects of invasive species on native flora and fauna [11], including endemic species [12–14], are increasing in Nepal.

In Nepal, of 182 alien flowering plants, 27 species are considered invasive [11]. Among these invasive species, four species, siam weed (*Chromolaena odorata*), lantana (*Lantana camara*), mikania, and water hyacinth, are considered among the World's 100 invasive species with the greatest negative impacts [15]. Water hyacinth is a perennial, mat-forming, and rooted macrophyte native to Brazil and can proliferate rapidly as an invasive species, limiting the growth of native species in polluted water bodies [16]. Its distribution outside of Brazil includes Africa, North America, New Zealand, and Southeast Asia, including Nepal [17], and is facilitated by human trade as an ornamental [18]. Water hyacinth was first reported in western Nepal in 1972 [18] and was likely introduced from India [19]. Water hyacinth has a high reproductive rate and can double in numbers within seven days [20]. Water hyacinth can degrade aquatic ecosystems displacement of native species and decreased water quality [16,21,22]. Decreased photosynthesis of phytoplankton, decreased dissolved oxygen, and increased water temperature can occur in areas with abundant water hyacinth [16,23,24], resulting in anoxic conditions with elevated concentrations of ammonia, iron, manganese, and sulphide [25].

Waterbirds are wetland dependent species, with wetlands providing habitat for many residential and migratory birds for foraging [26], nesting, and reproduction [27]. However, wetlands are highly vulnerable ecosystems, with the greatest loss of wetlands occurring in portions of Asia due to habitat degradation, habitat fragmentation, and biological invasion [22,27–29]. The invasion of water hyacinth in wetlands can reduce water quality and limit open water areas needed by some threatened bird species [22]. Consequently, many waterbirds populations are declining [29]. For example, migratory birds were absent in Lake Chapala Mexico when the lake surface was covered by water hyacinth [22].

Of the 886 bird species occurring in Nepal, 200 species are classified as waterbirds [30]. Waterbird populations in Nepal have declined in recent years, and about 25% of these species are at high risk for extinction [29]. Water hyacinth can decrease water quality and access to threatened waterbirds [22], which are critical to aquatic food webs [31–33]. However, we have little knowledge of the effects of water hyacinth on threatened waterbird distributions and abundance and the corresponding water quality, which is pre-requisite for developing invasive wetland species management strategies for the LCPV. We quantified the effects of water hyacinth on the abundance of waterbirds and the physicochemical parameters of water in the LCPV, Nepal, emphasizing globally threatened waterbirds.

2. Materials and Methods

2.1. Study Area

Pokhara Metropolitan City is one of the most rapidly urbanizing cities in Nepal and occurs within the Seti River watershed. The city encompasses the LCPV, which includes nine lakes (Phewa, Begnas, Rupa, Kamal Pokhari, Gunde, Khaste, Niureni, Dipang, and Maidi) of ecological importance and is identified as a Ramsar Site (Figure 1). The LCPV comprises 262 km^2 with the lakes covering 9 km^2. Among these lakes, Phewa is meso-eutrophic (i.e., moderate nutrient loading), Begnas is oligo-mesotrophic (i.e., low nutrient loading with clear, deep water), and the seven remaining lakes are eutrophic (i.e., high nutrient loading); all lakes contain diverse aquatic plants and animals [34]. Aquatic plant species in the LCPV include white cheesewood (*Alstonia scholaris*); yellow grass orchid (*Apostasia wallichii*); champak (*Michelia champaca*), satawari or asparagus (*Asparagus racemosus*), bulbophyllum (*Bulbophyllum plyrhiza*); cymbidium (*Cymbidium iridioides*), pineapple orchid (*Dendrobium densiflorum*), fringe-lipped dendrobium (*D. fimbriatum*), tree fern (*Cyathea spinulosa*), elephant's foot (*Dioscorea deltoidea*), oberonia or Nepal's orchid (*Oberonia nepalensis*), Sikkim's orchid (*O. iridifolia*), Indian trumpet flower (*Oroxylum indicum*), terete vanda (*Papilionanthe teres*), malabar gulbel (*Tinospora sinensis*), and common hornwort (*Ceratophyllum demersum*); and water chestnut (*Trapa natans*) and lesser Bulrush (*Typha angustifolia*) [34]. Bird species in the LCPV include spiny babbler (*Turdoides nepalensis*), Nepal wren babbler (*Pnoepyga immaculate*), comb duck (*Sarkidiornis melanotos*), Baer's pochard (*Aythya baeri*), ferruginous duck (*Aythya nyroca*) [35], and numerous other waterbirds.

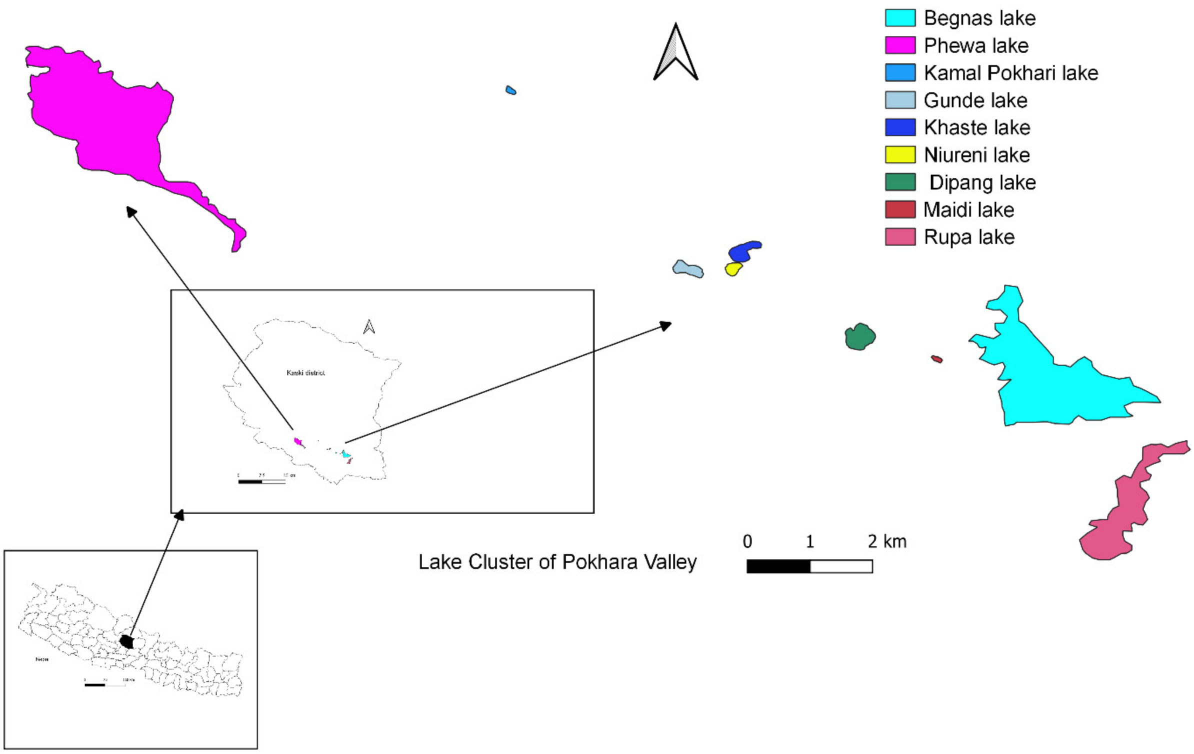

Figure 1. Lake Cluster of Pokhara Valley, Nepal.

2.2. Methods

Based on lake size and water hyacinth occurrence in lakes in the LCPV, we identified areas with water hyacinth presence (HP) and absence (HA). In larger lakes (i.e., Phewa, Begnas, and Rupa), we established three HP and three HA areas, and in each of the smaller remaining lakes, we established one HA area. In Phewa, Begnas, and Rupa, the distance between water hyacinth presence and absence areas was $\geq$500 m. During this study, water hyacinth coverage in HP areas was >90%, whereas water hyacinth was completely absent in HA areas in the last 10 years, confirmed through consultation with lake management committees. In HP and HA areas, we established a total of 24 samling plots each of 50-m radius and demarcated each to facilitate observations.

We recorded the coordinates of each plot center and measured the distance to the nearest lake edge and human settlement using a measuring tape. We conducted bird surveys during 7.00–11.00 h., using binoculars to record bird species composition and abundance at 5-min intervals for 30 min [36]. We initiated counts at each plot 5 min after arrival to reduce potential disturbance effects of observers. We observed birds on four occasions during winter (November 2019–February 2020) and summer (May–August 2020). We used the greatest number of birds and bird species counted during each observation period each season for analyses. We identified birds using field guides [28], and when uncertain, confirmed our observations through consultations with experts in bird identification.

At each plot, we measured physicochemical water parameters including depth, transparency, temperature, pH, total dissolved solids (TDS), turbidity, dissolved oxygen (DO), free carbon dioxide (CO_2), total alkalinity, and nitrate (NO_3). We established five 1- $\times$ 1- m^2 subplots, four along each plot edge and one at each plot center. We measured the water parameters in each subplot and averaged plot values for analyses. We determined water depth (cm) using a measuring tape and water transparency (cm) using a 20-cm diameter Secchi disc. We measured surface water temperature using a standard thermometer with 0.1 °C precision. We measured pH (4500-B, APHA), total dissolved solids (mg/L) (Instrumental), and turbidity (NTU) (3130 B, APHA, 1998). We measured dissolved oxygen (mg/L) using Winkler's method, free carbon dioxide (mg/L), total alkalinity (mg/L), and nitrate (mg/L) (4500-NO_3^- B, APHA) via the titrimetric method [37]. Laboratory work was performed at the Department of Zoology, Prithvi Narayan Campus, and Laboratory of Federal water Supply and Sewerage Management Project, Pokhara.

2.3. Data Analysis

We calculated Shannon–Weiner diversity (H′) [38], Pielou's species evenness (J) [39], and species richness (S) of waterbirds in HP and HA areas. We categorized waterbird species into four feeding guilds: piscivorous, insectivorous, omnivorous, and herbivorous [28,29]. We compared bird abundance, threatened bird abundance, feeding guilds of birds, and physicochemical parameters of water between HP and HA area using Mann–Whitney tests because our data were not normally distributed. We used generalized linear mixed models with Poisson distribution and lake as a random factor in the lme4 Package [40] in Program R [41] to identify factors affecting waterbird abundance and species richness at LCPV. We conducted model averaging using competing models with Akaike Information Criteria scores adjusted for small samples <2, estimated 95% confidence intervals for each variable, and accepted statistical significance at $\alpha = 0.05$.

3. Results

3.1. Abundance

We observed 4114 individual waterbirds overall, representing 32 species and 11 families (Figure 2, Table 1). Anatidae comprised the greatest number of species (35%, n = 11) followed by Ardeidae (16%, n = 5) and Rallidae (10%, n = 3).

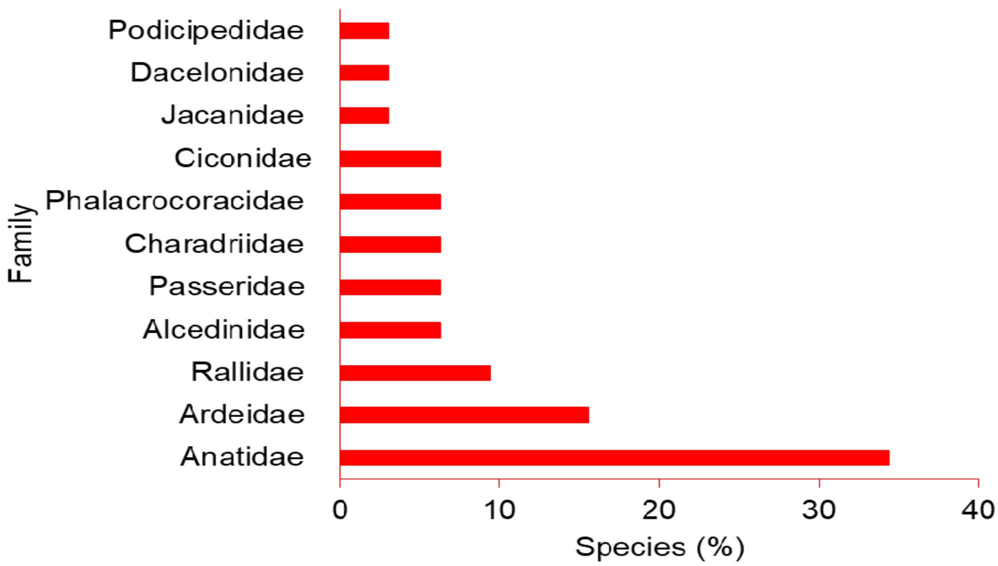

Figure 2. Families of waterbirds by species percentage, Lake Cluster of Pokhara Valley, Nepal, 2019–2020.

Bird abundance differed between the HP and HA habitats ($p = 0.03$; Table 2). The great cormorant, common pochard, ruddy shelduck, tufted duck, and lesser whistling duck were most abundant in HA areas, whereas purple swamphen, bronze wing jacana, cattle egret, Indian pond heron, and common moorhen were more abundant in HP areas. Among the birds observed, 73% were residential and 27% were migratory, with more residential birds in HP areas ($p = 0.004$; Table 1).

Table 1. Waterbirds recorded in Lake Cluster of Pokhara Valley, Nepal, 2019–2020. IUCN status: Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concerned (LC).

S.N.	Scientific Name	Common Name	Family	IUCN Status	Feeding Guild
1	*Phalacrocorax carbo* Linnaeus, 1758	Great Cormorant	Phalacrocoracidae	LC	Piscivore
2	*Aythya baeri* Güldenstädt, 1770	Baer's Pochard	Anatidae	CR	Omnivore
3	*Aythya ferina* Linnaeus, 1758	Common Pochard	Anatidae	VU	Omnivore
4	*Anser indicus* Latham, 1990	Bar-headed Goose	Anatidae	LC	Herbivore
5	*Mareca penelope* Linnaeus, 1758	Eurasian Wigeon	Anatidae	LC	Herbivore
6	*Aythya nyroca* Güldenstädt, 1770	Ferruginous Pochard	Anatidae	NT	Omnivore
7	*Anas platyrhynchos* Linnaeus, 1758	Mallard	Anatidae	LC	Omnivore
8	*Anas acuta* Linnaeus, 1758	Northern Pintail	Anatidae	LC	Omnivore
9	*Spatula clypeata* Linnaeus, 1758	Northern Shoveler	Anatidae	LC	Omnivore
10	*Tadorna ferruginea* Pallas, 1764	Ruddy Shelduck	Anatidae	LC	Omnivore
11	*Aythya fuligula* Linnaeus, 1758	Tufted Duck	Anatidae	LC	Omnivore
12	*Ciconia nigra* Linnaeus, 1758	Black Stork	Ciconiidae	LC	Piscivore
13	*Ciconia episcopus* Boddaert, 1783	Woolly Necked Stork	Ciconiidae	VU	Piscivore
14	*Ardea cinerea* Linnaeus, 1758	Grey Heron	Ardeidae	LC	Piscivore
15	*Dendrocygna javanica* Horsfield, 1821	Lesser Whistling duck	Anatidae	LC	Omnivore
16	*Tachybaptus ruficollis* Pallas, 1764	Little Grebe	Podicipedidae	LC	Insectivore
16	*Phalacrocorax niger* Gmelin, 1789	Little Cormorant	Phalacrocoracidae	LC	Piscivore
18	*Bubulcus ibis* Linnaeus, 1766	Cattle Egret	Ardeidae	LC	Insectivore
19	*Ardeola grayii* Sykes, 1832	Indian pond Heron	Ardeidae	LC	Insectivore
20	*Ardea intermedia* Wagler, 1829	Intermediate Egret	Ardeidae	LC	Insectivore
21	*Egretta garzetta* Linnaeus, 1766	Little Egret	Ardeidae	LC	Insectivore
22	*Fulica atra* Linnaeus, 1758	Common Coot	Ardeidae	LC	Omnivore
23	*Gallinula chloropus* Linnaeus, 1758	Common Moorhen	Rallidae	LC	Omnivore
24	*Porphyrio porphyrio* Linnaeus, 1758	Purple Swamphen	Rallidae	LC	Omnivore
25	*Metopidius indicus* Latham, 1790	Bronze-winged Jacana	Jacanidae	LC	Omnivore
26	*Alcedo atthis* Linnaeus, 1758	Common Kingfisher	Alcedinidae	LC	Piscivore
27	*Alcedo meninting* Horsfield, 1821	Blue-eared Kingfisher	Alcedinidae	LC	Piscivore
28	*Halcyon smyrnensis* Linnaeus, 1758	White Throated Kingfisher	Dacelonidae	LC	Piscivore
29	*Motacilla maderaspatensis* Gmelin, 1789	White-browed Wagtail	Passeridae	LC	Insectivore
30	*Motacilla alba* Linnaeus, 1758	White Wagtail	Passeridae	LC	Insectivore
31	*Charadrius dubius* Scopoli, 1786	Little Ringed Plover	Charadriidae	LC	Insectivore
32	*Vanellus indicus* Boddaert, 1783	Red Wattled Lapwing	Charadriidae	LC	Insectivore

Table 2. Comparison of bird abundance between water hyacinth presence (HP) and absence (HA) habitats of Lake Cluster of Pokhara Valley, Nepal, 2019–2020. Range of reported values are in parentheses. Bolded values are significant.

Parameters	HP Habitat	HA Habitat	Statistics
Abundance	Median = 33.5(9–160)	Median = 25.5(3–205)	Mann–Whitney test, U = 805.5; $p = 0.038$
Resident birds	Median = 33.5(9–131)	Median = 23(3–116)	Mann–Whitney test, U = 700.5; $p = 0.004$
Threatened birds	Median = 0(0–7)	Median = 0(0–14)	Mann–Whitney test, U = 1275; $p = 0.023$
Omnivore birds	Median = 15(0–82)	Median = 6(0–112)	Mann–Whitney test, U = 817; $p = 0.046$
Insectivore birds	Median = 20(7–58)	Median = 16(2–58)	Mann–Whitney test, U = 772.5; $p = 0.020$
Piscivore birds	Median = 4(1–21)	Median = 4.5(1–41)	Mann–Whitney test, U = 1064; $p = 0.906$

Bird diversity and evenness were greater in HA areas (H′ = 3.01; $J = 0.86$) than HP areas (H′ = 2.68; $J = 0.84$). The greatest species richness was found in HA areas (29 species) compared to HP areas (21 species). Using foraging niches, 41% of birds were omnivores, followed by insectivores (28%), carnivores (25%), and herbivores (6%) (Figure 3). The abundance of piscivorous birds was similar between HP and HA areas ($p = 0.90$), however, more insectivorous ($p = 0.01$) and omnivorous species ($p = 0.04$) birds were observed in HP areas.

Four observed species are threatened globally, including the critically endangered Baer's pochard (*Aythya baeri*), the vulnerable common pochard (*Aythya ferina*) and woolly necked stork (*Ciconia episcopus*), and near threatened ferruginous pochard (*Aythya nyroca*) (Table 1). More threatened birds occurred in HA areas than HP areas ($p = 0.023$; Table 2)

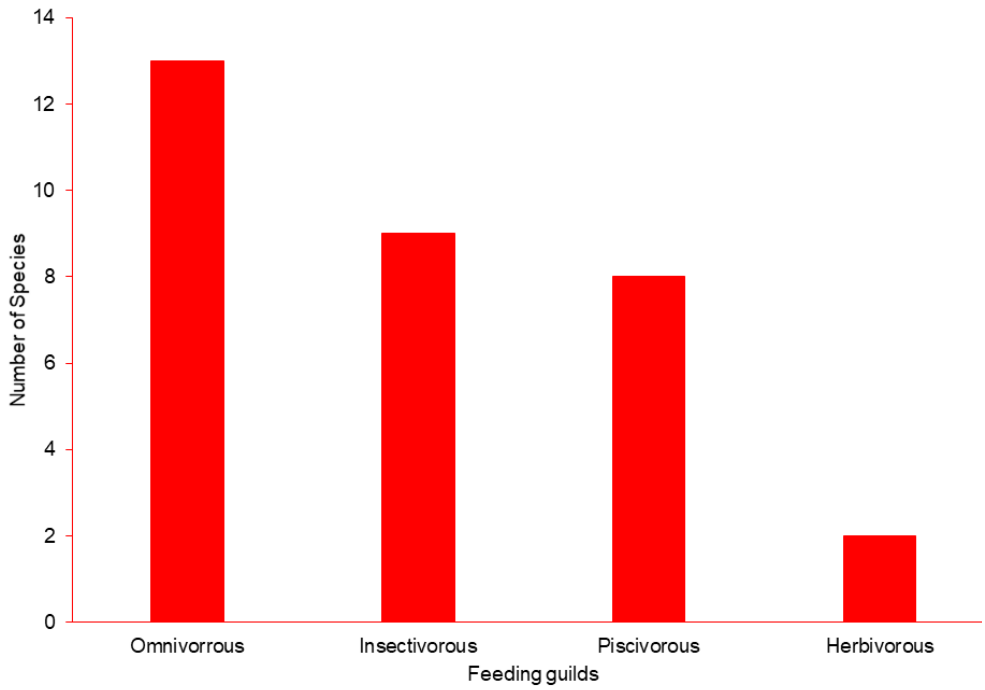

Figure 3. Number of waterbird species by feeding guild, Lake Cluster of Pokhara Valley, Nepal, 2019–2020.

3.2. Physicochemical Parameters

The water depth, transparency, pH, turbidity, total dissolved solid, dissolved oxygen, free carbon dioxide, total alkalinity, and nitrate differed between the HP and HA areas ($p < 0.001$; Table 3). The water temperature was similar between the HP and HA areas ($p > 0.05$).

Table 3. Comparison of physicochemical parameters of water in areas with water hyacinth present (HP) and absent (HA). Lake Cluster of Pokhara Valley, Nepal, 2019–2020. Bolded values are significant.

Parameters	HP Habitat	HA Habitat	Statistics
Depth (m)	Median = 3	Median = 4	Mann–Whitney test, U = 1817; *p* = <0.001
Temperature (°C)	Median = 25	Median = 24.5	Mann–Whitney test, U = 850.5; *p* = 0.082
Transparency(m)	Median = 0.77	Median = 1.4	Mann–Whitney test, U = 2057; *p* = <0.001
pH	Median = 6.6	Median = 7.4	Mann–Whitney test, U = 1825; *p* = <0.001
Turbidity (NTU)	Median = 3.05	Median = 3.05	Mann–Whitney test, U = 949; *p* = 0.323
TDS (mg/L)	Median = 23.5	Median = 19	Mann–Whitney test, U = 602; *p* = <0.001
DO (mg/L)	Median = 4	Median = 6.6	Mann–Whitney test, U = 1997; *p* = <0.001
Free CO_2 (mg/L)	Median = 11.85	Median = 6.8	Mann–Whitney test, U = 96.5; *p* = <0.001
Total alkalinity (mg/L)	Median = 126.5	Median = 149.5	Mann–Whitney test, U = 1677; *p* = <0.001
Nitrate (mg/L)	Median = 1.25	Median = 2.05	Mann–Whitney test, U = 1766; *p* = <0.001

3.3. Factors Affecting Waterbirds Abundance and Richness

Threatened water bird abundance was greater in HA areas and decreased with increasing distance to settlements (Table 4). Threatened waterbird abundance increased during winter and with increasing overall bird abundance. Bird species richness decreased with decreasing temperature.

Table 4. Model-averaged parameter estimates and 95% confidence limits (CL) describing the abundance of the waterbird species in Lake Cluster of Pokhara Valley, Nepal, during winter 2019–summer 2020. Model parameters include water hyacinth presence and absence, seasons (winter and summer), depth of water (m), temperature (°C), transparency of water (m), distance to lake edge (m) and distance to settlement (m), bird abundance and threatened bird abundance, and bird richness as response variables. Estimates were averaged from all models. Bolded values are significant.

	Parameters	Estimate	Lower CL	Upper CL	z	*p*
Threatened waterbird abundance	(Intercept)	−19.600	-5.27×10^{3}	5.23×10^{3}	−0.006	0.995
	Depth	0.447	0.00346	0.692	2.610	**0.009**
	Distance to settlement	2.70×10^{-5}	-2.23×10^{-3}	8.32×10^{-4}	0.022	0.982
	Bird abundance	1.23×10^{-2}	4.19×10^{-3}	2.18×10^{-2}	3.560	**0.000**
	Distance to edge	-1.01×10^{-3}	-2.23×10^{-3}	8.32×10^{-4}	−0.774	0.439
	Winter season	20.4000	-5.23×10^{3}	5.27×10^{3}	0.006	0.995
	Water hyacinth	−1.450	−3.170	0.185	−2.126	**0.033**
	Transparency	−1.380	−2.580	0.304×10^{-1}	−1.893	0.058
	Temperature	-6.25×10^{-2}	-2.16×10^{-1}	2.80×10^{-2}	−1.113	0.266
Threatened waterbird richness	(Intercept)	−22.200	-8.23×10^{3}	8.19×10^{3}	0.005	0.996
	Bird abundance	9.89×10^{-3}	2.19×10^{-3}	1.76×10^{-2}	2.517	**0.012**
	Depth	3.54×10^{-1}	9.06×10^{-2}	6.18×10^{-1}	2.633	**0.008**
	Winter season	20.400	-8.20×10^{3}	8.24×10^{3}	0.005	0.996
	Temperature	-9.80×10^{-2}	-2.67×10^{-1}	7.11×10^{-2}	1.136	0.256
	Water hyacinth	−0.631	−2.130	0.870	0.824	0.410
	Transparency	4.29×10^{-2}	−1.640	1.730	0.050	0.960
	Distance to settlement	1.96×10^{-4}	-1.67×10^{-3}	2.06×10^{-3}	0.205	0.837
	Distance to edge	2.70×10^{-4}	-1.67×10^{-3}	2.21×10^{-3}	0.273	0.785

4. Discussion

The LCPV supported abundant waterbird species with high species richness. High species diversity could be attributed to the wetlands providing shelter and foraging opportunities [22,42–44]. The community composition of waterbird in LCPV was dominated by the family Anatidae during winter in HA areas and purple swamphen, egrets and herons in HP areas during winter and summer. Similar dominant species composition including cormorants, herons, and egrets was observed in the Pulau Rambut Wildlife Sanctuary, Jakarta, Indonesia [27]. The high occurrence of Anatidae in the LCPV during winter is likely due to their migratory behavior and specific use of the LCPV as a wintering area. Anatidae were also the numerically dominant family in Lake Chapala, Mexico [22], and Beeshhazari Lake, Nepal [43]. The Anatidae, Ardeidae, and Jacanidae families were more abundant in HP areas than in HA areas, probably due to their respective migratory patterns, insectivorous foraging, and resident status, respectively. These families were also prevalent in other areas, including Phewa Lake Pokhara, Nepal, [35,45], Lake Chapala, Mexico [22], Beeshhazari Lake [43], and in the Khaste Lake Complex, Nepal [46].

In this study, threatened waterbird abundance was less in water hyacinth areas. This could be due to variation in vegetation structure, habitat heterogeneity, food resources, and foraging behavior of threatened waterbird species [16,22,47]. The greater abundance of these birds in HA habitats is probably due to open areas facilitating movement, swimming, and foraging. Generally, winter migratory birds such as ducks, geese, and cormorant species avoid dense emergent vegetation due to increased cover restricting movements and reduced foraging efficiency [26,33,48]. However, greater abundance of residential birds such as herons, common coots, common moorhens, and purple swamphens in HP habitat is probably due to their omnivorous feeding because HP habitat provides abundant insects and vegetation as potential food [22,48]. Most residential birds were insectivores and omnivores and the roots and leaves of water hyacinth support many macro-invertebrates including insects, mollusks, and worms [31,49]. In addition, the dense mats formed by water hyacinth are used by invertebrate prey, and we suggest can facilitate supporting the occurrence of more omnivorous bird species. Water hyacinth also likely provides nest sites and refugia for some species [22,50,51]. For example, the number of purple swamphens has increased in the LCPV, probably due to secure breeding in and around water hyacinth areas [52–54]. The number of migratory waterbirds has deceased in the LCPV during recent years [31], possibly due to increased water hyacinth presence in these lakes, which has altered the habitat. Similarly, fewer migratory birds were recorded in Lake Chapala, Mexico, corresponding with increased water hyacinth prevalence [22].

Threatened waterbird abundance and their richness were influenced by the overall bird abundance in the study area, possibly due to security afforded by other birds and higher availability of forage. Generally, the more-individuals hypothesis states that higher available energy supports the occurrence of individuals of species in a community, which in turn can increase the overall species richness [55]. The degraded water quality we detected in HP areas may also have contributed to the observed differences in threatened waterbird abundance and richness. Threatened water bird abundance and richness was greater in areas without water hyacinth, possibly due to greater water depth in areas without water hyacinth as these areas contain less detritus. In addition, the overall waterbird occurrence increased with increasing water depth in our study area. The greater abundance of wintering waterbirds in HA areas could be due to the presence of open shallow water areas [56] where they can dive and more easily catch fish. Furthermore, the abundance and diversity of waterbirds was greater in areas with reduced water hyacinth coverage [57], which could be attributed to reduced coverage, providing foraging areas for waterbirds. The decay of water hyacinth and accumulation of detritus likely resulted in lower observed pH, dissolved oxygen, low water transparency, short light penetration, and greater CO_2 and turbidity [21–23,58,59]. pH values greater than 9 and lower than 5 may kill aquatic life [44]. Thapa and Saund [44] found that pH and dissolved oxygen were positively associated with the abundance of birds; the higher abundance of waterbirds in HA areas of LCPV might be due to in part to the low free carbon dioxide and greater pH and dissolved oxygen in HA areas. Threatened waterbird abundance and richness decreased with increased temperature because these birds are migratory and more abundant during winter.

5. Conclusions

We were unable to monitor bird use of all catchment areas of the LCPV. However, the extent of water hyacinth coverage had demonstrable adverse effects on waterbird abundance as well as the physicochemical parameters of water in the LCPV. We expect more species of waterbirds to occupy the LCPV and improved water quality if managed to emphasize habitat restoration, particularly through removal of water hyacinth. We recommend that habitat restoration be integrated with a multidisciplinary research and monitoring strategy to further our understanding of the effects of water hyacinth and other invasive plant species on waterbird populations.

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ORIGINAL RESEARCH ARTICLE

The Invasion of Water Hyacinth and Its Impact on Diversity of Macro-Invertebrates in the Lake Cluster of Pokhara Valley, Nepal

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ABSTRACT
Invasion of Alien Invasive Plant species (IAPs) is one of the major drivers for the wetland ecosystem degradation and aquatic biodiversity loss. Among the wetland ecosystems, the freshwater habitats including lakes and streams are more susceptible to species extinction. In the Lake Cluster of Pokhara Valley (LCPV), many aquatic species have been threatened by an abundant occurrence of water hyacinth (*Eichhornia crassipes*). Thus, this study aims to identify the invasion effect of water hyacinth on abundance and diversity of macro-invertebrates, and association of this weed with different water parameters,. Water hyacinth is not only correlated with depth, transparency, pH and dissolved oxygen negatively, it is also correlated with temperature and free carbon dioxide positively. A total of 29 species and 26 genera from 21 families and 15 orders of macro-invertebrates were recorded. Among the macro-invertebrates, haplotaxida and diptera were found to be less abundant in the water hyacinth presence (HP) habitat than the water hyacinth absence (HA) habitat. However, the macro-invertebrates were found more abundant and diverse in the HP habitat than the HA habitat (Ranged: HP: 177 to 666; HA: 46 to 483). The abundance of orders like ephemeroptera, odonata, coleoptera, sphaeriida and caenogastropoda was significantly higher in the HP habitats. The direct and indirect effect of water hyacinth on the occurrence of macro-invertebrates and abundance can change the faunal structure of LCPV. Therefore, it is recommended to develop a plan of LCPV to manage the water hyacinth.
KEYWORDS: *Eichhornia crassipes*, habitats, IAPs, invasion, physico-chemical

INTRODUCTION
The invasion of Alien Invasive Plant species (IAPs) causes the wetland habitat degradation, which is now a global problem for conservationists (Coetzee et al., 2014;

Lamelas-López et al., 2021; Pathak et al., 2021). Basically, IAPs modify the ecosystem

of invaded areas, and reduce the abundance and diversity of native floral and faunal species. Due to high competition, predation and hybridization, they change in the community structure (Blackburn et al., 2011; Gentili et al., 2021). Among the various ecosystems, the freshwater ecosystems including lakes and streams are more susceptible to species extinction due to the habitat change and its IAPs invasion (Havel et al., 2015; Shrestha, 2016; Shrestha & Shrestha, 2021). Out of six aquatic invasive plant species of Nepal (Shrestha, 2016; Shrestha & Shrestha, 2021), five species: water hyacinth (*Eichhornia crassipes*), bush morning-Glory (*Ipomoea cornia*), southern cut grass (*Leersia hexandra*), water lettuce (*Pistia stratoides*) and alligator weed (*Alternanthera philoxeroides*) are reported from the lakes of Pokhara valley (Pathak et al., 2021). According to Lowe et al. (2000), the water hyacinth is under the category of 100 worst invasive species in the world.

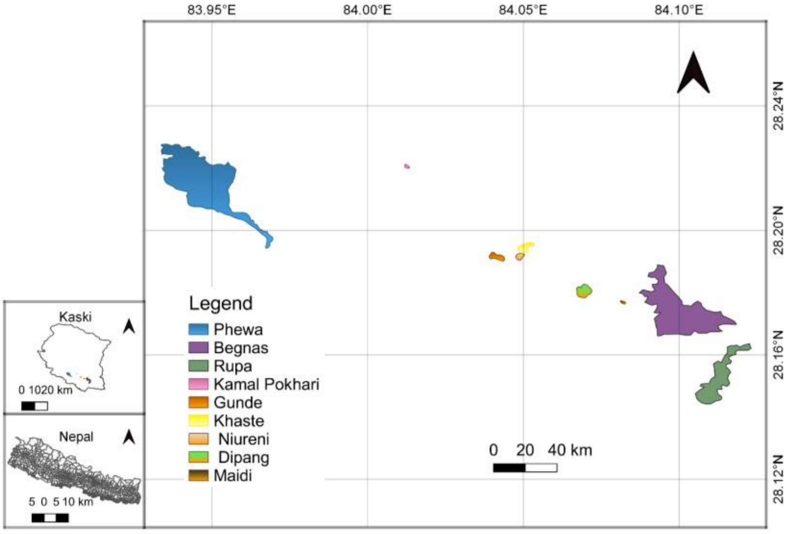
The water hyacinth is widely distributed in freshwater bodies of about 50 countries in the regions of tropic and subtropic (Bartodziej & Weymouth, 1995; Brendonck et al., 2003; Villamagna, 2009), generally in eutrophic lakes having 30°C of average water temperature, which is suitable for water hyacinth (Harun et al., 2021). It is abundantly found in Africa, North America, Nigeria, New Zealand and Southeast Asia (Ndimele et al., 2011). Probably, the water hyacinth was introduced in Nepal from India, which was first reported in Nepal in 1972 in the western part (Khatri et al., 2018). Its distribution out of its native range was facilitated by human activities such as decoration in gardens, trade and transportation (Villamagna, 2009). The free-floating invasive water hyacinth is native in Brazil and South America; it has a massive reproductive output and rapid growth in polluted water bodies due to lack of natural enemies (Khatri et al., 2018). Due to the rapid proliferation capacity of water hyacinth, its number could be doubled within a week at high temperature (25°C-35°C) (Gunnarsson & Petersen, 2007; Villamagna, 2009). The invasion of water hyacinth in freshwater ecosystem is one of the major causes for the degradation of aquatic ecosystem, deterioration of water quality and the decline of native species (Hailu et al., 2020; Villamagna, 2009; Villamagna et al., 2012). Furthermore, the water hyacinth causes serious ecological and socio-economic problems including water quality degradation and difficulties in recreational activities like boating, swimming and water transport, and obstructing in fisheries as well as agricultures (Assefa & Yigermal, 2019; Njiru et al., 2007; Villamagna & Murphy, 2010).

The decreased rate of photosynthesis by the phytoplankton under the dense mat of water hyacinth results in a decrease in dissolved oxygen and an increase in water temperature (Mangas-Ramírez & Elías-Gutiérrez, 2004; Mironga et al., 2012; Villamagna, 2009). Consequently, the aquatic system changes to anoxic conditions with increasing the concentration of ammonia, iron, manganese and sulphide, and change the whole aquatic ecosystem in terms of structure and function (Yongo & Outa, 2015). However, the varying length and complex structure of root of invasive water hyacinth (Gopal, 1987) forms the dense mats of interlocking roots and provides a novel habitat heterogeneity for many epiphytic macro-invertebrates such as amphipods (Rocha-Ramírez et al., 2007; Toft et al., 2003), snails and arachnids (Brendonck et al., 2003). Furthermore, the macro-invertebrates like insects, crustaceans and fish get benefited from the mat of water hyacinth as a nursery ground (Brendonck et al., 2003; Villamagna, 2009). Bailey and Litterick (1993) reported a higher density of macro-invertebrates within six metres of open water from the water hyacinth edge other than that of the non-vegetated habitat. Similarly, more abundant and diverse macro-invertebrates were reported from the water hyacinth edge than that of the rooted emergent vegetation in Lake Victoria (Masifwa et al., 2001). Further, the habitat complexity may be altered due to the introduction of invasive species in the freshwater lakes and streams resulting in a shift in the macro-invertebrate community structure (Wahl et al., 2021). Moreover, the larger lakes of LCPV (Phewa, Begnas and Rupa) are exceedingly invaded by the water hyacinth and had adverse effects on the occurrence and abundance of threatened water birds (Basaula et al., 2021). Similar effects can be found in other faunal species including the macro-invertebrates in LCPV due to a presence of the water hyacinth. The macro-invertebrates are one of the major components in the ecological process to transfer energy from the detritus to consumers. However, little knowledge is available on the invasion effect of water hyacinth on diversity and abundance of macro-invertebrates in LCPV; therefore, this study aimed to explore the invasion effects of water hyacinth on the diversity and abundance of macro-invertebrates in LCPV, which have provided the baseline data for developing a plan.

MATERIALS AND METHODS

Study Area

Figure 1
The Lake Cluster of Pokhara Valley



Pokhara, the largest and speedily urbanizing Metropolitan City of Nepal, is located in Kaski District of Gandaki Province in the watershed of Seti River. The area of LCPV is expanded from Pokhara Metropolitan City to a small portion of Rupa and Annapurna Village Municipalities. It consists of three large lakes (Phewa, Begnas and Rupa) and six small lakes (Kamal Pokhari, Gunde, Khaste, Niureni, Dipang and Maidi) (Figure 1). On 2 February 2016, the LCPV was listed as the tenth Ramsar site with an area of 262 square kilometres, including 9 squqre kilometres of the water body, which is the largest Ramsar site of Nepal. Among these lakes, the Phewa lake, the largest lake of the cluster, fluctuates between mesotrophic and eutrophic while the Begnas lake, the second-largest lake of the cluster, fluctuates between oligotrophic and mesotrophic, and the remaining are eutrophic lakes (MoFE, 2018). The LCPV is regarded as the hotspot of biodiversity; its southern part is covered with sub-tropical to the tropical broad-leaved forest of Sal (*Shorea robusta*) whereas the northern and western parts are covered with the forest of Chilaune-katus (*Schima-Castanopsis*). More than 360 species (plants), 32 species (mammals), 140 species (birds), 24 species (reptiles), 27 species (fish) and 11 species (amphibians) are found in LCPV (MoFE, 2018). The globally threatened fauna such as clouded leopard (*Neofelis nebulosa*) and leopard (*Panthera pardus*), yellow-breasted bunting (*Emberiza aureola*), baer's pochard (*Aythya baeri*), common pochard (*Aythya ferina*), woolly necked stork (*Ciconia episcopus*) and ferruginous pochard (*Aythya nyroca*) were reported from LCPV (MoFE, 2018; Basaula et al., 2021). In addition, some invasive animals and plants are found in LCPV. For examples, tilapia (*Tilapia nilotica*), African cuttlefish (*Clarias gairiepinus*), giant African land snail (*Achatina fulica*) parthenium (*Parthenium hysterophorus*), mikania (*Mikania micrantha*), water hyacinth, cut grass and water lettuce (MoFE, 2018; Adhikari et al., 2020).

Methods

A total of 24 sampling plots, each of 50 m × 50 m, were established based on the size of the lakes and the presence of water hyacinth (HP) or absence of water hyacinth (HA) areas in the lakes of LCPV. Three HP and three HA plots were established in the larger lakes (Phewa, Begnas and Rupa) and a single HA plot was established in each of the smaller lakes. During the study period, the water hyacinth coverage percentage in HP areas was > 90%, but the water hyacinth was completely absent in HA areas in the past ten years, which was confirmed through the personal communication with the members of fishing community and lake management committees. The distance between HP and HA plots in large lakes was ≥500 m. The coordinates of each plot were recorded using GPS (Garmin eTrex Touch 35). The data collection was carried out seasonally during the autumn 2019 (September to November), the winter 2020 (December to February), the spring 2020 (March to May) and the summer 2020 (June to August).

Macro-Invertebrates Survey

Each 50 m × 50 m plot was again divided into 1 m × 1 m of sub-plot for the study of macro-invertebrates. Five sub-plots (four at the corners and one at the center) were selected from each plot to collect the macro-invertebrates. Peterson's Grab Sampler (0.0289 m) was used to collect the samples from each sub-plot. All the contents of Grab samples were collected in the polythene bags and were screened by using a standard sieve of 40 mesh size/inch. The water hyacinth plants from each subplot were taken out and the epiphytic macro-invertebrates found in stem and leaf were manually picked up using entomological forceps and brush, but the root was combed in a bucket containing 70% ethanol. The sieved samples and manually picked samples were transferred to plastic bottles containing 5% formalin and kept the details of sampling site and date following Toft et al. (2003). The samples of macro-invertebrates were taken to the laboratory and poured into a white enamel tray. All the macro-invertebrate taxa were identified up to possible taxonomic level (species) following the taxonomic manuals (Budha, 2016; Edmondson, 1959; Macan, 1977; Pennak, 1953; Subramanian & Sivaramakrishnan, 2007; Tonapi, 1980). All the individuals were counted from each sub-plot and its average was calculated for analysis. The abundance of macro-invertebrate taxa was expressed as individuals per square meter.

The Parameters of Water

The water samples were collected from a 1m × 1m sub-plot (four from the edge and one from the center of each plot). A measuring tape was used to measure the depth (m) and then Sechhi disc of 20 cm diameter was used to measure the transparency (cm). The disc was immersed from the water surface downward till it disappeared and the depth was noted. Then, the disc was lifted upward till it reappeared and again the depth was noted. The water temperature of the lake was measured in the field using a standard mercury thermometer graduated as 0-50ºC, having a precision of 0.1ºC. The pH was measured using (4500- B, APHA), total dissolved solids (mg/l) were measured

(Instrumental) and turbidity (NTU) was measured using (3130 B, APHA). In addition, the titration method APHA (1998) was used to measure the dissolved oxygen (mg/l), free carbon dioxide (mg/l), total alkalinity (mg/l) and Nitrate (mg/l).

Data Analysis

Diversity indices (H'), species evenness (J) and species richness (S) of macro-invertebrates were calculated following Shannon-Wiener et al. (1949) and Pielou (1966). Our data were not normally distributed. We compared the abundance of macro-invertebrates between HP and HA areas using Mann–Whitney U test. The association between water hyacinth coverage with physico-chemical parameters was measured. A simple linear regression was performed between macro-invertebrate abundance and species richness with dissolved oxygen, free carbon dioxide and water hyacinth coverage percentage. In addition, a multivariate ordination analysis was used between biological variables (most abundant 14 macro-invertebrate taxa) and environmental variables (physico-chemical parameters and water hyacinth coverage). Detrended Correspondence Analysis (DCA) was applied to find whether our data support for Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA). Our data supported RDA because the lengths of all four axes were less than two in DCA (Yang et al., 2020) to identify the variation in the dominant macro-invertebrates compared with the environmental variables. Moreover, Monte-Carlo permutation test under the reduced model of 999 permutations at (α = 0.05) (Powell, 2019) was used to perform the significance testing of ordination. The function decorana was used for DCA and the function rda was used for RDA in the vegan package of R statistical software. The data analysis was carried out using program R (R Core Team, 2020).

RESULTS

Abundance and species diversity of macro-invertebrates

The researchers identified 29 species and 26 genera of macro-invertebrates belonging to 21 families, 15 orders and three phyla (Annelida, Arthropoda and Mollusca) during the study period in LCPV (Table 1 and Table 2). The highest relative abundance of macro-invertebrates 16.47% (n=4644) was found in HP habitat in autumn 2019 and 16.25% (n=4583) in HA habitat in the spring 2020. Similarly, the lowest relative abundance 9.85% (n=2776) was found in HP habitat and 10.57% (n=2981) in HA habitat during the winter 2020 (Table 1). The highest mean abundance of macro-invertebrates (102.35±7.04) was recorded in bottom sediments, followed by (50.38±3.67) in the root of water hyacinth and the lowest abundance (7.47±0.33) was in the stem of water hyacinth and sand (Figure 2).

Table 1

Seasonal abundance of Macro-Invertebrates in Water Hyacinth Presence Habitat (HP) and Water Hyacinth Absence Habitat (HA) of Lake Cluster of Pokhara Valley between 2019 and 2020.

Seasons	HP	Relative abundance%	HA	Relative abundance%
Autumn 2019	4644	16.47	3889	13.79
Winter 2020	2776	9.85	2981	10.57
Spring 2020	3860	13.69	4583	16.25
Summer 2020	3006	10.66	2457	8.71

Figure 2

Mean Abundance of Macro-Invertebrates in Different Habitats of Lake Cluster of

Pokhara Valley between 2019 and 2020

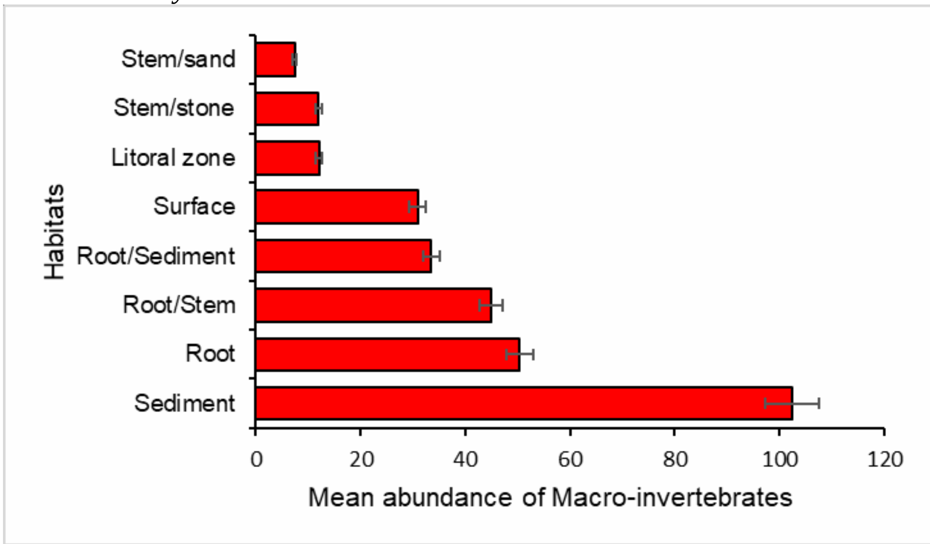


Table 2

Mean Abundance ± Standard Error of the Macro-Invertebrates Recorded in Water Hyacinth Presence Habitat (HP) and Water Hyacinth Absence Habitat (HA) of Lake Cluster of Pokhara Valley between 2019 and 2020

SN	order	Family	Scientific name	Mean ± SE (HP)	Mean ± SE (HA)
1	Haplotaxida	Tubificidae	*Limnodrilus hoffmeisteri* Claparède, 1862	11.42±1.15	20.93±2.41
2			*Branchiura sowerbi* Beddard, 1892	13.25±1.47	21.27±2.14
3		Naididae	*Tubifex tubifex* Mueller, 1774	5.61±0.94	16.95±1.75
4			*Stylaria lacustris* Linnaeus, 1767	5.83±0.55	13.47±1.72
5	Hirudinida	Hirudinidae	*Hirudinaria granulosa* Lucknow, 1941	2.64±0.4	2.75±0.59
6			*Hirudo medicinalis* Linnaeus, 1758	5.33±0.67	3.55±0.45
7	Rhynchobdellida	Glossiphonidae	*Helobdella stagnalis* Linnaeus, 1758	6.81±0.97	2.95±0.47
8			*Placobdella parsitica*S ay, 1824	5.36±0.77	2.18±0.4
9	Diptera	Chironomidae	*Chironomus* sp. Meigen, 1803	21.69±4.44	37.52±4.07
10	Ephemeroptera	Leptophlebiidae	*Leptophlebia marginata* Linnaeus, 1767	39.92±2.95	11.75±0.92
11	Odonata	Libellulidae	*Diplacodes* sp. Rambur, 1842	13.67±0.98	4.85±0.83
12			*Brachythemis contaminate* Fabricius, 1793	15.69±0.98	4.23±0.71
13		Gomphidae	*Gomphidia* sp. Selys, 1854	10.31±0.8	4.03±0.68
14		Hemiphlebiidae	*Schnura heterosticta* Burmeister, 1842	15.75±1.09	8.77±0.69
15	Hemiptera	Gerridae	*Aquarius remiges* Say, 1824	31.81±2.19	30.3±1.92
16		Nepidae	*Nepa cinerea* Linnaeus, 1758	0.08±0.08	1.67±0.48
17	Coleoptera	Dytiscidae	*Thermonectus* sp. Dejean,1833	72.69±4.79	16.67±1.66
18	Araneae	Pisauridae	*Dolomedes tenebrosus* Hentz, 1844	2.08±1.07	0.25±0.1
19	Decapoda	Paleomonidae	*Paleomon* Weber, 1795	1.89±0.56	0.53±0.23
20	Sphaeriida	Sphaeriidae	*Sphaerium* sp. Scopoli, 1777	64.06±3.53	15.2±1.99
21	Acroloxoidea	Acroloxidae	*Acroloxus lacustris* Linnaeus, 1767	12.64±1.03	2.03±0.49
22	Unionoida	Unionidae	*Lamellidens* sp. Simpson, 1900	3.08±0.76	2.3±0.46
23		Veneridae	*Radiatula* sp. Simpson, 1900	0.75±0.37	1.17±0.35

24	Basommatophora	Planorbidae	*Helisoma* sp. Brown, 1967	1.64±0.91	
25			*Segmentina* sp. Fleming, 1817	0.97±0.35	0.28±0.12
26	Caenogastropoda	Thiaridae	*Thiara requeti* Grateloup, 1840	1.47±0.86	
27			*Thiara tuberculata* Mueller, 1774	10.97±1.52	2.05±0.35
28			*Thiara granifera* Lamarck, 1822	6±1.51	1.25±0.41
29		Viviparoidae	*Bellamya bengalensis* Lamarck, 1822	13.42±1.39	2.93±0.42

The highest mean abundance for *Chironomus* sp. (37.52±4.07) was recorded in the study and was followed by *Aquarius remiges* (30.30±1.92) in the HA habitat whereas the lowest abundance was found for *Dolomedes tenebrosus* (0.25±0.10). Similarly, the highest mean abundance for *Thermonectus* sp. (72.69±4.79) was recorded in the HP habitat, followed by *Sphaerium* sp. (64.06±3.53) whereas the lowest abundance was found for *Nepa cenerea* (0.08±0.08) (Table 2). The abundance of macro-invertebrate was varied between HP and HA habitats (Ranged: HP: 177 to 666; HA: 46 to 483, p <0.001). The abundance of orders ephemeroptera, odonata, coleoptera, sphaeriida and caenogastropoda was significantly higher in the HP habitats whereas orders haplotaxida and diptera were lower in the HA habitat (Table 3).

Table 3

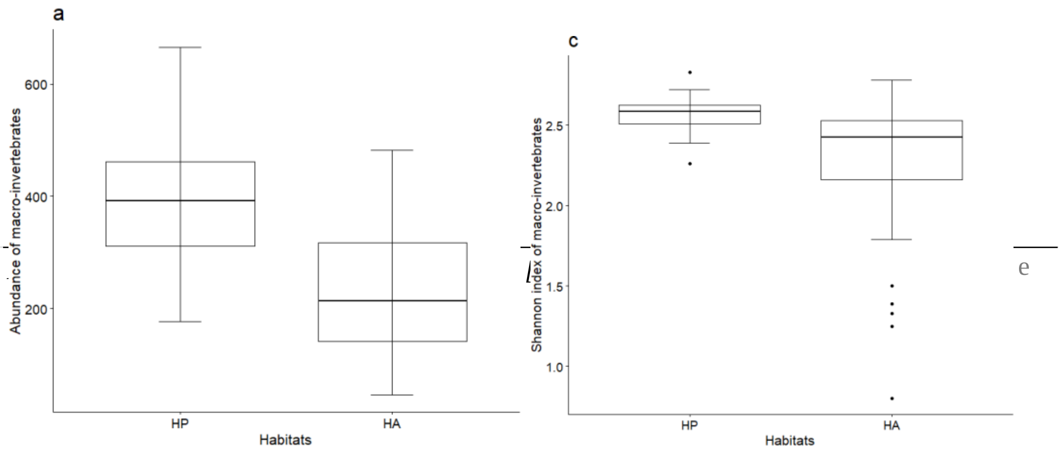
Comparison of Different Variables in Water Hyacinth Presence Habitat (HP) and Water Hyacinth Absence Habitat (HA) of Lake Cluster of Pokhara Valley between 2019 and 2020

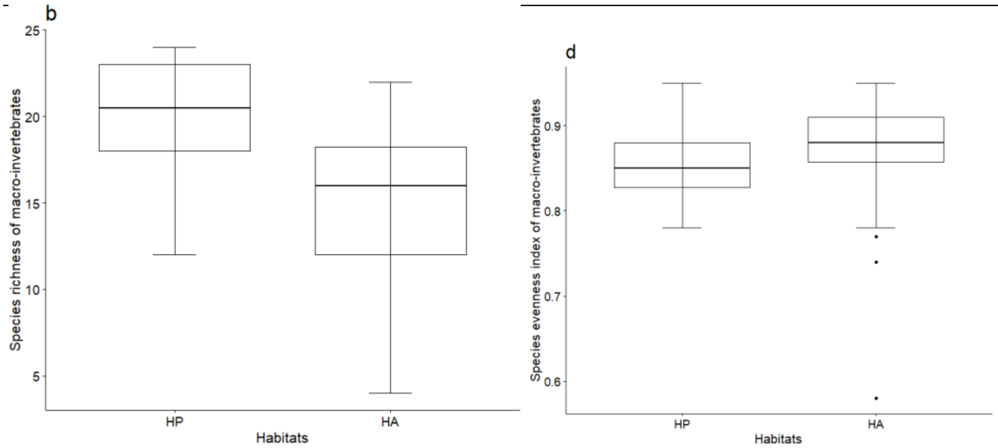
Variables	HP habitat	HA habitat	Statistics
Abundance	Median = 392	Median = 231	Mann Whitney test, U = 358, p = <**0.001**
Orders			
Haplotaxida	Median = 27.5	Median = 66	Mann Whitney test, U = 1390, p = **0.01**
Diptera	Median = 10.5	Median = 26.5	Mann Whitney test, U = 1499.5, p = **0.001**
Ephemeroptera	Median = 37	Median = 11	Mann Whitney test, U = 92, p = <**0.001**
Odonata	Median = 52.5	Median = 13	Mann Whitney test, U = 263.5, p =<**0.001**
Coleoptera	Median = 68.5	Median = 18	Mann Whitney test, U = 65.5, p = <**0.001**
Sphaeriida	Median = 60.5	Median = 14	Mann Whitney test, U = 64.5, p = <**0.001**
Caenogastropoda	Median = 23.5	Median = 5	Mann Whitney test, U = 110, p = <**0.001**

Macro-invertebrate diversity and species richness were greater in the HP habitat (H' = 2.58; S = 24) than the HA habitat (H' = 2.42; S = 22). Greater species evenness was found in the HA habitat (J = 0.87) than in the HP habitat (J = 0.85) (Table 3, Figure 3).

Figure 3

Abundance and Species Diversity of Macro-Invertebrates in HP and HA Areas in Lake Cluster of Pokhara Valley between 2019 and 2020. HP=Water Hyacinth Presence Habitat and HA=Water Hyacinth Absence Habitats





Relation of water hyacinth with physico-chemical parameters

The water hyacinth coverage had a significant moderate negative correlation with depth, transparency, pH, dissolved oxygen and nitrate ($r = -0.51$, $r = -0.73$, $r = -0.63$, $r = -0.77$ and $r = -0.63$, respectively, $p < 0.001$) while it had a significant positive correlation with the water temperature and free carbon dioxide ($r = 0.86$ and $r = 0.84$, respectively, $p < 0.001$) (Table 4).

Table 4

Correlation Matrix among the Environmental Variables of Lake Cluster of Pokhara Valley between 2019 and 2020, Bold Values Represent Significant (P < 0.05)

Variables	Depth	Temp	Transp	pH	DO	Free CO_2	Total Alk	Nitrate	Whcov
Depth	1								
Temp.	-0.62	1							
Transp.	**0.82**	-0.76	1						
pH	0.68	-0.64	0.75	1					
DO	0.69	**-0.80**	0.78	0.61	1				
Free CO_2	-0.78	**0.80**	**-0.93**	**-0.81**	-0.74	1			
Total Alk	0.30	-0.30	0.30	0.48	0.27	-0.40	1		
Nitrate	0.45	-0.67	0.61	0.58	0.66	-0.61	0.51	1	
Whcov	**-0.51**	**0.86**	**-0.73**	**-0.63**	**-0.77**	**0.84**	-0.46	**-0.63**	1

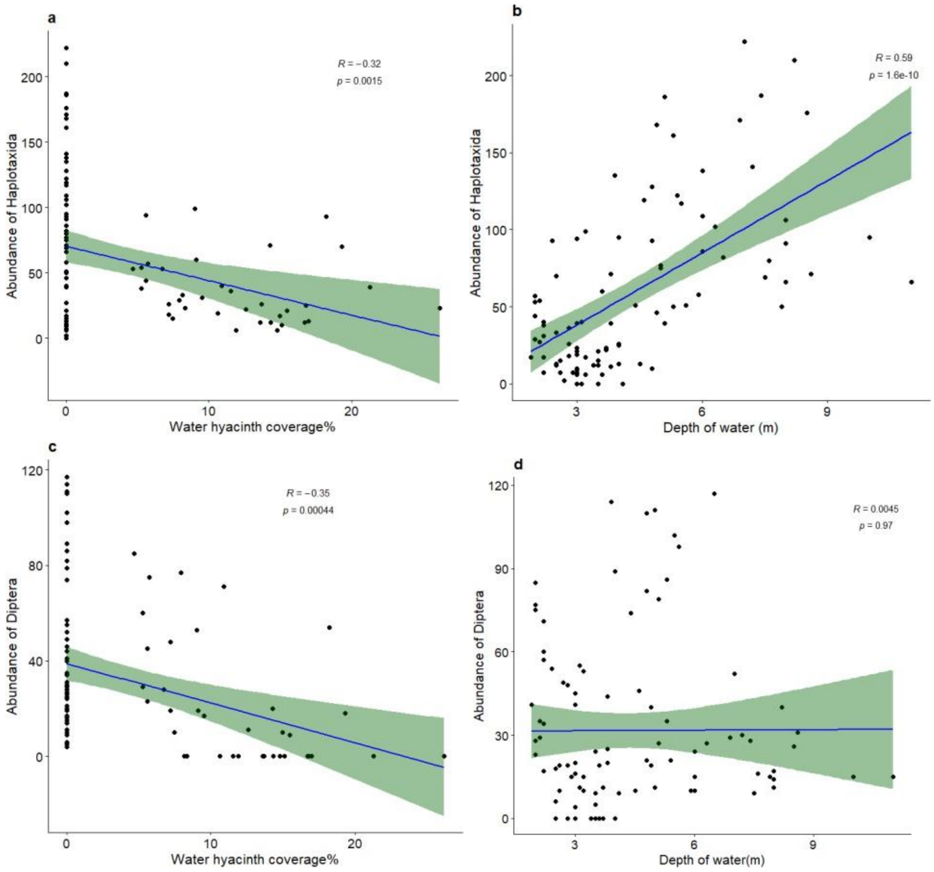
*Temp. = Water temperature, Transp. = Transparency, Alk. = Alkalinity and Whcov = Water hyacinth coverage.

Correlation of Macro-Invertebrates with Environmental Variables

The correlation of order haplotaxida's abundance with the water hyacinth coverage was negative and significant ($r = -0.32$, $p < 0.001$) whereas positive and significant with depth ($r = 0.59$, $p < 0.001$). Similarly, the abundance of order diptera was negative, but significantly correlated with the water hyacinth coverage ($r = -0.3$, $p < 0.001$) and positively correlated with depth ($r = 0.0045$, $p = 0.09$) (Figure 4). For other taxa, the researchers performed RDA analysis.

Figure 4

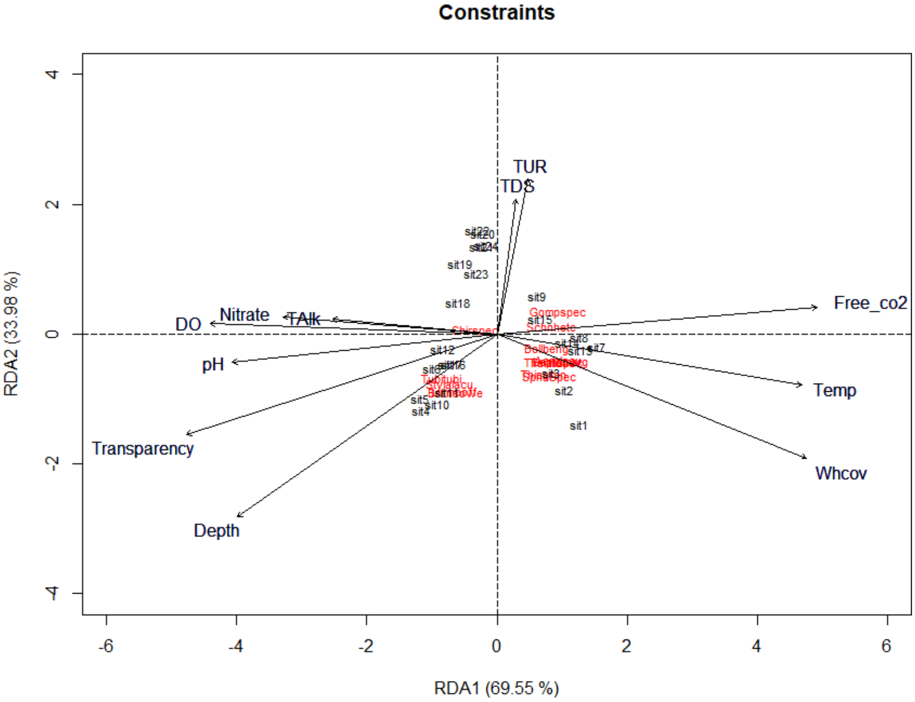
Abundance of Haplotaxida and Diptera with Water Hyacinth Coverage and Depth of Water



The temperature and free carbon dioxide were closely associated with the water hyacinth coverage whereas other variables were negatively correlated (Figure 5). Similarly, the macro-invertebrates such as *Leptophlebia marginata*, *Gomphidia* sp., *Schnurahete rosticta*, *Thermonectus* sp. and *Sphaerium* sp. were closely associated with the water hyacinth coverage, but *Limnodrilus hoffmeisteri*, *Branchiura sowerbi*, *Tubifex tubifex*, *Stylariala custris* and *Chironomus* sp. were negatively associated (Figure 5). The canonical axes of RDA analysis explained the variance in the macro-invertebrates and environmental variables interaction (F = 5.83, p = 0.001), and the first and second axis accounted for 69.55% and 33.98%, respectively (Figure 5).

Figure 5

RDA of Macro-Invertebrates after Constraining Variation by Environmental Variables



DISCUSSION

The present study identified the effect of water hyacinth invasion on the diversity and abundance of macro-invertebrates in the Lake Cluster of Pokhara valley. Marginally, more macro-invertebrates and higher abundance were recorded in the water hyacinth presence habitat than the absence habitat. Higher diversity of maco- invertebrates in the HP habitat might be due to the presence of substrate structures such as roots, stem and leaves (da Silva & Henry, 2020; Hansen et al., 1971; O'Hara, 1967; Toft et al., 2003; Villamagna & Murphy, 2010). The diversity and abundance of macro- invertebrates were varied according to the season in the HP and HA habitat. In the winter season, the low abundance of macro-invertebrates in the study area might be due to low water temperature, which will not be suitable for the seasonal invertebrates especially for mayfly and other ephemeroptera. The wetland vegetation along with the water hyacinth start to grow during the summer and become dense during late summer and autumn, and provide suitable habitats for the macro-invertebrates. Therefore, the researchers assumed abundant macro-invertebrates and water hyacinth coverage in the autumn season in the study area, which have a positive relationship. Not only in the study area, the macro-invertebrates and water hyacinth had a positive relation in the reservoir of the western part of Ecuador (Thi Nguyen et al., 2015). At the time of higher abundance of water hyacinth, the body structure of plants becomes flat, cover a larger area and provides a shelter for more species such as larvae of mayfly, dragonfly and diving beetles (Villamagna, 2009); therefore, the researchers assume abundant macro-invertebrates in the summer and autumn seasons. The morphological characteristics and structural complexity of plants determine the abundance of macro-invertebrates (Villamagna, 2009; da Silva and Henry, 2020). The root and leaves of floating water hyacinth offer the complex habitat for the colonization of macro-invertebrates like snails, arachnids and amphipods (Brendonck et al., 2003; Toft et al., 2003; Villamagna, 2009). The greater abundance of macro-invertebrates including mayfly, arachnids and snails in the HP areas might be due to the presence of water hyacinth for providing spaces. However, an abundance of macro-invertebrates was reported higher in the artificially constructed HA habitats than the HP habitats (Coetzee et al. 2014). It might be due to the substrate variation and age of habitats.

The higher abundance of omnivore and insectivore waterbirds in the HP habitat of LCPV was reported by Basaula et al. (2021), which could be due to the higher abundance of macro-invertebrates in the HP areas of LCPV. However, the abundance of macro-invertebrates from two orders haplotaxida (*Limnodrilus hoffmeisteri, Branchiura sowerbi, Tubifex tubifex, Stylaria lacustris*) and diptera (*Chironomous sp*.) were found significantly lower in the HP habitats than in the HA habitats. The abundance of haplotaxida and diptera decreased with the increasing water hyacinth coverage, indicating that the water hyacinth has negative effects on the macro-invertebrates in LCPV. The taxa in these orders have a high tolerance to the toxic substances (Mandaville, 2002). The higher abundance of haplotaxida and diptera in the HA habitat at LCPV was found in higher depth in the large lakes and the bottom sediments of small lakes where decomposed/sediment materials were found. The water hyacinth is floating and buoyant and move from one location to another, and their deposition of parts may not be in the same location of lake. Additionally, oligochaetes and chironomid larvae were abundantly found in the HA habitats, having a higher decomposition of organic materials, which were high tolerance taxa (Shah et al., 2011). Furthermore, the HP habitat was more diversified than the HA habitat in LCPV. In this way, all three indices were consistently higher at the HP habitats (Brendon et al., 2003). Moreover, these studies supported that the water hyacinth can provide a suitable and novel habitat for the macro-invertebrates when it is in a fragmented patch in waterbodies.

The analysis of physico-chemical parameters revealed that all the parameters were found significantly different in the HP and HA habitats except water temperature and turbidity; however, it was also slightly more in the HP habitats (Basaula et al., 2021). This slight increase in temperature in the HP habitats might be due to the prevention of sunlight penetration by the mats of water hyacinth. Consequently, the rate of photosynthesis will be decreased below the dense mat of water hyacinth by the phytoplankton, resulting a decrease in dissolved oxygen and an increase in water temperature (Mangas-Ramírez & Elías-Gutiérrez, 2004; Mironga et al., 2012; Villamagna, 2009). The pH of the water was found higher in the HA habitat in all seasons, which indicated that the water hyacinth makes the habitat slightly acidic (Dersseh et al., 2019; Mironga et al., 2012) probably due to higher production of free carbon dioxide in the HP habitat. The eutrophic level of the lake is increased due to the secondary pollution and due to the decay of dead water hyacinth, and finally it may cause the serious problems in the lake ecosystem (Chen et al., 2021). Consequently, the aquatic system changes to anoxic conditions with increasing the concentration of ammonia, iron, manganese and sulphide, which change the whole aquatic ecosystem in terms of structure and function (Yongo & Outa, 2015). A depth of water was recorded higher in the HA habitat than in the HP habitat because the water hyacinth cannot grow in the water more than six-meter depth (Dersseh et al., 2019). The water hyacinth can absorb the nutrients including nitrates from the water body (Masifwa et al., 2001; Villamagna et al., 2010), and therefore the low content of nitrate was found in the HP habitat than the HA habitat. The low value of dissolved oxygen in the HP habitat with high free carbon dioxide might be due to the metabolic activities of the epiphytic organism (Masifwa et al., 2001; Villamagna et al., 2010). The correlation matrix showed that the water temperature and free carbon dioxide increased with the increase of water hyacinth coverage, but it was opposite to depth, transparency, dissolved oxygen, etc. The multivariate RDA ordination showed that the species like *Leptophlebia marginata*, *Gomphidia* sp., *Schnurahete rosticta, Thermonectus* sp. and *Sphaerium* sp. were found as dominant species in the water HP habitats and these species were closely associated with the water hyacinth coverage percentage, free carbon dioxide and water temperature, but the species like *Limnodrilus hoffmeisteri, Branchiura sowerbi, Tubifex tubifex, Stylaria lacustris* and *Chironomus* sp. were found as dominant species in the HA habitats.

CONCLUSION

In conclusion, the variation on the diversity and abundance of macro-invertebrates in LCPV between the HP and HA habitat is mainly due to changes in the structural composition and physico-chemical properties. The increased in water temperature and carbon dioxixe with decreased dissolved oxygen in the increased water hyacinth coverage indicates the probability of macro-invertebrate variation, which was reported in this study. The water hyacinth supports the occurrence of some species with higher abundences while it has negative effects on others including haplotaxida and diptera. The consequences of increased water hyacinth coverage is noticed in some water birds. Therefore, based on these findings, the researchers recommended multidisciplinary research approaches in the field of effects of invasive water hyacinth and other weeds on the structure and function of aquatic ecosystem, and implementation of an integrated approach to managing the water hyacinth to conserve aquatic biodiversity in LCPV.

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Effects of invasive water hyacinth on fish diversity and abundance in the Lake Cluster of Pokhara Valley, Nepal

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ABSTRACT

Water hyacinth (*Pontederia crassipes*) invasion is a growing global issue which poses a threat to aquatic life. As in the global context, the water hyacinth invasion is increasing rapidly in the lakes of Pokhara valley which is listed in Ramsar sites. Its effect on aquatic life is little known. This study examined the effects of water hyacinth invasion on the diversity and abundance of fishes in lakes of Pokhara Valley. We estimated the seasonal fish diversity and abundance in water hyacinth presence (HP) and absence (HA) habitats between 2019 and 2020. The study found that water hyacinth invasion has a negative impact on fish diversity and abundance in the lakes of Pokhara Valley. Twenty fish species were recorded, with family Cyprinidae being the most abundant. Fish abundance was higher in the winter season and in habitats with water hyacinth presence. The invasive *Oreochromis niloticus* was the most abundant fish in both habitats, but more so in habitats with water hyacinth presence. The exotic fish species *Ctenopharyngodon idella* had greater abundance in habitats with water hyacinth presence while *Ciprinus carpio* had greater abundance in habitats without water hyacinth. Exotic fish species abundance was positively correlated with water hyacinth coverage. The findings suggest that water hyacinth invasion negatively impacts native fish species, with the diversity of native species being low in habitats with water hyacinth presence. The study recommends regular stock enhancement programs for native fish species, control and management of invasive weeds, and further study to support the conservation of native fisheries resources and the livelihood of the fishery-dependent community.

1. Introduction

The biotic composition of an ecosystem is determined by the community structure of flora and fauna (Jewel et al., 2018). Among the fauna the fish are one of the major components of aquatic ecosystem, and there is over 35,100 species globally (Froese and Pauly, 2023). Nepal has a rich freshwater resource with varying altitudes, supporting a wide range of fish species and about 230 species of fish are found in these water resources (Rajbanshi, 2002; Shrestha, 2019; Khatri et al., 2020). The occurrence and abundance of fish species

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is influenced by physical and chemical parameters, habitat connectivity and can be negatively impacted by invasive aquatic weeds (Jin et al., 2019; Mamilov et al., 2021; Virgilio et al., 2022). In addition, the aquatic ecosystem is also severely affected by the aquatic weed invasion (Mailu, 2001; Villamagna, 2009; Ongore et al., 2018). Their impacts are noticed on the deterioration of water quality, increased evapotranspiration, enhanced flooding, siltation and loss of native species (Mailu, 2001; Ongore et al., 2018). Water hyacinth (*Pontederia crassipes*) is one of the 27 invasive weed species in Nepal and has been found to threaten the ecosystem and livelihood of people by reducing fish catch, causing transportation problems, and deteriorating water quality (Mailu, 2001; Kateregga and Sterner, 2009; Villamagna, 2009; Yongo and Outa, 2015; Asmare, 2017; Ongore et al., 2018; Shrestha and Shrestha, 2021).

In Nepal, the Lake Cluster of Pokhara Valley (LCPV) encompasses nine ecologically significant lakes and is recognized as a major Ramsar site (MoEF, 2018; Paudel et al., 2018). The lakes of LCPV are plagued by five invasive aquatic plant species, with water hyacinth being the most dominant (Pathak et al., 2021). This invasive species has had a detrimental impact on the abundance of globally threatened water birds in the LCPV (Basaula et al., 2021). The lakes of Pokhara are home to 34 fish species, including 27 native and seven exotic species (Timilsina et al., 2019). Among these species are numerous threatened species, such as sahar (*Tor putitora*), falame sahar (*Tor tor*), katlae (*Neolissochilus hexagonolepi*), rewa (*Chagunius chagunio*) and bhitti (*Danio devario)* (Husen and Sherpa, 2017). However, the impact of water hyacinth invasion on the diversity and abundance of ichthyofauna in the LCPV remains unstudied. To develop an effective conservation and management plan for the Ramsar site lakes of LCPV, it is crucial to gather information on the fish assemblage in freshwater systems. Thus, this study aimed to investigate the effect of the invasive water hyacinth on the diversity and abundance of fish in the lakes of LCPV, providing baseline data for future conservation efforts.

2. Materials and methods

2.1. Study area

The study was conducted in the Lake Cluster of Pokhara Valley (LCPV) in Nepal, which is situated in the Kaski district of Gandaki Province. The LCPV is comprised of nine lakes, including three major lakes (Phewa, Begnas, and Rupa) and six smaller lakes (Kamal Pokhari, Gunde, Khaste, Niureni, Dipang, and Maidi; Fig. 1). On February 2, 2016, it was designated as the 10th Ramsar Site of international importance due to its rich biodiversity, including more than 360 plant species, 32 mammal species, 140 bird species, 24 reptile species, 27 fish species, and 11 amphibian species. The LCPV is also home to four globally threatened waterbird species and a

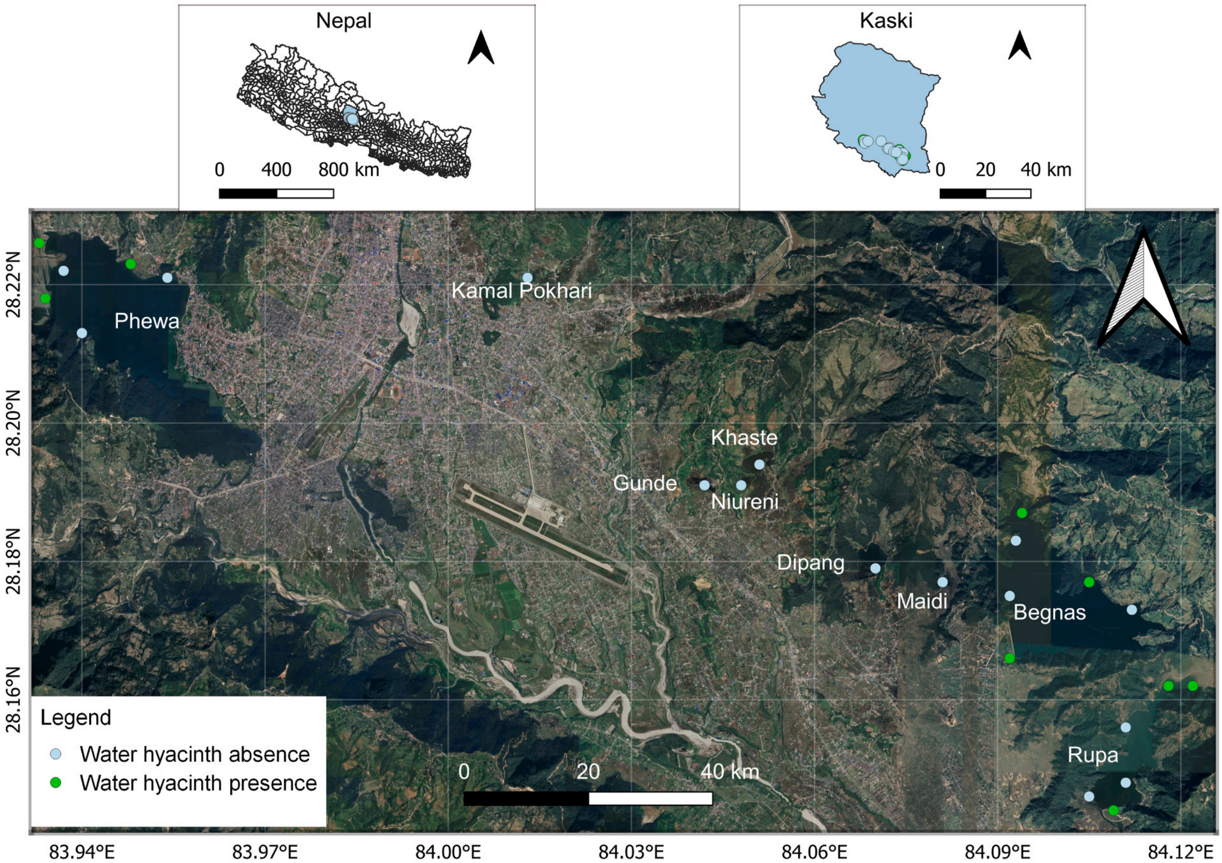

Fig. 1. Lake Cluster of Pokhara Valley, Nepal.

number of invasive plants and animals. The lakes in the LCPV vary in terms of trophic level, with Phewa lake fluctuating between mesotrophic and eutrophic, Begnas lake fluctuating between oligotrophic and mesotrophic, and the rest being eutrophic lakes (MoEF, 2018). Additionally, globally threatened waterbird species such as the Baer's pochard (*Aythya baeri*), common pochard (*Aythya ferina*), ferruginous pochard (*Aythya nyroca*), and woolly necked stork (*Ciconia episcopus*) have been reported in the LCPV (Basaula et al., 2021). The presence of invasive species, including tilapia (*Oreochromis nilotica*), African cuttlefish (*Clarias gairiepinus*), giant African land snail (*Achatina fulica*), parthenium (*Parthenium hysterophorus*), mikania (*Mikania micrantha*), water hyacinth, cut grass (*Leersia hexandra*), water lettuce (*Pistia stratiotes*), alligator weed (*Alternanthera philoxeroides*), and bush morning glory (*Ipomoea carnea* subsp. *fistulosa*) has also been documented (Adhikari et al., 2020; MoEF, 2018; Pathak et al., 2021).

2.2. Methods

The study was conducted in the water hyacinth presence and absence habitats of the lakes of LCPV. We demarcated habitats water hyacinth presence and absence based on the occurrence of water hyacinth. Only three lakes, such as Phewa, Begnas, and Rupa had water hyacinth. On the other hand, the absence of water hyacinth (HA) was confirmed through the lake management committees, where the water hyacinth was totally absent in past 10 years and was verified through field study. Three 50 m x 50 m sampling plots were established in each of the HP and HA habitats of the large lakes (Phewa, Begnas, and Rupa), and a single HA plot was established in the smaller lakes, resulting in a total of 24 sampling plots. The coverage of water hyacinth in each HP plot was calculated using the measuring tape. The distance between the HP and HA plots in the large lakes was recorded as being greater than or equal to 500 m. The coordinates of each plot were recorded using a GPS device (Garmin eTrex Touch 35).

We conducted a seasonal survey of fish during Autumn 2019 (September to November), Winter 2020 (December to February), Spring 2020 (March to May), and Summer 2020 (June to August) utilizing experienced local fishermen and rectangular gill-net enclosures of 20 mm mesh size (1 m x 50 m) deployed overnight. The collected fish were identified using reference keys or expert assistance and following the taxonomic monographs (Shrestha, 2019). The total species number and total individuals of fish from each species per catch were recorded.

During the study period, the physicochemical parameters of water were recorded in each plot. This included depth, transparency, temperature, pH, total dissolved solids (TDS), turbidity, dissolved oxygen (DO), free carbon dioxide (CO2), total alkalinity and nitrate (NO_3). Five subplots of 1 m × 1 m were established in each plot, four at the edge and one in the center, to collect water samples. The depth and transparency of water were measured using a measuring tape and a 20-cm diameter Secchi disk, respectively. The surface water temperature was recorded using a standard thermometer with 0.1 °C precision. The pH, TDS, and turbidity were measured following the methods outlined in the American Public Health Association's Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Dissolved oxygen was measured using Winkler's methods, and free carbon dioxide, total alkalinity, and nitrate were measured via the titrimetric method. All the physicochemical parameter measurements were performed in the Laboratory of the Department of Zoology at Prithvi Narayan Campus and the Federal Water Supply and Sewerage Management Project in Pokhara. In addition, we established a 1 m X 1 m plot, four at the corners and one at the centre of 50 m X 50 m plots and measured the percent coverage by water hyacinth and averaged it for that plot.

Table 1
The fish species recorded in Lake cluster of Pokhara Valley of Nepal between 2019 and 2020.

Orders	Family	Scientific name	Common name	IUCN Status
Cypriniformes	Cyprinidae	*Puntius sophore* Hamilton, 1822	Bhitta	LC
Cypriniformes	Cyprinidae	*Puntius chola* Hamilton, 1822	Bhurluk	LC
Cypriniformes	Cyprinidae	*Danio devario* Hamilton, 1822	Sera (Sil-Bhitta)	LC
Cypriniformes	Cyprinidae	*Tor putitora* Hamilton, 1822	Sahar	**EN**
Cypriniformes	Cyprinidae	*Naziritor chelynoides* McClelland, 1839	Karange	LC
Cypriniformes	Cyprinidae	*Labio rohita* Hamilton, 1822	Rahu	LC
Cypriniformes	Cyprinidae	*Labio angra* Hamilton, 1822	Gardi	LC
Cypriniformes	Cyprinidae	*Cirrhinus mrigala* Hamilton, 1822	Naini	LC
Cypriniformes	Cyprinidae	*Chagunius chagunio* Hamilton, 1822	Rewa	LC
Cypriniformes	Cyprinidae	*Catla catla* Hamilton, 1822	Bhakur	LC
Cypriniformes	Cyprinidae	*Ctenopharyngodon idella* Valenciennes, 1844	grass carp	LC*
Cypriniformes	Cyprinidae	*Cyprinus carpio* Linnaeus, 1758	Common Carp	LC*
Siluriformes	Bagridae	*Mystus bleekeri* Day, 1877	Junge	LC
Siluriformes	Clariidae	*Heteropneustes fossilis* Bloch, 1794	Balim (Chille)	LC
Siluriformes	Clariidae	*Clarias batrachus* Linnaeus, 1758	Magur	LC
Beloniformes	Belonidae	*Xenentodon cancila* Hamilton, 1822	Chuche Bam	LC
Synbranchiformes	Mastacembelidae	*Mastacembelus armatus* Lacepede, 1800	Dhunge Bam	LC
Channiformess	Channidae	*Channa punctatus* Bloch, 1794	Bhoti	LC
Perciformes	Cichlidae	*Oreochromis niloticus* Linnaeus, 1758	Tilapia	LC*
Perciformes	Ambassidae	*Chanda nama* Hamilton, 1822	Glass fish	LC

LC = Least Concern, EN = Endangered, * = Exotic species

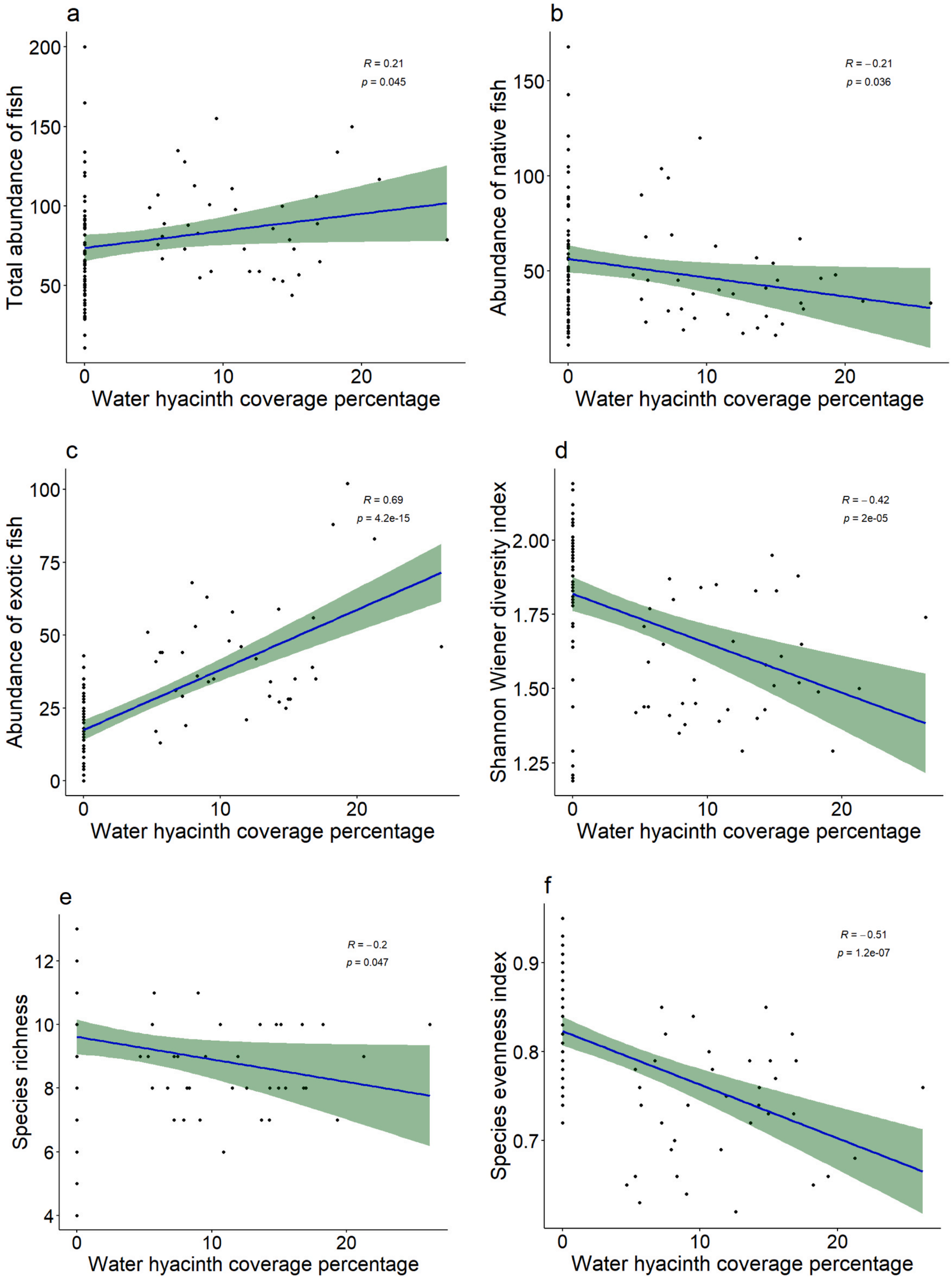

Fig. 2. Relation of water hyacinth coverage on the abundance and diversity of fish in Lake cluster of Pokhara Valley of Nepal between 2019 and 2020.

2.3. Data analysis

We quantified the diversity of fish species in both water hyacinth presence (HP) and absence (HA) habitats by computing the Shannon-Wiener diversity index (H′) (Shannon and Weaver, 1949), species evenness (J), (Pielou, 1966) and species richness using the package vegan. The seasonal variation in fish abundance was analyzed using the Kruskal-Wallis test, and the difference in fish abundance between HP and HA habitats was evaluated using the Mann-Whitney U test, as our data were not normally distributed. The relationship between water hyacinth coverage and fish abundance, species richness, and Shannon-Wiener index was assessed using simple linear regression and Spearman's correlation.

We employed multivariate ordination analysis to examine the relationship between fish species and environmental variables, including water hyacinth coverage and various physicochemical parameters of water. Detrended Correspondence Analysis (DCA) was used to determine the suitability of either Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA) for our data set. The data supported RDA, as indicated by the lengths of all four axes being less than two in DCA (Yang et al., 2020). RDA was utilized to examine the relationship between fish species and environmental variables, highlighting the inter-species distance in biplot scaling. Monte Carlo permutation test, with 999 permutations at a significance level of alpha = 0.05 (Powell, 2019), was conducted to test the significance of the ordination results. The data analysis was performed using R statistical software and its vegan package (R Core Team, 2020), with the functions decorana and rda applied for DCA and RDA, respectively.

We also used a generalized linear model to identify factors affecting on the abundance of native fish species in the Lake Cluster of Pokhara Valley between 2019 and 2020. Model parameters include Water hyacinth presence and absence, Transparency (%), temperature, Water hyacinth coverage (%) and dept (m). To avoid over dispersion on the model, we used negative binomial distribution. All analysis are performed in R Program (R Core Team, 2020).Table 1.

3. Results

3.1. Abundance and diversity of fish

We documented 20 fish species, consisting of 17 native and three exotic species, from the lakes of LCPV. Our findings revealed that 60 % of the species belonged to the Cyprinidae family, followed by Clariidae (10 %) and one species each from Bagridae, Belonidae, Mastacembelidae, Channidae, Cichlidae, and Ambassidae (Fig. 2). Our seasonal survey indicated that the highest fish catch was recorded during the winter season in both habitats (HP = 12.79 % and HA = 9.48 %), followed by spring (HP = 11.8 % and HA = 16.36 %), and the lowest catch was found during summer in both habitats (HP = 7.71 %, HA = 9.54 %) (Table 2).

The most abundant fish species observed in both the HP and HA habitats during all seasons was *Oreochromis niloticus*, followed by *Chanda nama, Puntius chola*, and *P. sophore*. The overall abundance of fish was found to be highest during the winter season ($H = 30.06$; $p < 0.001$; Table 3). Among the top eight abundant fish species, *P. chola* and *P. sophore* were found to be most abundant only during the winter season ($H = 22.87$, $p < 0.001$; 23.98, $p < 0.001$, respectively; Table 4).

There was no significant seasonal variation in the diversity indices of fish in the lakes of LCPV. However, the HA habitat had a slightly higher level of diversity ($H' = 2.45$; $J = 0.81$ and species richness) than HP habitat ($H' = 1.79$; $J = 0.63$ and $S = 17$). The total fish abundance was found higher in the HP habitat than HA habitat, however, the abundance of native fish was higher in HA habitat than HP habitat and opposite is true for the abundance of exotic fish species (Table 5).

3.2. Factors affecting the abundance and diversity of fish

We found a significant positive association between water hyacinth coverage and total abundance as well as abundance of exotic fish species ($r = 0.21$, $df = 94$, $p = 0.04$ and $r = 0.67$, $df = 94$, $p < 0.001$, respectively). However, there was a significant negative association between water hyacinth coverage and the abundance of native fish species ($r = -0.36$, $df = 94$, $p < 0.001$). Additionally, the results showed significant negative associations between water hyacinth coverage and the Shannon-Wiener diversity index ($r = -0.42$, $df = 94$, $p < 0.001$), species richness ($r = -0.2$, $df = 94$, $p = 0.04$), and species evenness index ($r = -0.51$, $df = 94$, $p < 0.001$).

Based on the retained eigenvalues 39 %, the variables are more correlated with them are considered the most important for the patterns of species abundance and composition. It indicated that the close associations between water temperature and free carbon dioxide and water hyacinth coverage. However, other water parameters had a negative association with water hyacinth coverage. Furthermore, some fish species such as *O. niloticus, C. iIdella*, and *C. nama* had close association with water hyacinth coverage, while other species like *N. chelinoides, C. chagunio, H. fossilis*, and *T. putitora* had negative associations (Fig. 3).

Table 2
Total abundance and relative abundance of fish in Lake cluster of Pokhara Valley of Nepal between 2019 and 2020.

Seasons	Abundance (HP)	RA% (HP)	Abundance (HA)	RA% (HA)
Autumn 2019	761	10.10	920	12.21
Winter 2020	964	12.79	1468	19.48
Spring 2020	889	11.80	1233	16.36
Summer 2020	581	7.71	719	9.54

Table 3
Abundance and relative abundance of each species of fish in Lake cluster of Pokhara Valley of Nepal between 2019 and 2020.

Abundance and Relative abundance of fish												
Taxa	Autumn	RA%	Winter	RA%	Spring	RA%	Summer	RA%	HP	RA%	HA	RA%
Puntius sophore	148	8.80	289	11.88	263	12.39	120	9.23	241	6.66	579	14.79
Puntius chola	228	13.56	346	14.23	281	13.24	169	13.00	293	8.10	731	18.67
Danio devario	130	7.73	216	8.88	192	9.05	90	6.92	194	5.36	434	11.08
Tor putitora	4	0.24	7	0.29	13	0.61	2	0.15	3	0.08	23	0.59
Naziritor chelinoides	27	1.61	29	1.19	20	0.94	21	1.62	7	0.19	90	2.30
Labio rohita	16	0.95	14	0.58	14	0.66	10	0.77	0	0.00	54	1.38
Labio angra	21	1.25	22	0.90	15	0.71	14	1.08	0	0.00	72	1.84
Cirrhinus mrigala	7	0.42	5	0.21	12	0.57	5	0.38	4	0.11	25	0.64
Chagunius chagunio	14	0.83	20	0.82	17	0.80	18	1.38	3	0.08	66	1.69
Catla catla	37	2.20	29	1.19	23	1.08	26	2.00	102	2.82	13	0.33
Ctenopharyngodon idella	26	1.55	19	0.78	12	0.57	23	1.77	45	1.24	35	0.89
Cyprinus carpio	3	0.18	34	1.40	27	1.27	9	0.69	0	0.00	73	1.86
Mystus bleekeri	23	1.37	19	0.78	19	0.90	21	1.62	17	0.47	65	1.66
Heteropneustes fossilis	88	5.23	75	3.08	56	2.64	63	4.85	5	0.14	277	7.07
Clarias batrachus	35	2.08	187	7.69	147	6.93	32	2.46	96	2.65	305	7.79
Xenentodon cancila	71	4.22	73	3.00	67	3.16	53	4.08	76	2.10	188	4.80
Mastacembelus armatus	54	3.21	67	2.75	50	2.36	38	2.92	117	3.23	92	2.35
Channa punctatus	14	0.83	29	1.19	25	1.18	8	0.62	7	0.19	69	1.76
Orechromis niloticus	603	35.87	737	30.30	636	29.97	418	32.15	1506	41.61	888	22.68
Chanda nama	132	7.85	215	8.84	233	10.98	160	12.31	471	13.01	269	6.87
Grand Total	1681		2432		2122		1300		3187		4348	

Table 4
Seasonal comparison of abundance and diversity of fishes in Lake cluster of Pokhara Valley of Nepal between 2019 and 2020.

Variables	Autumn Median	Winter Median	Spring Median	Summer Median	Kruskal Wallis test
Total abundance	68.5	95	86	53	H = 30.06, p < **0.001**
Native species	38	58	55.5	31	H = 19.5, p = **< 0.001**
Exotic species	21	29	23.5	15	H = 7.51, p = **0.05**
Abundant species					
Puntius chola	8	14	11	6	H = 22.87, p = < **0.001**
Puntius sophore	6	11.5	10.5	4.5	H = 23.98, p = < **0.001**
Chagunius chagunio	0	0	0	0	H = 0.67, p = 0.8
Heteropneustes fossilis	0	0.5	1	0	H = 2.59, p = 0.4
Chanda nama	0	0	0	2	H = 1.94, p = 0.5
Oreochromis niloticus	21	28.5	22	14.5	H = 5.98, p = 0.1
Ctenopharyngodon idella	0.5	0	0	0	H = 5.82, p = 0.12
Cyprinus carpio	0	0	0	0	H = 1.09, p = 0.77

Table 5
Comparison of abundance and diversity of fishes in HP and HA areas in Lake cluster of Pokhara Valley of Nepal between 2019 and 2020.

Variables	HP habitat Median (Range)	HA habitat Median (Range)	Mann Whitney test
Total abundance	84.5 (44–155)	66 (11–200)	U = 721.5, p = **0.006**
Native species	39 (16–120)	49 (11–168)	U = 1294, p = 0.1
Exotic species	40 (13–102)	15 (0–43)	U = 182, p < **0.001**
Abundant species			
Puntius chola	8.5 (2–15)	11 (0–37)	U = 1481, p = **0.002**
Puntius sophore	6.5 (1–15)	8.5 (0–31)	U = 1292, p = 0.1
Chagunius chagunio	0 (0–2)	1(0–5)	U = 1637.5, p < **0.001**
Heteropneustes fossilis	0 (0–2)	2(0–30)	U = 1666, p < **0.001**
Chanda nama	0 (0–61)	0 (0–32)	U = 844, p = **0.03**
Oreochromis niloticus	39.5 (13–102)	15 (0–39)	U = 172.5, p < **0.001**
Ctenopharyngodon idella	1 (0–5)	0 (0–6)	U = 736.5, p = **0.002**
Cyprinus carpio	0 (0–0)	0 (0–17)	U = 1224, p = **0.02**

The abundance of native fish species decreased in water hyacinth habitat (Table 6). In addition, the native fish species' abundance also decreased at low water transparency and depth of lake, while the native fish abundance was higher with increasing abundance of exotic fish species (Table 6).

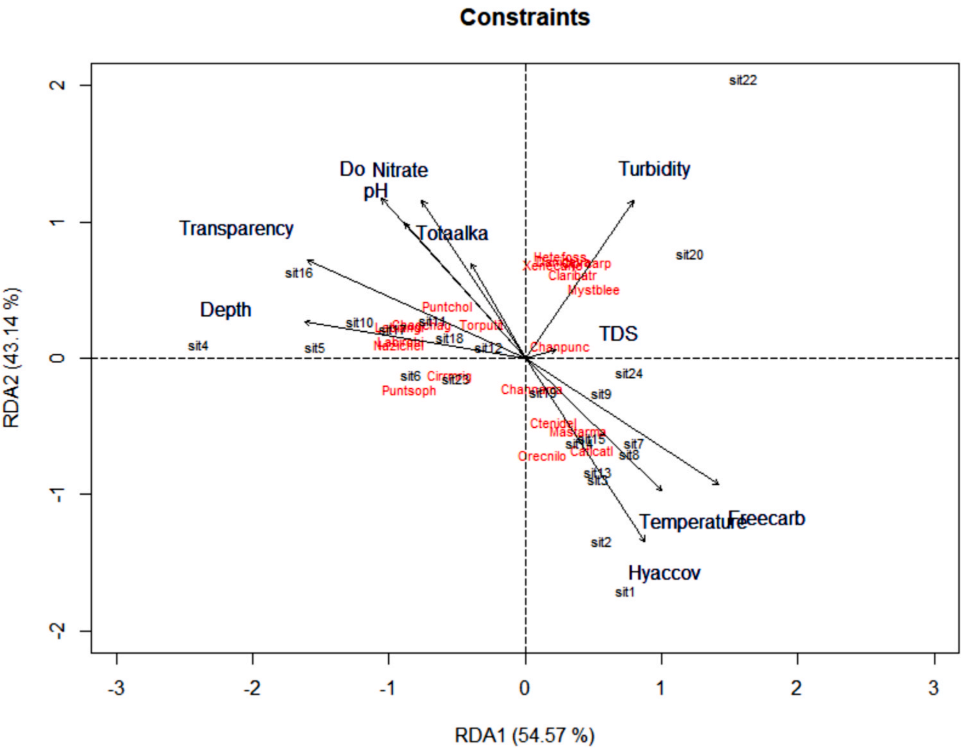

Fig. 3. RDA of fish species after constraining variation by environmental variables in Lake Cluster of Pokhara Valley of Nepal between 2019 and 2020. Abbreviation for fish species: Torputit = *Tor putitora*, Chagchag = *Chagunius chagunio*, Puntchol = *Puntius chola*, Puntsoph = *Puntius sophera*, Cirrmrig = *Cirrhinus mrigala*, Nazichel = *Naziritor chelynoides*, Labirohi = *Labio rohita*, Labiangr = *Labio angra*, Danideva = *Danio devario*, Clarbatr = *Clarias batrachus*, Xenecanc = *Xenentodon cancila*, Hetefoss = *Heteropneustes fossilis*, Cyprcarp = *Cyprinus carpio*, Mystblee = *Mystus bleekeri*, Channama = *Chanda nama*, Ctenidel = *Ctenopharyngodon Idella*, Mastarma = *Mastacembelus armatus*, Catlcalt = *Catla catla*, Orecnilo = *Oreochromis niloticus***.** Abbreviation for environmental variables: Hyaccov = Water hyacinth coverage, Freecarb = Free carbon dioxide, TDS = Total dissolved solid, Totaalka = Total alkalinity, DO = Dissolve oxygen.

Table 6
Generalized linear model describing the effects of given predictors on the abundance of native fish species Lake cluster of Pokhara Valley of Nepal between 2019 and 2020. Model parameters include Water hyacinth presence and absence, Transparency (%), abundance of exotic fish, and depth of lake (m). *Significant effects are in bold.

Parameters	Estimate	SE	z	p
(Intercept)	4.3848	0.2092	20.96	**<0.001**
Water hyacinth presence	–0.9117	0.1946	–4.686	**0.001**
Transparency (%)	–0.2661	0.1828	–1.456	0.145
Exotic fish abundance	0.0136	0.0032	4.288	**0.000**
Depth (m)	–0.1033	0.0357	–2.893	**0.004**

4. Discussion

Our study highlights the impact of water hyacinth on the fish species diversity and abundance in the LCPV lakes. Water hyacinth has a significant positive association with the total abundance and the abundance of exotic fish species, but it has a significant negative association with the abundance of native species. The RDA analysis showed that water hyacinth has a close association with water temperature and free carbon dioxide and has a negative association with other water parameters. The high abundance of certain fish species in the lakes of LCPV could be attributed to the favorable water temperature conditions that are suitable for the widespread fish species to thrive (Swar, 2002). Moreover, the study revealed that the abundant fish species in the lakes of LCPV, such as *O. niloticus*, *C. nama*, etc., have a close association with water hyacinth while other species like *N. chelinoides*, *H. fossilis* etc., have a negative association. The study findings suggest that water hyacinth might have an impact on the fish species occurrence, especially native species, in the LCPV lakes. The results also emphasize the importance of monitoring the spread of water hyacinth in aquatic ecosystems and its impact on the fish community.

The rapid spread of ornamental fish species, *C. nama* and exotic *H. fossilis*, has been observed in the lakes of the study area, with C. *nama* being recorded only in Begnas and Phewa lakes while, both species were recorded in Begnas lake (Husen et al., 2019). The method of introduction of these fish into the lake remains unclear, but it is possible that it occurred due to the accidental release of fry into the lakes. The recently introduced specis in pokhara, *C. nama* is a facultative feeder and feeds on scales and hatchlings of other fish species (Chakraborty et al., 2018), which could potentially harm native species in the lake. For instance, in the Kulekhani reservoir, 66.66 % and 33.33 % of native fish mortalities were reported to be due to scale fall out in cultivated carp fish and the presence of *C. nama*, respectively (Dahal et al., 2012). The high catch rate of *C. nama* in Begnas and Phewa lakes, as well as the decline of other fish species, may be related to its impact on these bodies of water.

The winter season in the lakes of LCPV is characterized by higher fish abundance, which could be attributed to the sparse coverage of water hyacinth. This aquatic plant provides an ideal nursery habitat for fish and increases food availability through the greater abundance of macro-invertebrates (Basaula et al., 2022; Villamagna, 2009). As a result, higher fish abundance was observed in the habitat with more water hyacinth coverage (HP) compared to the habitat with less water hyacinth coverage (HA).

Despite this trend, the endangered fish species *T. putitora* had similar abundance in both HP and HA habitats, suggesting that its feeding habits on insects, algae, and macrophytes play a more important role in determining its abundance (Bhatt and Pandit, 2016). The cultivable exotic fish species *C. idella* showed a preference for the HP habitat, which can be attributed to its herbivorous feeding habits and use in controlling aquatic weeds (Pipalova, 2006). In contrast, the commonly growing exotic fish species *C. carpio* had a strong preference for the HA habitat, as it is a benthivore with a larger bottom-up effect (Rahman, 2015).

Further our findings showed that certain fish species exhibit a preference for either the HP or HA habitats. The fish species *O. niloticus* and *C. nama* were found to prefer the HP habitat, while *P. chola*, *P. sophore*, *Naziritor chelinoides, C. chagunio*, and *H. fossilis* preferred the HA habitat. Although a similar pattern of fish diversity was observed throughout the seasons, the HA habitat was found to be more diverse, potentially due to the greater presence of native fish species.

The greater abundance of fish in the HP habitat can be attributed to the structural complexity and increased food availability provided by the fragmented mat of water hyacinth (Njiru et al., 2002, 2004; Toft et al., 2003; Troutman et al., 2007; Villamagna, 2009). Recent studies have shown that invasive species such as *O. niloticus* are becoming increasingly abundant in the lakes of Pokhara, leading to a decline in the abundance of native fish species (Husen, 2014; Husen et al., 2016, 2019). The fragmented mat of water hyacinth may provide a suitable habitat for *O. niloticus*, it could be attributed for the changes its feeding habits from omnivorous to herbivorous as its body size increases (Tesfahun and Temesgen, 2018). The water hyacinth facilitates the survival and excessive increase in the abundance of invasive *O. niloticus* in the lakes of LCPV may be supported by the invasional meltdown (Simberloff and Von Holle, 1999). It could be attributed for the decline in the catch of native fish in the lakes of LCPV, and also the shifting of exotic fish species towards water hyacinth coverage areas as found for Nile perch (*Lates niloticus*) which shifted towards the water hyacinth covered habitat in Lake Victoria, Uganda, where the mat of water hyacinth supports their invertebrate diets (Njiru et al., 2004).

The winter water temperature remains cool and the pH and free carbon dioxide levels increase, resulting in a decrease in fish abundance. However, the abundance is higher in the HP habitat compared to the HA habitat, possibly due to the presence of fragmented water hyacinth that provides breeding, nursery, and feeding grounds for fish. (Toft et al., 2003; Villamagna, 2009; Villamagna and Murphy, 2010). Dense water hyacinth mats can cause hypoxia, leading to a decrease in dissolved oxygen and reduced fish catch, with only two hypoxic species recorded (Ongore et al., 2018). The abundance of native fish species is increased by nitrate levels, which water hyacinth absorbs, resulting in higher fish abundance in the HA habitat (Gichuki et al., 2012), it could be the reason for the higher abundance of the native fish species in HA. More native species were recorded in HA habitat than that of the HP habitat, it could be attributed the lower species richness of fish in HP habitat. The Shannon-Wiener diversity index, evenness index, and species richness are negatively affected by water hyacinth coverage, and more native fish species are recorded in the HA habitat.

Our RDA ordination showed that water temperature and free carbon dioxide were closely associated with water hyacinth cover, while other water parameters were more distantly associated. The water hyacinth mat decreases dissolve oxygen concentration by blocking the transfer of sunlight and inhibiting photosynthesis in phytoplankton and other plants (Mangas-Ramírez and Elías-Gutiérrez, 2004; Masifwa et al., 2001; McVea and Boyd, 1975; Villamagna and Murphy, 2010). The low dissolve oxygen levels enhance phosphorus release from the bottom and increase temperature, carbon dioxide, and decrease pH, leading to eutrophication and decreased water quality (Bicudo et al., 2007; Dersseh et al. (2019); Ndimele et al. (2011) Dense water hyacinth mats also cause hypoxia due to a large amount of dead plant matter that sinks to the bottom and consumes dissolve oxygen during decomposition (Bicudo et al., 2007). The species *O. niloticus, C. idella*, and *C. nama* are supported by water hyacinth and thus have a higher catch rate in the lakes of LCPV. However, the invasion of *O. niloticus* and water hyacinth has dramatically reduced the catch of native species (Husen, 2014; Husen et al., 2016, 2019). The catch of *O. niloticus* increased from 0.6 metric tons in 2006–58.1 metric tons in 2011 (Husen, 2014). The invasion of *O. niloticus* also negatively impacts the growth and development of native mud carp (Gu et al., 2015). The negative association between fish species such as *Naziritor chelinoides*, *C. chagunio*, *H. fossilis*, *Danio devario*, *T. putitora*, *C. carpio*, *P. chola*, and *P. sophore* and water hyacinth cover was mainly due to the avoidance of native fish species in water hyacinth presence habitat.

5. Conclusion

The lakes in the LCPV are crucial freshwater sources and significant habitats for fish diversity in central Nepal. Although fish abundance was found to be higher in winter, the diversity of fish remained consistent throughout the seasons. However, the invasive weed water hyacinth provided support for the invasive fish species *O. niloticus*, the rapidly spreading fish species *C. nama*, and the exotic species *C. idella*. On the other hand, the abundance of native fish species was negatively impacted by the presence of water hyacinth. The close association between carbon dioxide levels and water hyacinth coverage, as well as the inverse relationship between transparency, pH, and dissolved oxygen levels, suggested a decline in water quality. The decline in native fish species in the LCPV lakes may be attributed to the deterioration of water quality, the invasion of water hyacinth, and the introduction of *O. niloticus* and the scale-feeding *C. nama*. Regular monitoring of native fish catch is crucial to understand the impact of invasive species on the lakes. To preserve the native fish species, it is recommended to implement regular stock enhancement programs, control and manage invasive weeds, and conduct further studies on the status of exotic fish and the impact of invasive weeds across the LCPV.

Ethical statement

Data for this research was collected without handing the animals so there was no loss or damage of fish, we just identified and released fish in the respective lakes so no need to take ethical permission on animal handling.

CRediT authorship contribution statement

RB, HPS and KS conceptualized the research. RB conducted field research, data curation, formal analysis, and drafted manuscript. HPS, KS supervised the project. HPS, KS, BRP and PSK revised and edited the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix II: Participation in Seminars

a. Oral presentation in first National Conference organized by CDZ, 28 to 30 November, 2020 **("Variation on macro-invertebrates' abundance in presence and absence areas of invasive species at Lake Cluster of Pokhara Valley, Nepal")**

b. Oral Presentation on Conference May 24 and 25, 2021, HIMALAYAN KNOWLEDGE CONCLAVE **("A variation on the abundance of waterbirds in presence and absence areas of invasive species at Lake Cluster of Pokhara Valley, Nepal.")**

c. Oral presentation in International Conference organized by CDZ, 29 Nov to 01 Dec 2021 **("Effects of the invasive water hyacinth on globally threatened waterbird abundance and diversity at Lake Cluster of Pokhara Valley, Nepal.")**

55th Anniversary

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organized by

Central Department of Zoology & Alumni Association of CDZ, Tribhuvan University

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Prof Dr Tej Bahadur Thapa Conference Chair and Head Central Department of Zoology	**Prof Dr Ram Prasad Khatiwada** Dean, Institute of Science & Technology Tribhuvan University	**Prof Dr Dharma Kanta Baskota** Vice Chancellor Tribhuvan University

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Lumbini Buddhist University

Sharada Poudel, PhD
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Central Department of Zoology

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Appendix III: List showing the macro-invertebrates deposited in Central Department Zoology Museum of Tribhuvan University (CDZMTU) with their ID and acronyms or codes

SN	Scientific name	ID in Museum of CDZ	Acronym
1	*Limnodrilus hoffmeisteri*	CDZMTUM1	Limnhoff
2	*Branchiura sowerbi*	CDZMTUM2	Bransowe
3	*Tubifex tubifex*	CDZMTUM3	Tubitubi
4	*Stylaria lacustris*	CDZMTUM4	Stylalacu
5	*Hirudinaria granulosa*	CDZMTUM5	Hirugran
6	*Hirudo medicinalis*	CDZMTUM6	Hirumedi
7	*Helobdella stagnalis*	CDZMTUM7	Helostag
8	*Placobdella parsitica*	CDZMTUM8	Placpars
9	*Chironomus* sp.	CDZMTUM9	Chirspec
10	*Leptophlebia marginata*	CDZMTUM10	Leptomarg
11	*Diplacodes* spp.	CDZMTUM11	Diplaspec
12	*Brachythemis contaminata*	CDZMTUM12	Braccont
13	*Gomphidia* sp.	CDZMTUM13	Gompspec
14	*Schnura heterosticta*	CDZMTUM14	Schnhete
15	*Aquarius remigis*	CDZMTUM15	Aquaremi
16	*Nepa cinerea*	CDZMTUM16	Nepacine
17	*Thermonectus* sp.	CDZMTUM17	Thermspec
18	*Dolomedes tenebrosus*	CDZMTUM18	Dolotene
19	*Paleomon* sp.	CDZMTUM19	Palespec
20	*Sphaerium* sp.	CDZMTUM20	SphaSpec
21	*Acroloxus lacustris*	CDZMTUM21	Acrolacu
22	*Lamellidens* spp.	CDZMTUM22	Lamespec
23	*Radiatula* spp.	CDZMTUM23	Radispec
24	*Helisoma* spp.	CDZMTUM24	Helispec
25	*Segmentina* spp.	CDZMTUM25	Segmspec
26	*Thiara requeti*	CDZMTUM26	Thiarequ
27	*Thiara tuberculata*	CDZMTUM27	Thiatube
28	*Thiara granifera*	CDZMTUM28	Thiagran
29	*Bellamya* bengalensis	CDZMTUM29	Bellbeng

Appendix IV: List showing the seasonal mean abundance of macro-invertebrates in HP and HA habitats of the lakes of Pokhara Valley from 2019 to 2020

HP habitats		Autumn 2019		Winter 2020		Spring 2020		Summer 2020	
SN	Taxa	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
1	*Limnodrilus hoffmeisteri*	7.22	1.33	13.9	2.6	20.89	2.97	3.67	1.13
2	*Branchiura sowerbi*	10.33	1.68	15.1	2.7	21.44	3.15	6.11	1.14
3	*Tubifex tubifex*	2.11	0.42	6.6	1.8	12.00	1.77	1.78	0.43
4	*Stylaria lacustris*	5.11	0.42	4.4	0.6	10.67	0.69	3.11	0.31
5	*Hirudinaria granulosa*	4.56	0.60	2.0	0.5	-	-	4.00	0.78
6	*Hirudo medicinalis*	8.22	0.72	5.6	1.2	-	-	7.56	0.85
7	*Helobdella stagnalis*	11.33	2.26	5.4	0.9	3.67	1.86	6.78	1.71
8	*Placobdella parsitica*	9.00	1.69	4.1	1.2	3.33	1.67	5.00	1.00
9	*Chironomus* sp.	-	-	33.2	8.5	**50.22**	7.12	3.33	1.67
10	*Leptophlebia marginata*	**60.00**	5.93	24.6	3.0	32.11	3.27	43.00	3.01
11	*Diplacodes* spp.	15.33	1.97	10.7	1.6	17.00	1.56	11.67	2.17
12	*Brachythemis contaminata*	22.00	1.94	11.4	1.1	15.78	1.56	13.56	1.24
13	*Gomphidia* sp.	12.22	1.61	5.2	1.1	14.00	0.94	9.78	1.04
14	*Schnura heterosticta*	21.67	1.86	10.2	1.7	16.00	1.53	15.11	1.91
15	*Aquarius remigis*	46.89	5.17	23.3	2.2	27.33	1.88	29.67	2.81
16	*Nepa cinerea*	-	-	-	-	-	-	0.33	0.33
17	*Thermonectus* sp.	**94.22**	11.13	55.1	8.6	**73.78**	7.65	67.67	6.75
18	*Dolomedes tenebrosus*	3.56	1.26	4.8	4.0	-	-	-	-
19	*Paleomon*	3.33	1.74	0.9	0.6	2.00	1.05	1.33	0.73
20	*Sphaerium* sp.	**80.89**	8.07	48.4	6.4	**63.56**	3.32	63.33	5.97

21	*Acroloxus lacustris*	19.67	1.47	7.0	1.3	12.67	1.25	11.22	1.61
22	*Lamellidens* spp.	2.89	1.33	1.0	0.5	7.33	2.16	1.11	0.45
23	*Radiatula* spp.	0.67	0.67	-	-	-	-	2.33	1.21
24	*Helisoma* spp.	5.78	3.37	0.6	0.4	-	-	0.22	0.22
25	*Segmentina* spp.	2.56	1.07	0.1	0.1	0.11	0.11	1.11	0.65
26	*Thiara requeti*	5.89	3.10	-	-	-	-	-	-
27	*Thiara tuberculata*	20.33	3.35	4.1	1.2	8.00	2.13	11.44	2.32
28	*Thiara granifera*	19.11	2.69	2.2	1.2	2.67	1.46	-	-
29	*Bellamya*bengalensis	21.11	3.16	8.4	1.1	14.33	1.76	9.78	2.68
HA habitats									
1	*Limnodrilus hoffmeisteri*	17.60	3.05	23.87	5.36	**29.67**	6.61	12.60	2.18
2	*Branchiura sowerbi*	16.93	3.26	25.87	4.94	**30.53**	4.69	11.73	2.20
3	*Tubifex tubifex*	13.13	2.40	18.47	4.01	**27.00**	3.84	9.20	1.54
4	*Stylaria lacustris*	10.53	2.10	15.93	3.75	**20.40**	4.68	7.00	1.43
5	*Hirudinaria granulosa*	7.67	1.72	0.33	0.21	-	-	3.00	0.31
6	*Hirudo medicinalis*	7.40	0.73	1.40	0.31	0.07	0.07	5.33	0.51
7	*Helobdella stagnalis*	7.47	0.82	0.80	0.34	1.53	0.86	2.00	0.49
8	*Placobdella parsitica*	6.47	0.58	-	-	1.20	0.65	1.07	0.38
9	*Chironomus* sp.	30.07	10.58	37.67	6.21	**53.20**	7.37	29.13	6.92
10	*Leptophlebia marginata*	18.07	1.88	5.67	1.16	14.13	1.50	9.13	0.84
11	*Diplacodes* spp.	7.93	2.41	0.67	0.67	6.73	1.36	4.07	1.16
12	*Brachythemis contaminata*	6.93	2.03	0.53	0.53	6.13	1.15	3.33	1.03
13	*Gomphidia* sp.	4.73	1.87	0.20	0.20	8.67	0.68	2.53	0.96
14	*Schnura heterosticta*	12.67	1.42	3.87	0.75	11.80	0.81	6.73	1.07

15	*Aquarius remigis*	22.60	1.34	34.13	3.53	**42.07**	5.12	22.40	1.33
16	*Nepa cinerea*	3.53	1.41	0.53	0.41	1.60	1.13	1.00	0.38
17	*Thermonectus* sp.	19.40	3.76	16.00	3.17	20.60	3.63	10.67	2.20
18	*Dolomedes tenebrosus*	0.80	0.33	-	-	-	-	0.20	0.20
19	*Paleomon*	1.53	0.83	0.13	0.13	0.13	0.13	0.33	0.19
20	*Sphaerium* sp.	24.20	5.39	7.93	1.92	14.73	3.82	13.93	3.11
21	*Acroloxus lacustris*	3.40	1.48	0.33	0.23	2.93	0.98	1.47	0.68
22	*Lamellidens* spp.	2.60	0.67	0.87	0.39	4.27	1.54	1.47	0.40
23	*Radiatula* spp.	0.53	0.38	1.20	0.65	2.27	1.10	0.67	0.33
24	*Helisoma* spp.	-	-	-	-	-	-	-	-
25	*Segmentina* spp.	0.87	0.42	0.13	0.13	0.13	0.13	0.00	0.00
26	*Thiara requeti*	-	-	-	-	-	-	-	-
27	*Thiara tuberculata*	5.13	0.72	-	-	-	-	3.07	0.41
28	*Thiara granifera*	5.00	1.24	-	-	-	-	-	-
29	*Bellamya*bengalensis	2.07	0.30	2.20	0.52	5.73	1.31	1.73	0.40

Appendix V: List showing the overall analysis of water parameters in lakes of Pokhara during winter 2020 (Similar types analysis was performed in all seasons)

Plots	Depth (m)	Temp. (°C)	Trans. (m)	pH	TUR (NTU)	TDS mg/L	DO mg/L	CO_2 mg/L	TAlk mg/L	NO_3 mg/L
1	2.5	16	0.5	7.1	3	23	3.24	15	132	1.06
2	3.8	16.8	0.6	6.7	2.9	21	4.1	11	121	0.9
3	3.6	16.5	0.8	6.3	2.7	19	4	12.2	124	1.02
4	7.4	16	1.9	7.3	1.2	20	7	6.42	168	2.88
5	8.5	15.2	1.2	7.6	1.1	17	6.8	6.2	155	1.88
6	5.4	15.5	1.4	7.4	0.9	19	6.02	4.8	142	1.07
7	2.2	15.6	0.6	7.2	3.1	31	4	11.5	131	1.32
8	2.8	15.8	0.7	6.6	2.6	25	3.7	10.3	124	1.83
9	2.6	15.6	0.8	6.3	2.1	27	4.3	11	127	2.05
10	5.3	16	1.8	7.4	3	19	7.14	6.24	180	3.09
11	7.2	15.6	1.4	7.7	4	18	7.2	5.5	168	2.4
12	6.3	15.4	1.3	7.5	3.6	17	6.8	4.7	162	2.02
13	2.2	16	0.5	6	5	22	4.26	13.45	122	2.02
14	2	16.9	0.7	6.2	5.1	23	4.1	12.2	126	1.2
15	2.8	16.8	0.6	6.5	4.8	19	4.02	11.8	130	0.92
16	4.6	15.8	1.6	6.6	5.8	21	6.1	7.31	153	3.2
17	5.5	15.3	1.4	7.2	5	22	5.7	6.9	140	1.85
18	4.8	15.5	1.6	7.6	4.7	21	6.2	7	135	2.01
19	3	15.5	0.8	6.4	4.2	23	7.2	12.04	170	1.88
20	2.6	15	1	6.7	4.6	17	7	11.21	135	2.6
21	3.8	16	1.1	7	8	21	7.6	9.12	129	2.2
22	2.7	16.4	0.7	7.5	9.6	47	4.5	11.25	298	2.8
23	4.8	15.5	1.4	7.2	2	99	7	6.05	137	1..9
24	2.2	16	1	6.8	1.4	35	2.4	9.01	142	1.22
Avg.	**4.11**	**15.86**	**1.06**	**6.9**	**3.77**	**26.08**	**5.43**	**9.26**	**147.96**	**1.89**

Appendix VI: List showing the field datasheet of waterbirds count in the HP areas of Phewa lake during winter 2020 (Similar types of data sheet were used in all the plots for the respective season)

SN	Common name of birds	Plot-1 (HP)	Plot-2 (HP)	Plot-3 (HP)
1	Great Cormorant	10	7	3
2	Little Cormorant	0	0	0
3	Cattle Egret	11	12	7
4	Indian pond Heron	15	12	7
5	Intermediate Egret	7	5	3
6	Little Egret	8	6	4
7	Common Coot	8	4	2
8	Common Moorhen	10	6	4
9	Purple Swamphen	26	11	8
10	Common Kingfisher	3	3	1
11	White Throated Kingfisher	6	8	4
12	White-browed Wagtail	4	3	2
13	Black Kite	6	6	4
14	Baer's Pochard	0	0	0
15	Bar-headed Goose	0	0	0
16	Comb Duck	0	0	0
17	Common Goldeneye	0	0	0
18	Comman Pochard	4	2	0
19	Common Shelduck	0	0	0
20	Common Teal	2	0	0
21	Cotton Pygmy-goose	0	0	0
22	Eurasian Wigeon	0	0	0
23	Falcate Duck	0	0	0
24	Ferruginous Pochard	0	0	0
25	Gadwall	0	0	0
26	Garganey	0	0	0
27	Mallard	4	2	0
28	Northern Pintail	0	0	0
29	Northern Shoveler	0	0	0
30	Red Crested Pochard	0	0	0
31	Ruddy Shelduck	8	4	2
32	Tufted Duck	0	0	0
33	Lesser Whistling Duck	6	5	2

34	Bronze-winged Jacana	13	7	3
35	Little Ringed Plover	0	0	0
36	Red Wattled Lapwing	4	4	2
37	White Wagtail	6	4	4
38	Greater Painted Snipe	0	0	0
39	Common Sandpiper	0	0	0
40	Marsh Sandpiper	0	0	0
41	Great Crested Grebe	0	0	0
42	Little Grebe	0	0	0
43	Darter	0	0	0
	Total	164	113	62
	Sampling date	Time	Time	Time
	20th Jan 2020	7.00-7.20(AM)	7.40-8.00(AM)	8.30-850(AM)

Appendix VII: List showing the datasheet of macro-invertebrates count/m^2 in one HP plot of Phewa lake during winter 2020 (Similar types of data sheet were used in all the plots for the respective season): I, II, III and IV (sub plots in edges) and V (sub plot at center) of the plot

SN	Acronym	Plot No-1	I	II	III	IV	V	Average	Round
1	Limnhoff	HP	25	27	24	26	26	25.6	26
2	Bransowe	HP	28	25	23	21	25	24.4	24
3	Tubitubi	HP	15	13	12	11	12	12.6	13
4	Stylalacu	HP	8	10	5	7	3	6.6	7
5	Hirugran	HP	3	2	2	1	5	2.6	3
6	Hirumedi	HP	7	6	4	4	7	5.6	6
7	Helostag	HP	3	2	3	4	2	2.8	3
8	Placpars	HP	7	6	6	2	9	6	6
9	Chirspec	HP	21	16	19	15	20	18.2	18
10	Leptomarg	HP	43	47	46	41	35	42.4	42
11	Diplaspec	HP	13	11	11	12	10	11.4	11
12	Braccont	HP	12	8	9	12	10	10.2	10
13	Gompspec	HP	2	1	0	3	4	2	2
14	Schnhete	HP	8	5	7	7	6	6.6	7
15	Aquaremi	HP	19	18	21	19	17	18.8	19
16	Nepacine	HP	0	0	0	0	0	0	0
17	Thermspec	HP	59	54	61	51	58	56.6	57
18	Dolotene	HP	5	2	1	2	3	2.6	3
19	Palespec	HP	0	0	0	0	0	0	0
20	SphaSpec	HP	65	56	54	64	57	59.2	59
21	Acrolacu	HP	12	11	9	6	8	9.2	9
22	Lamespec	HP	0	0	0	0	0	0	0
23	Radispec	HP	0	0	0	0	0	0	0
24	Helispec	HP	4	4	1	3	2	2.8	3
25	Segmspec	HP	0	0	0	2	1	0.6	1
26	Thiarequ	HP	0	0	0	0	0	0	0
27	Thiatube	HP	7	6	5	8	5	6.2	6
28	Thiagran	HP	8	6	7	7	5	6.6	7
29	Bellbeng	HP	5	6	5	3	4	4.6	5

Appendix VIII: List showing the field datasheet of fish in the HP areas of Begnas lake during winter 2020 (Similar types of data sheet were used in all the plots for the respective season)

SN	Species	Plot 7 (HP)	Plot 8 (HP)	Plot 9 (HP)
1	Bhitta	23	17	13
2	Bhurluk	19	17	11
3	Sera (Sil Bhitta)	15	11	9
4	Sahar	0	0	0
5	Karange	0	0	0
6	Rahu	0	0	0
7	Gardi	0	0	0
8	Naini	0	0	0
9	Reba	0	0	0
10	Bhakur	0	0	0
11	Grass Carp	4	1	2
12	Scale-Co. Carp	0	0	0
13	Junge	0	0	0
14	Magur	0	0	0
15	Balim (Chille)	16	11	8
16	Chuche Bam	1	1	1
17	Dhunge Bam	4	2	1
18	Bhoti	0	0	0
19	Tilapia	35	29	19
20	Glass fish	55	46	34

Appendix IX: The list showing the field visit schedule in the lakes of Pokhara Valley from 2019 to 2020

Seasons	Autumn 2019	Winter 2020	Spring 2020	Summer 2020
Months	Sep, Oct, Nov	Dec, Jan, Feb	Mar, April, May	June, July, Aug
Data collection	Oct 16-Nov 15	Jan 16-Feb 15	April 16-May15	July 16-Aug15
Phewa	Oct 16,17,18	Jan 16,17,18	April 28,29,30	July 29,30,31
Begnas	Oct 21,22,23	Jan 21,22,23	May 2,3,4	Aug 2,4,5
Rupa	Oct 25,26, 27	Jan 25,26,27	May 6,7,8	Aug 7,8,9
Other lakes	Nov 2,3,4,5	Feb 3, 4, 5, 6	May 10,11,12,13	August 11,12,13,14
Field revisit	Nov 7,8,9,11	Feb 8,9,10,11	May 15,16,17,18	Aug 14,15,16,17
Total days	16	16	16	16

Appendix X: The protocols and methods followed during present study

1. Aquatic Insects of India-A field guide (Subramanian & Sivaramakrishnan, 2007)
2. Effect of water hyacinth on structure of habitat, assemblages of invertebrate, and diets of fishes (Toft *et al.*, 2003)
3. AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling (Parker, 2018)
4. Evaluating gillnetting protocols to characterize lacustrine fish communities (Alexander *et al.*, 2015)
5. Bird census techniques (Bibby *et al*., 2000)

Appendix XI: The morphological identification keys of macro-invertebrates recorded in the lakes of Pokhara Valley from 2019 to 2020

SN	Taxa	Keys for identification
1	*Limnodrilus hoffmeisteri*	• Red in colour due to presence of hemoglobin • Tapering end, 25-40mm long, conical shaped head • No eye spots, 55-95 body segments, lives in sandy sediments
2	*Branchiura sowerbi*	• Presence of dorsal and ventral gill pairs in posterior segments • Head buried in the mud, body is 10-15 cm long • Presence of dorsal and ventral setae • Clitellum lies in 10, 11 and 12 segments
3	*Tubifex tubifex*	• Long about 20 cm, and thin, 34 -120 segments, red in colour • Eye spot absent, upper and lower setae on each side, tapering
4	*Stylaria lacustris*	• Small in size, identified by the dorsal bundle with • hair chaetae in VI, ventral chaetae close to proximal end and ventral chaetae with upper and lower tooth.
5	*Hirudinaria granulosa*	• Body with several ring-like structures, long about 20cm, one to • four pairs of eyes, posterior and anterior suckers
6	*Hirudo medicinalis*	• body cylindrical, 30-34 segments, dorsal dark brown and • Ventral speckled, five pairs of eyes, posterior and anterior suckers
7	*Helobdella stagnalis*	• Crawls on aquatic plants or other objects, lack of jaw • Body flat, 10mm long and 2mm width, a pair of eye spots
8	*Placobdella parsitica*	• Smooth dorsal surface, unpigmented median yellow line present

		• Medial and paramedial marble patches, 8-12 stripes in ventrum
9	*Chironomus* sp.	• Red in colour, head bears a pair of small tubercules, 5 to 13 mm long, • Usually with tubes on the sides
10	*Leptophlebia marginata*	• Nymphs are about 18 mm long, well developed lateral spines on abdominal segments with dark marks, large tracheal gills • Short antennae, fast swimmers, feeds on detritus in sediments
11	*Diplacodes* spp.	• The larvae with protruding eyes, labial palps lacking teeth • Jaw like structure for capturing prey, lack of spines on dorsal surface
12	*Brachythemis contaminata*	• Usually drab, six segmented legs, large eyes • Small wings bud on the back of the thorax, gills inside the rectum
13	*Gomphidia* sp.	• Antennae with four segments, the third is larger than other • Prementum and palpal lobes are flattened, body is broad and extremely flattened
14	*Schnura heterosticta*	• Slender and elongated body, colour green to brown • Legs are long and end with two claws, well camouflaged
15	*Aquarius remigis*	• About 0.5 inches in size, dark brown in colour • Sharp rostrum, move continuously in water surface
16	*Nepa cinerea*	• Oval body with raptorial front legs, presence of a pair of non-retractable breathing tubes on the terminal abdominal segment • Easily distinguish from other insects
17	*Thermonectus* sp.	• Finely emarginate metatibial spurs • Mesofemur with hind margin setae longer than femur

18	*Dolomedes tenebrosus*	• Female 15-26mm, male 7-13mm, legsn50-90mm, body is pale or dark brown in colour • Several marker and lighter stripes around the legs, femora are banded with brown/black and tibia are banded with reddish-brown/black annulation
19	*Paleomon* sp.	• Body is compressed laterally and divisible into anterior cephalothorax and posterior abdomen • 19 pairs of appendages
20	*Sphaerium* sp.	• Aquatic Bivalvia pale yellow in colour • Long and strong muscular foot helps to move and climb on aquatic plants under the water
21	*Acroloxus lacustris*	• Hat shaped limpet of about 7 mm long, 3mm wide and 2mm heigh • Shell is laterally compressed, yellowish-grey to brown in colour
22	*Lamellidens* spp.	• Body is laterally flattened and bilaterally symmetrical • Outline of anterior side is roughly oval with slightly narrower posterior end
23	*Radiatula* spp.	• The soft body is surrounded by two hanging shells formed in single piece • Shell small, greenish, broader posterior end, transverse line prominent
24	*Helisoma* spp.	• Unlike other most of the molluscs, this snail respires through lungs • The foot and head are small but thread like tentacles are long coiled shells in flat lacking elevated spires
25	*Segmentina* spp.	• Smooth and small shells with lower brightness which are glossy and discoidal • Aperture heart-shaped, two external flagella on penial sac
26	*Thiara (Sermyla) requeti*	• Axial ribs strongly curved on spire whorls • Apical half in last whorl and spiral ribs in basal half

27	*Thiara (Melanoides) tuberculata*	• Elongated and conical shell dextrally (clockwise or right-handed) coiled in 8-12 whorls, strongly pointed spire • Length of shell is about 30-36mm, colour dark reddish
28	*Thiara (Tarebia) granifera*	• Clear rows of nodules in elongated and conical shell, height of flat body whorls > half of total height of the shell • Length of the shell about 8-12 mm
29	*Bellamya* bengalensis	• Large snail with a gill and an operculum. • Colour bands ≥ 3 in smooth and thin shell, rows of chaetae in the embryonic shell = 3

Appendix XII: The morphological identification keys of fish species recorded in the lakes of LCPV between 2019 and 2020

SN	Taxa	Keys for identification
1	*Puntius sophore*	• Scales on lateral line (25-25), pored lateral line scales (24-24) • Scales rows above lateral line (4.5-5.5), scale rows below lateral line (4.5-4.5), scales around caudal peduncle (10-10), barbels (0). • Total soft rays- Dorsal fin (11-12), caudal fin (forked), anal fin (8-8), paired fins – pectoral (14-16), pelvic- (9-9) • Dorsal fin inserted equidistant between tip of snout and base of caudal fin; its last unbranched rays osseous and smooth. • Fin formula: D. 3/8; P_1.15; P_2. 1/8; A. 3/5
2	*Puntius chola*	• Scales on lateral line (24-28), pored lateral line scales (24-24) • Scales rows above lateral line (4.5-6), scale rows below lateral line (3.5-4), scales around caudal peduncle (11-14), barbels (maxillary single pair).

		• Total soft rays- Dorsal fin (11-11), caudal fin (forked), anal fin (8-8), paired fins – pectoral (14-17), pelvic- (9-9) • Fin formula: D. 3/8; P_1. 1.14; P_2. 1/8; A 2/5
3	*Danio devario*	• Scales on lateral line (44-46) • Scales around caudal peduncle (16), barbels (0). • Total soft rays- Dorsal fin (16-17), caudal fin (forked), anal fin (16-17), paired fins – pectoral (14-17), pelvic- (9-9) • Fin formula: D. 2-3/15-16; P_1.12; P_2. 8; A. 2-3/17
4	*Tor putitora*	• Scales on lateral line (25-28), pored lateral line scales (24-24) • Scales rows above lateral line (4.5-4.5), scale rows below lateral line (2.5-2.5), scales around caudal peduncle (11-14), barbels (2). • Total soft rays- Dorsal fin (8-8), spines (4-4), caudal fin (forked,), anal fin (5-5), spines (2-2), paired fins – pectoral (16-17), pelvic- (8-8) • Fin formula: D. 2/9; P_1. 15; P_2. 9; A. 2/5
5	*Naziritor chelynoides*	• It has thick lips with horny covering which help feed on algae. • The mouth is transverse cleft which is situated towards the ventral side, behind the lip of the snout. • Gill rakers were short and stumpy, a typical case of omnivorous type
6	*Labio rohita*	• Scales on lateral line (40-62), barbels (0). • Dorsal fin with 12-14 (1/2) branched rays; lower profile of head conspicuously arched; short dorsal fin with anterior branched rays shorter than head; 12-16 pre-dorsal scales; snout without lateral lobe • Fin formula: D. 15-16; P_1. 16-17; P_2. 9; A. 7
7	*Labio angra*	• Scales on lateral line (42), barbels (short a pair). • Dorsal fin with 13 (3/10) branched rays; lower profile of head conspicuously arched; short dorsal fin with anterior branched rays shorter than head; 12-16

		pre-dorsal scales; pectoral fin as long as head and caudal fin deeply forked • Fin formula: D. 13(3/10); P_1 10; P_2 9(1/8); A. 7(5/2)
8	*Cirrhinus mrigala*	• Scales on lateral line (40-45), barbels (short a pair), pharyngeal teeth in three rows • Color of the body is silvery, dark gray along the back, sometimes coppery. Pectoral, ventral and anal fins are tinged with black. Eyes are golden. • Fin formula: D. 16 (3/13); P_1. 17; P_2. 9 (1/8); A. 8(3/5)
9	*Chagunius chagunio*	• Scales on lateral line (44-47), scale rows below lateral line (6-6), Scales around caudal peduncle (23-25), barbels (0). • Total soft rays- Dorsal fin (13-13), spines (0-0), caudal fin (forked,), anal fin (8-8), spines (0-0), paired fins – pectoral (16-16), pelvic- (9-9) • Fin formula: D. 11(3/8); P_1. 15; P_2. 9; A. 8 (3/5)
10	*Catla catla*	• Scales on lateral line (40-43), barbels (0). • Dark gray above, silvery on sides and abdomen. Fins are blackish and pectoral pale • Fin formula: D. 18; P_1. 20; P_2. 9; A. 8
11	*Ctenopharyngodon idella*	• Scales on lateral line (40-45), barbels (0). • Total soft rays- Dorsal fin (7-8), spines (3-3), caudal fin (forked,), anal fin (7-11), spines (3-3), paired fins – pectoral (15-20), pelvic- (6-8) • Body olive to brassy green above, silvery white to yellow below; pharyngeal teeth usually in two rows • Fin formula: D. 3/7; P_1. 1/17; P_2. 1/8; A. 3/7-8
12	*Ciprinus carpio*	• Scales on lateral line (36-37), barbels (2 pairs). • Total soft rays- Dorsal fin (17-23), spines (3-4), caudal fin deeply emarginate, anal fin (5-6), spines (2-30) • Body grey to brown, pharyngeal teeth usually in two rows • Fin formula: D. 3-4/14-19; P_1. 1/16-18; P_2. 1/7-8; A. 2-3/5

13	*Mystus bleekeri*	• It has four pairs barbel, its maxillary barbels long up to anal fin sometimes larger than anal fin. • Dorsal spine smooth. Adipose fin present. Caudal fin forked. The least height of caudal peduncle about 2 times its height • Fin formula: D. I/7; P_1-1/9-10; P_2. 6; A.9-10
14	*Heteropneustes fossilis*	• It has four pairs barbel, its maxillary pairs extend to end of pectorals or to commencement to anal and mandibular pairs extend up to base of pelvics but nasal pair considerably shorter than mandibular pairs • Body color reddish brown or purplish brown but in mature stage of specimens it shows black in color • Fin formula: D. 6-7; P_1. 1/6-7; P_2. 6; A. 62-70
15	*Clarias batrachus*	• Barbels (0). • Total soft rays- Dorsal fin (60-76), spines (0-0), caudal fin more or less truncate, anal fin (47-58), spines (0-0), paired fins – pectoral (8-11), spine (1), pelvic- (6-6) • Fin formula: D. 64-70; P_1. 1/9-10; P_2. 6. A. 45-52
16	*Xenentodon cancila*	• Barbels (0). • Total soft rays- Dorsal fin (15-18), spines (0-0), caudal fin more or less truncate, anal fin (16-18), spines (0-0), paired fins – pectoral (11), pelvic- (6) • Green-silvery dorsally, grading to whitish below • Fin formula: D. 15-16; P_1. 10-11; P_2. 6; A. 17-18
17	*Mastacembelus armatus*	• Barbels (0). • Total soft rays- Dorsal fin (67-82), spines (33-40), caudal fin more or less truncate, anal fin (67-83), spines (0-0), paired fins – pectoral (24-26) • Body dull brown with 1-3 darker, longitudinal zigzag lines, more or less connected to form a reticulated pattern, more or less distinct and restricted to the dorsal two thirds of the body • Fin formula: D. 32-39/74-90, P_1. 23, A. 3/75-88
18	*Channa punctatus*	• Barbels (0).

		• Pectoral just above pelvic, caudal large and rounded. Pelvic is about 75% of pectoral fin length • scales on lateral line • Body color generally yellowish to brown on back and lighter below • Fin formula: D. 29-32; P_1. 17; P_2. 6; A. 21-23; C. 12
19	*Orechromis niloticus*	• Lateral line (2), scales on lateral line (32-34), barbels (0). • Total soft rays- Dorsal fin (12-13), spines (16-18), caudal fin deeply emarginate, anal fin (10), spines (3-3) • about 9 narrow dark cross-bars on sides; soft dorsal and caudal fin sharply barred; anal fin faintly barred; dark blotch at angle of operculum • Fin formula: D. 15-16/10-12; P_1. 14-15; P_2. I/5; A. 3/10-12
20	*Chanda nam*a	• Lateral line is partly distinct, partly absent. Second dorsal spine is longest. • Caudal fin forked, black and orange in colour. Body is transparent yellowish white with numerous tiny black dots. • A small black spot is found at the origin of the base of anal fin. • Lower jaw is longer than upper jaw • Fin formula: D1. 8; D2. 1/16-17; P_1. 12-13; P_2. I/5; A. 3/16-17