Flora and Fauna
An International Research Journal of Biological Sciences
Journal recognised by DST New Delhi (NAAS RATING 4.74)
ISSN 2456-9364(Online) 0971-6920(Print)

WATERSHED MICROBIOMES AND HABITAT SUITABILITY OF THE ORIENTAL DARTER IN A UNIVERSITY CAMPUS ECOSYSTEM

Biswa Jyoti Singha[1], P. Nicolas Enghee[1], Joydeep Ghosh[2], Lima Hazarika [1]*

[1]Department of Zoology, Assam Don Bosco University, Sonapur, 782402, Assam, India
[2]GRED Institute of Geoinformatics, Guwahati, 781007, Assam, India

*Corresponding author: Dr. Lima Hazarika (lima.hazarika@dbuniversity.ac.in)

Abstract
Freshwater ecosystems in semi-urban landscapes are highly sensitive to changes in water quality, biological processes, and land-use patterns. The present study evaluates the habitat suitability of the Oriental Darter (Anhinga melanogaster), a Near Threatened aquatic bird, within the Assam Don Bosco University (ADBU) Tapesia Campus by integrating physicochemical water analysis, microbial community profiling, avian observations, and GIS-based spatial assessment. Physicochemical parameters including pH, dissolved oxygen (DO), free carbon dioxide (CO_2), alkalinity, and hardness were analyzed across selected aquatic sites. Systematic field surveys were conducted to record Oriental Darter sightings and behavioural patterns. Metagenomic analysis of water from preferred habitats was carried out using 16S rRNA gene sequencing, followed by bioinformatic processing to characterize microbial community composition. GIS tools were employed to map land use, bird sightings, and habitat suitability gradients across the campus. The results showed that the Oriental Darter preferentially occupied lake habitats exhibiting neutral pH, higher dissolved oxygen, and lower carbon dioxide levels. These sites supported balanced freshwater microbial communities involved in nutrient cycling, while sites with poorer water quality showed microbes associated with organic enrichment and ecological stress. GIS-based habitat suitability mapping corroborated field observations, identifying spatial clusters of high suitability aligned with favourable water quality and biological conditions. The study highlights the value of integrating water biochemical parameters, microbiome data, avian bioindicators, and geospatial analysis for assessing freshwater ecosystem health. The findings provide baseline ecological information for campus-level conservation planning and demonstrate a replicable framework for monitoring semi-urban aquatic ecosystems.

Keywords: Oriental Darter (*Anhinga melanogaster*); Aquatic Microbiome; Habitat Suitability; GIS; Semi-Urban Ecosystems, 16S rRNA Metagenomics, Avian Bioindicator.

1. Introduction
Freshwater ecosystems are among the most sensitive components of the environment, responding rapidly to changes in physicochemical conditions, biological interactions, and anthropogenic pressures. Water quality parameters such as pH, dissolved oxygen (DO), carbon dioxide (CO_2), alkalinity, and hardness play a critical role in regulating aquatic biodiversity and ecosystem stability (Omer, 2019; Verma et al., 2022). Alterations in these parameters can cascade through trophic levels, affecting microorganisms, fish communities, and higher vertebrates dependent on aquatic habitats.
Aquatic birds are widely recognized as effective bioindicators of wetland health due to their position at higher trophic levels and their reliance on water quality and prey availability (Kovacs, 1992; Mishra et al., 2023). Among them, the Oriental Darter (*Anhinga melanogaster*), a Near Threatened species according to the IUCN Red List (BirdLife International, 2016), is particularly sensitive to changes in freshwater ecosystems. The species prefers clean, well-oxygenated water bodies rich in fish and suitable perching sites, making it an important indicator of wetland integrity (Ali & Ripley, 1978; Surya Babu & Raju Thomas, 2003). Recent advances in environmental microbiology have emphasized the role of aquatic microbiomes as early responders to environmental stress and water quality changes. Microbial community composition reflects nutrient status, organic load, and oxygen availability, providing valuable insights into ecosystem health (Lange-Bertalot,

1979; Verma et al., 2022). However, studies integrating microbiome structure with avian habitat use and spatial analysis remain limited, particularly at local scales in Northeast India.

The Assam Don Bosco University (ADBU) Tapesia Campus represents a semi-urban green landscape with multiple aquatic habitats and high avian diversity, offering a unique opportunity to study ecological interactions in a controlled landscape. Therefore, the present study aimed to evaluate the habitat suitability of the Oriental Darter at ADBU Tapesia Campus by integrating water quality assessment, watershed microbiome profiling, and GIS-based spatial analysis within a single environmental monitoring framework.

2. Materials and Methods

2.1 Study Area: The study was conducted at the Assam Don Bosco University (ADBU) Tapesia Campus, Kamrup Metropolitan District, Assam, India (26.1285° N, 91.9021° E). The campus comprises lakes, ponds, and seasonal water channels surrounded by semi-natural vegetation and limited built-up areas. Three sites were selected: two lakes (Site 1 and Site 3) and one pond (Site 2), based on size, disturbance level, and preliminary bird presence. (**Figures 1 and 3**)

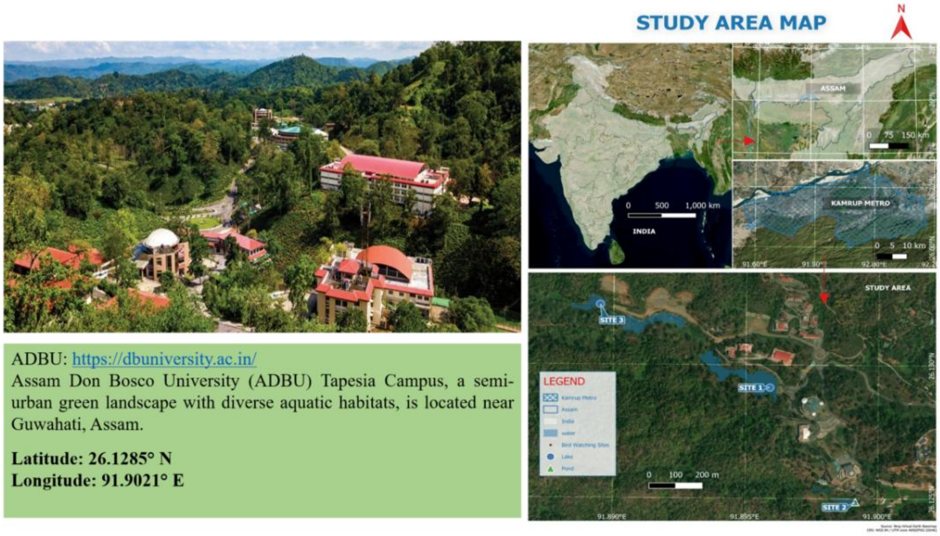


Figure 1: Map of the Study area in the ADBU campus and the water body Site

2.2 Bird Observation: Oriental Darter sightings were recorded twice weekly during early morning (06:00–08:30 h) and late afternoon (16:00–18:00 h) (**Figures 2 and 3**). Observations included habitat type, behaviours (basking, resting, flight), and frequency. Species identification was confirmed using standard field guides (Ali & Ripley, 1978).

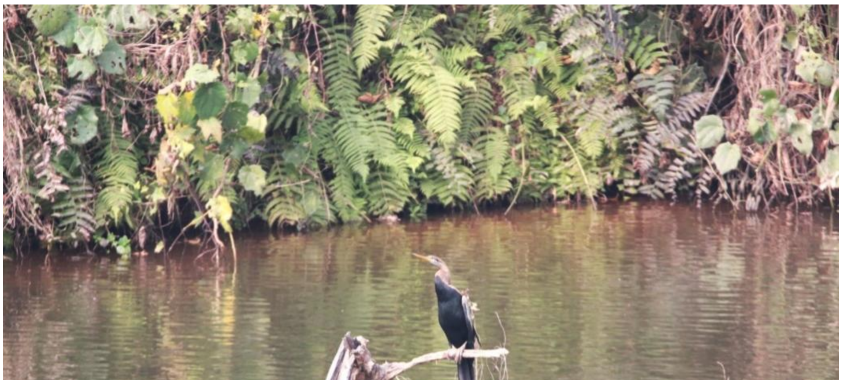


Figure 2: Oriental Darter (*Anhinga melanogaster*) observed perching on a submerged log at a freshwater lake within the ADBU Tapesia Campus, exhibiting typical basking behaviour indicative of suitable aquatic habitat conditions.

2.3 Physicochemical Analysis of Water: Physicochemical analysis of water was carried out monthly at all three sites. Parameters analyzed included pH (portable digital pH meter), dissolved oxygen (Winkler titration method), free CO_2 (titrimetric method), alkalinity, and hardness following standard protocols (APHA, 2017; Omer, 2019).

2.4 GIS-Based Habitat Mapping: Spatial analysis was conducted using QGIS (version 3.28). Layers included water quality parameters, land-use/land-cover, vegetation distribution, and bird sighting coordinates. Habitat suitability maps and heatmaps were generated using weighted overlay analysis (Lillesand et al., 2015; Wu, 2017).

2.5 Metagenomic Analysis: Water samples from habitats with high Oriental Darter activity were selected for microbiome analysis. DNA extraction was followed by amplification of the V3–V4 region of the 16S rRNA gene and sequencing on the Illumina MiSeq platform. Bioinformatic processing, taxonomic classification, and diversity analysis were performed using QIIME2 (Bolyen et al., 2019) and the SILVA reference database. Alpha diversity indices (Shannon, Chao1) were calculated using the vegan R package (**Figure 3**).

Figure 3: Workflow of the integrated methodology adopted for the study. Step 1-4: Stepwise methodological approach illustrating physicochemical analysis of water, Oriental Darter observation, GIS-based habitat mapping, and metagenomic analysis.

3. Results

3.1 Water Quality: Lake sites exhibited neutral to slightly alkaline pH, higher DO, and lower CO_2 compared to the pond site. Site 3 showed the most stable and favourable conditions, while Site 2 (pond) recorded lower DO and higher buffering alkalinity, indicating reduced water quality (**Table 1 and Figure 4**). Similar mean values observed for Sites 1 and 3 reflect comparable seasonal water quality conditions across the two lake habitats.

Table 1: Mean physicochemical parameters of water from three aquatic sites at ADBU campus.

Sl. No.	Site ID	pH	Free CO_2 (mg L^{-1})	DO (mg L^{-1})	Alkalinity (mg L^{-1}as $CaCO_3$)	Water Hardness (mg L^{-1} as $CaCO_3$)
1	Site 2: Pond Water	5.70 ± 0.15	0.97± 0.08	1.08± 0.10	0.70± 0.06	3.43± 0.21
2*	Site 1: Lake Water	6.39± 0.12	0.85± 0.07	0.79± 0.09	0.98± 0.05	3.79 ± 0.18

3*	Site 3: Lake Water	6.41± 0.11	0.83± 0.06	0.81± 0.08	0.97± 0.04	3.80± 0.17

"*" Values represent seasonal mean concentrations ± standard deviation.

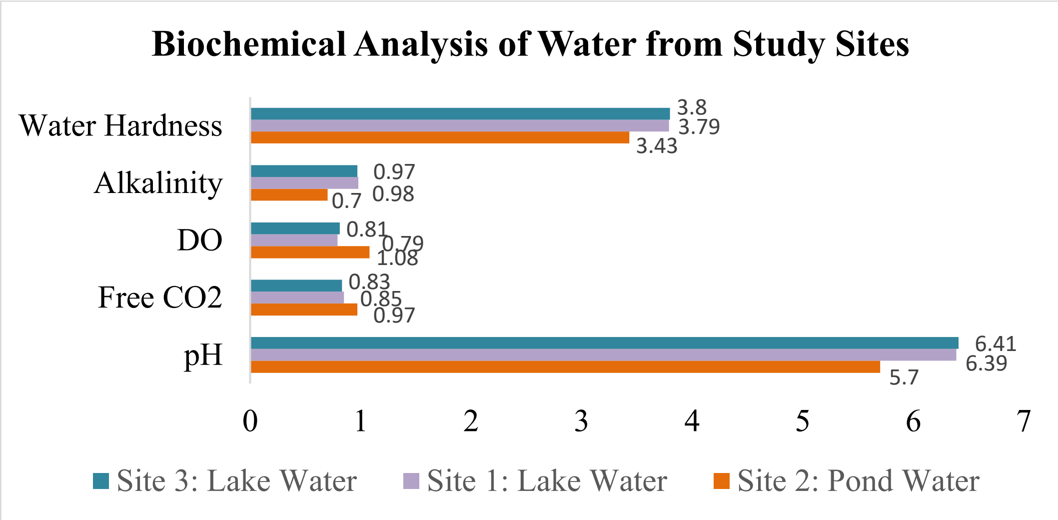


Figure 4: Physicochemical comparison among the water samples collected from Sites 1, 2, and 3.

3.2 Oriental Darter Distribution and Behaviour: Out of 19 sightings, 13 were recorded in lake habitats. Active behaviours (basking, resting, flight) occurred exclusively in lakes, while pond sites showed either inactivity or the absence of the species.

Site 1: lake water

Sighting ID	Date	Time	Latitude	Longitude	Habitat Type	Activity	pH	DO (mg L^{-1})	CO2(mg L^{-1})	Alkalinity (mg L^{-1})	Hardness (mg L^{-1})
WB_1Site	19/09/2024	13:15	26° 7'47.87"N	91°53'37.98"E	Lake	resting	6.66	0.82	0.85	0.86	3.4
WB_1Site	27/.09/2024	14:15	26° 7'47.06"N	91°53'39.65"E	Lake	flight	6.65	0.84	0.93	0.97	3.2
WB_1Site	15/10/2024	13:33	26° 7'48.83"N	91°53'36.15"E	Lake	sun basking	6.68	0.83	2.2	1.56	3.4
WB_1Site	6/11/2024	14:23	26° 7'44.95"N	91°53'45.28"E	Lake	sun basking	6.72	0.92	1	1.23	4.2
WB_1Site	8/11/2024	13:15	26° 7'48.15"N	91°53'36.66"E	Lake	flight	6.71	0.89	0.93	1.07	4.1
WB_1Site	11/11/2024	14:07	26° 7'48.83"N	91°53'36.15"E	Lake	resting	6.69	0.91	0.89	0.89	4.4
WB_1Site	16/11/2024	12:47	26° 7'47.06"N	91°53'39.65"E	Lake	sun basking	6.69	0.93	0.86	0.53	4.6
WB_1Site	23/11/2024	13:47	26° 7'48.15"N	91°53'36.66"E	Lake	sun basking	6.68	0.95	0.85	0.67	4.4
WB_1Site	30/11/2024	13:33	26° 7'48.83"N	91°53'36.15"E	Lake	resting	6.72	0.97	0.84	0.71	4.3
WB_1Site	3/12/2024	13:15	26° 7'44.95"N	91°53'45.28"E	Lake	sun basking	6.68	1.12	0.81	0.75	4.1
WB_1Site	9/12/2024	6:45	26° 7'47.48"N	91°53'38.28"E	Lake	flight	6.67	1.23	0.83	0.73	4.2
WB_1Site	14/12/2024	12:33	26° 7'44.95"N	91°53'45.28"E	Lake	sun basking	6.68	1.24	0.86	0.75	4.2
WB_1Site	15/01/2025	6:30	26° 7'48.15"N	91°53'36.66"E	Lake	sun basking	6.72	1.32	0.78	0.77	3.45
WB_1Site	28/01/2025	13:23	26° 7'48.83"N	91°53'36.15"E	Lake	resting	6.65	1.53	0.73	0.80	2.43
WB_1Site	14/02/2025	6:52	26° 7'44.95"N	91°53'45.28"E	Lake	flight	6.67	1.16	0.85	0.83	3.56

Table 2 (a): Frequency of Oriental Darter (*Anhinga melanogaster*) sightings recorded at Site 1 during the study period.

Site 2: Pond Water

Sighting ID	Date	Time	Latitude	Longitude	Habitat Type	Activity	pH	DO (mg L^{-1})	CO2(mg L^{-1})	Alkalinity (mg L^{-1})	Hardness (mg L^{-1})
WB_2site	6/11/2024	15:07	26° 7'29.56"N	91°53'56.98"E	Pond	None	6.47	0.86	1.16	1.61	4.5
WB_2site	16/11/2024	13:15	26° 7'29.56"N	91°53'56.98"E	Pond	None	6.35	0.81	0.85	0.63	4.7
WB_2site	9/12/2024	7:15	26° 7'29.56"N	91°53'56.98"E	Pond	None	6.47	0.56	0.83	0.66	4.5
WB_2site	20/12/2024	8:00	26° 7'29.56"N	91°53'56.98"E	Pond	None	6.37	0.61	1	0.71	3.89
WB_2site	15/01/2025	7:12	26° 7'29.56"N	91°53'56.98"E	Pond	None	6.27	0.73	0.75	0.74	3.17
WB_2site	28/01/2025	8:12	26° 7'29.56"N	91°53'56.98"E	Pond	None	6.35	0.86	0.66	0.96	1.96
WB_2site	14/02/2025	7:15	26° 7'29.56"N	91°53'56.98"E	Pond	None	6.28	1	0.66	0.86	2.46

Table 2 (b): Frequency and behavioural activity of Oriental Darter (*Anhinga melanogaster*) observed at Site 2 during the study period.

Site 3: lake water

Sighting ID	Date	Time	Latitude	Longitude	Habitat Type	Activity	pH	DO (mg L^{-1})	CO2(mg L^{-1})	Alkalinity (mg L^{-1})	Hardness (mg L^{-1})
WB_3Site	19/09/2024	7:17	26° 7'53.95"N	91°53'32.68"E	Lake	sun basking	7.19	0.84	0.86	0.83	3
WB_3site	30/09/2024	7:24	26° 7'53.79"N	91°53'31.45"E	Lake	resting	7.24	0.85	0.93	0.91	3.3
WB_3Site	15/10/2024	11:03	26° 7'52.55"N	91°53'25.61"E	Lake	flight	7.21	0.86	1.3	1.23	3.5
WB_3Site	29/10/2024	7:15	26° 7'54.06"N	91°53'29.94"E	Lake	sun basking	7.23	0.85	0.83	1.63	4.6
WB_3Site	6/11/2024	8:15	26° 7'56.09"N	91°53'22.17"E	Lake	resting	7.22	0.82	0.80	1.5	3.7
WB_3Site	14/11/2024	7:31	26° 7'53.79"N	91°53'31.45"E	Lake	flight	7.18	0.79	0.8	0.98	4.3
WB_3Site	26/11/2024	15:21	26° 7'52.55"N	91°53'25.61"E	Lake	sun basking	7.17	0.77	0.82	0.63	4.9
WB_3site	5/12/2024	7:22	26° 7'53.95"N	91°53'32.68"E	Lake	resting	7.21	0.70	0.83	0.61	4.5
WB_3Site	13/12/2024	7:17	26° 7'53.79"N	91°53'31.45"E	Lake	resting	7.21	0.56	0.86	0.53	4.52
WB_3Site	20/12/2024	7:34	26° 7'56.09"N	91°53'22.17"E	Lake	resting	7.24	0.68	0.76	0.58	3.73
WB_3Site	15/01/2025	12:37	26° 7'54.06"N	91°53'29.94"E	Lake	flight	7.21	0.83	0.79	0.61	3.12
WB_3Site	28/01/2025	7:15	26° 7'53.79"N	91°53'31.45"E	Lake	flight	7.24	1.68	0.63	0.66	2.53
WB_3Site	14/02/2025	7:43	26° 7'56.09"N	91°53'22.17"E	Lake	sun basking	7.19	1.6	0.73	0.73	3.8

Table 2 (c): Frequency and behavioural activity of Oriental Darter (*Anhinga melanogaster*) recorded at Site 3 during the study period.

Table 2(a-c): The records show clear differences in Oriental Darter activity across sites, with active behaviours restricted to lake habitats.

3.3 Microbiome Composition: Metagenomic analysis revealed dominance of *Proteobacteria*, *Bacteroidota*, *Firmicutes*, and *Actinobacteriota*. Beneficial freshwater genera such as *Limnohabitans* and *Nitrosomonas* were more abundant in lake samples, whereas pond samples showed higher proportions of pollution-associated taxa. (Table 3) The identified species are further discussed (**Table 4**) showing the ecological significance with respect to bird habitat in both sites 2 and 3.

Table 3: Relative abundance of the fifteen most dominant bacterial species detected in the water samples from Site 2 and Site 3 of the ADBU campus using 16S rRNA (V3–V4) metagenomic sequencing.

Sl. No.	Species Identified	Site 2	Site 3
1	*Bacteroides_H 1174 768*	1174	768
2	*Pelagibacter_A_533952*	826	590
3	*Akkermansia*	1131	569
4	*Aquabacterium_B_592457*	0	553
5	*Prevotella*	942	534
6	*Metamycoplasma*	1066	519
7	*Phocaeicola_A_858004*	513	436
8	*Fuerstia spp*	169	432
9	*UBA3015*	48	302
10	*Armatimonas*	95	301
11	*Streptococcus*	631	273
12	*Klebsiella_724518*	518	250
13	*Escherichia_710834*	526	201
14	*Methanobrevibacter_A smithii_A_1174*	318	291
15	*Limnohabitans_A*	1102	166

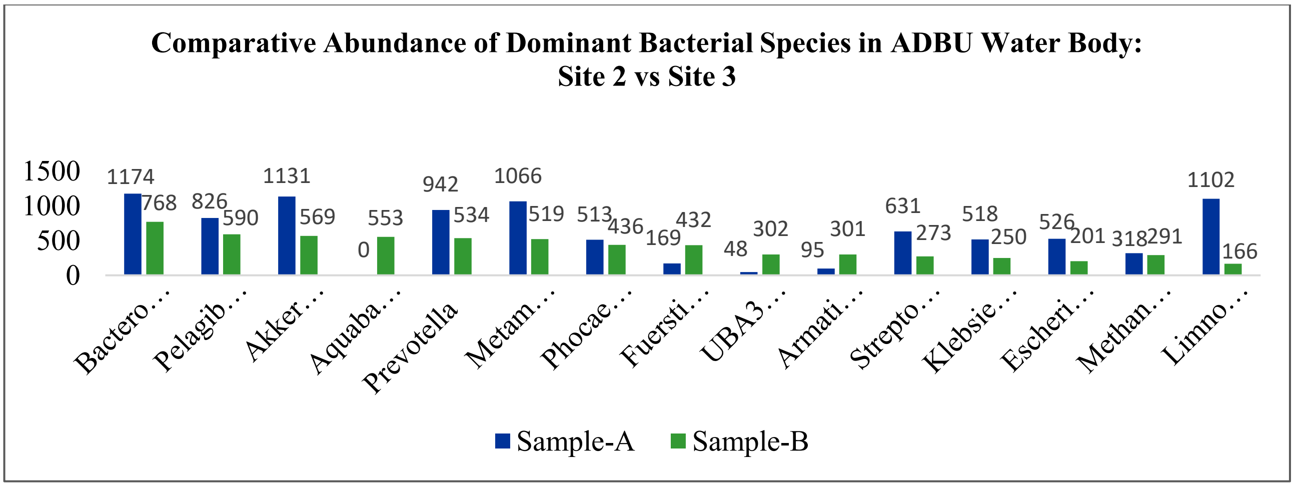


Figure 5: Comparative abundance of dominant bacterial species identified in the water samples from Site 2 and Site 3 of the ADBU campus. The bar plot shows differences in the relative abundance of selected taxa based on 16S rRNA metagenomic sequencing. **Table 3** and **Figure 5** present a comparative overview of

species-level differences between the two sites, reflecting localized ecological conditions and microbial responses.

3.4 GIS-Based Habitat Suitability: GIS maps identified Site 3 as highly suitable, Site 1 as moderately suitable, and Site 2 as unsuitable for Oriental Darter habitation. These spatial patterns closely matched field observations and water quality data and are shown in **Figures 6.1 to 8**. The habitat preference map (**Figure 6**.1) showed that Site 3 was categorized as a highly suitable habitat, characterized by low anthropogenic disturbance, stable water quality conditions, and dense surrounding vegetation. Site 1 was identified as moderately suitable, where darter presence was recorded despite proximity to human activity. In contrast, Site 2 was classified as poorly suitable, corresponding to degraded water quality and higher levels of disturbance, which aligned with the absence or minimal activity of the species. Land use and land cover analysis (**Figure 7**) indicated that vegetation cover was dominant across most of the campus, supporting its role as a biodiversity-rich landscape. However, barren and built-up areas were more prominent around Sites 1 and 2, whereas Site 3 was located within a relatively undisturbed vegetated zone, providing favourable perching, foraging, and resting conditions for the Oriental Darter. This spatial distribution highlights the influence of surrounding land cover in shaping habitat suitability.

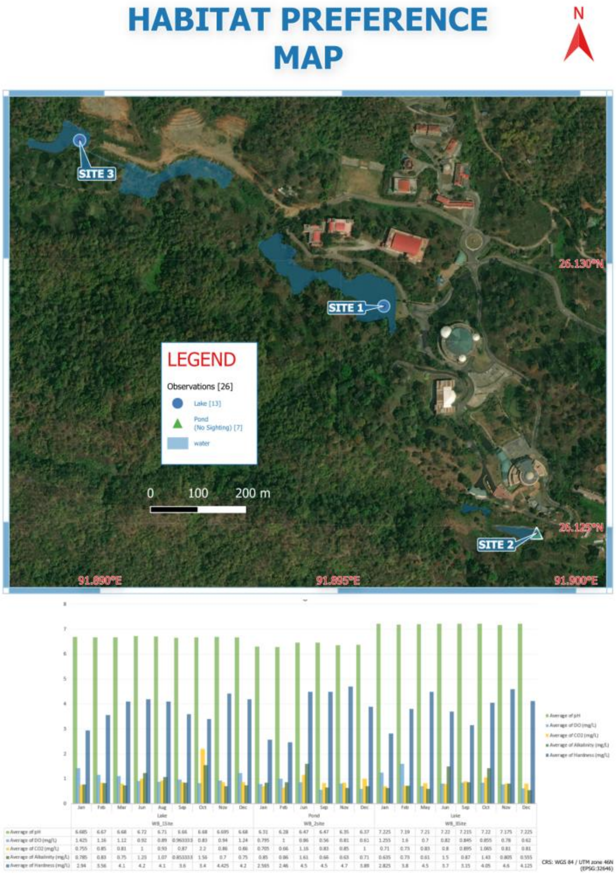


Figure 6.1: GIS-based habitat preference map of the Oriental Darter at ADBU, Tapesia Campus, showing sites categorized as highly suitable, moderately suitable and poorly suitable based on water quality, habitat structure and disturbance levels.

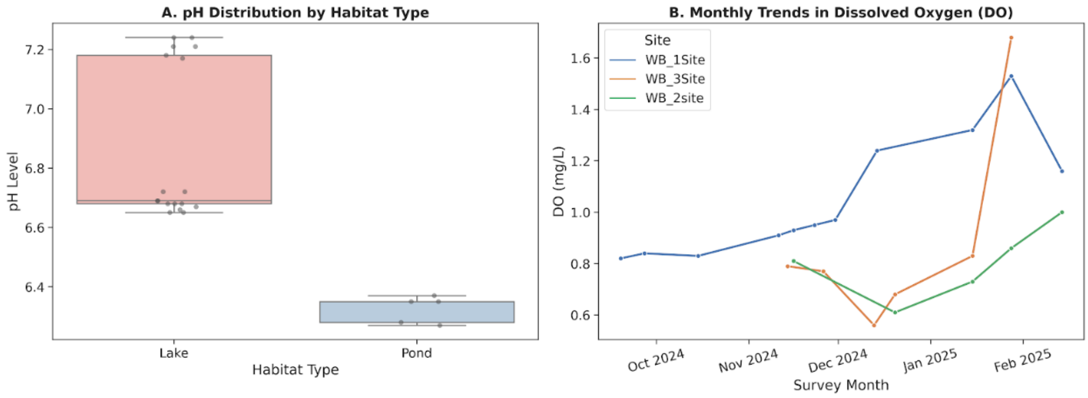


Figure: 6.2: Variation in water quality parameters across habitats, showing pH differences between lake and pond systems and monthly trends in dissolved oxygen across surveyed sites influencing Oriental Darter habitat suitability.

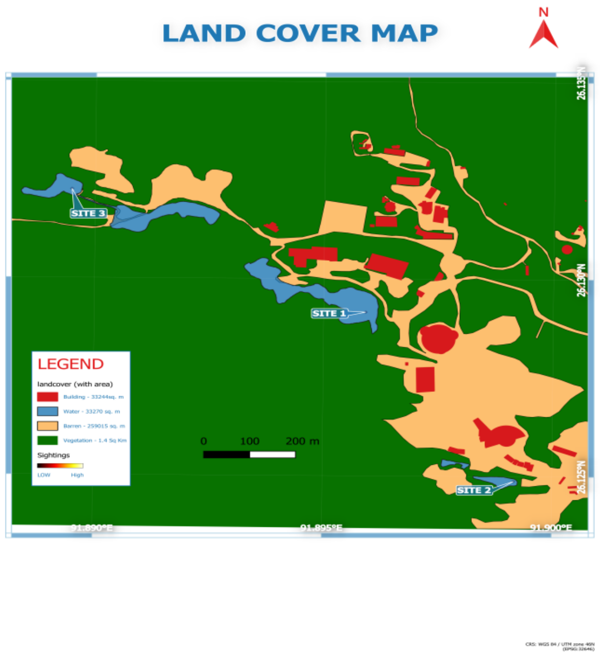
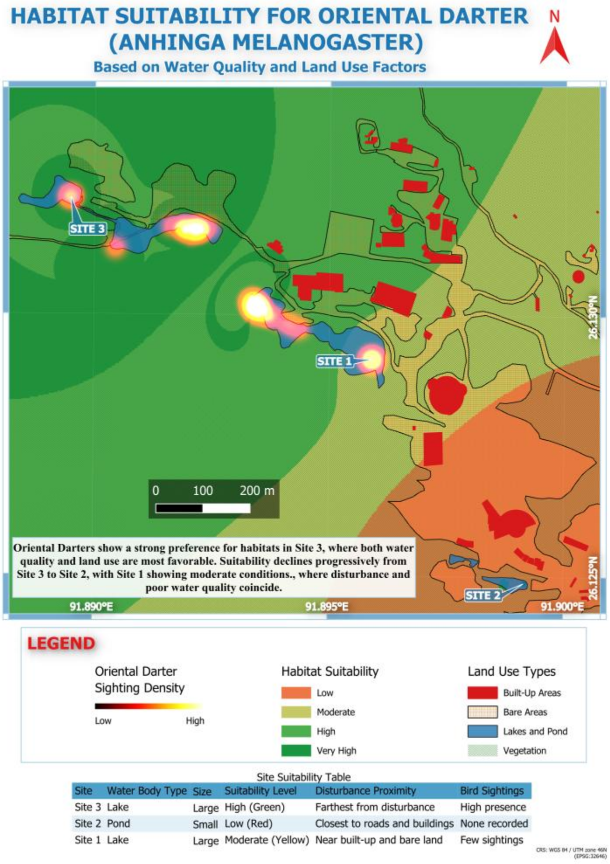


Figure 7: Land use and land cover (LULC) map of the ADBU, Tapesia Campus, illustrates the vegetation-dominated areas, built-up zones, and barren land in relation to the studied aquatic sites.



Site Suitability Table

Site	Water Body Type	Size	Suitability Level	Disturbance Proximity	Bird Sightings
Site 3	Lake	Large	High (Green)	Farthest from disturbance	High presence
Site 2	Pond	Small	Low (Red)	Closest to roads and buildings	None recorded
Site 1	Lake	Large	Moderate (Yellow)	Near built-up and bare land	Few sightings

Figure 8: Integrated habitat suitability map generated by overlaying water quality parameters, Oriental Darter sighting locations, and land use patterns, highlighting Site 3 as the most suitable habitat.

The integrated habitat suitability map (**Figure 8**), generated by overlaying water quality parameters, bird sighting locations, and land-use data, further validated field observations. Site 3 emerged as the most suitable habitat, consistently overlapping with frequent Oriental Darter sightings, while Site 2 showed negligible suitability, corresponding to poor water quality and lack of bird presence. The spatial patterns observed in the GIS outputs clearly demonstrate that habitat suitability for the Oriental Darter at ADBU Tapesia Campus is strongly influenced by a combination of water quality conditions, habitat structure, and land-use characteristics.

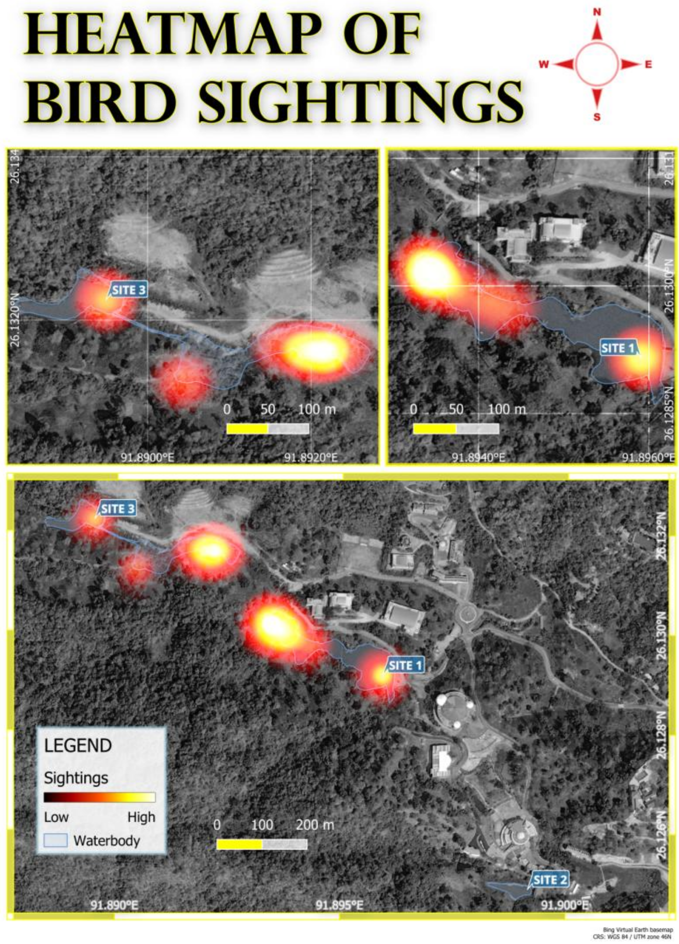


Figure 9: The Heatmap of the bird sightings is generated by recording the coordinates of the Oriental Darter sightings with the help overlaying water quality parameters, and land use patterns, highlighting Site 1 and 3 as the most suitable habitat, which are both lakes.

Figure 9 illustrates the spatial distribution and intensity of Oriental Darter sightings across the study sites using a heat map approach. High-density clusters of sightings were observed around Site 3 and Site 1, indicating frequent use of these water bodies by the species. In contrast, Site 2 exhibited little to no clustering, reflecting minimal or absent bird presence. The heat map visually confirms the non-uniform distribution of sightings and highlights preferred habitats in relation to water bodies within the ADBU Tapesia Campus.

4. Discussion:

The present study demonstrates the importance of integrating water physicochemical parameters, microbial community structure, avian habitat preference, and GIS-based spatial analysis to achieve a holistic understanding of freshwater ecosystem health in a semi-urban university campus. The microbial taxa identified at Site 2 and Site 3 (Table 3) were studied for the ecological significance and understood the clear ecological contrasts between habitats that were avoided and preferred by the Oriental Darter (*Anhinga melanogaster*) (Table 4), thereby reinforcing the role of aquatic microbiomes as sensitive indicators of water quality and habitat suitability. From the study, it was observed that Oriental Darter showed a clear preference for habitats with near-neutral to slightly alkaline pH and relatively higher dissolved oxygen levels, indicating that stable pH and adequate oxygen availability play a key role in determining its habitat suitability (**Figure 6.2**).

In context of microbial communities of the aquatic environment, several dominant genera, such as *Bacteroides*, *Prevotella*, *Streptococcus*, *Klebsiella*, and *Escherichia,* were present in higher relative abundance at Site 2, which coincided with lower dissolved oxygen levels and limited bird activity. These taxa are commonly associated with organic enrichment, anthropogenic inputs, and reduced water quality (Palmer, 1969; Omer, 2019; Verma et al., 2022). Elevated abundance of such microbes suggests increased organic loading and potential eutrophication, conditions that negatively affect fish populations and, consequently, piscivorous birds like the Oriental Darter. In contrast, Site 3, which was identified as highly suitable through GIS-based habitat mapping, supported higher abundances of freshwater-associated and ecologically beneficial taxa such as *Limnohabitans*, *Aquabacterium*, *Fuerstia*, and *Armatimonas*. These genera are frequently linked to stable, oxygen-rich freshwater systems and efficient nutrient cycling (Kalmbach et al., 1999; Hahn et al., 2010; Tamaki et al., 2011), conditions that promote healthy planktonic communities and sustain fish availability. The presence of *Pelagibacter* (SAR11 clade), a key bacterium involved in carbon cycling in oligotrophic waters, further suggests relatively balanced nutrient conditions at the preferred habitat site (Morris et al., 2002). The detection of methanogenic archaea such as *Methanobrevibacter smithii* at both sites indicates localized anaerobic microzones associated with organic matter decomposition; however, their comparable abundance suggests that anaerobic processes alone did not dictate habitat suitability but acted in conjunction with broader water quality conditions (Stumm and Morgan, 1981).

Table 4: Dominant microbial taxa detected at Site 2 and Site 3 and their ecological significance in relation to Oriental Darter habitat suitability.

Sl. No.	Species Identified	Site 2	Site 3	Ecological significance with respect to bird habitat	References
1	*Bacteroides* (H)	1174	768	Indicative of organic matter decomposition; elevated abundance may reflect nutrient enrichment influencing aquatic food webs	Omer, 2019; Verma et al., 2022
2	*Pelagibacter* (SAR11 clade)	826	590	Dominant freshwater bacterium involved in carbon cycling; reflects relatively stable oligotrophic conditions	Morris et al., 2002; Verma et al., 2022
3	*Akkermansia*	1131	569	Associated with organic matter utilization; indirectly reflects availability of microbial substrates in water	Omer, 2019
4	*Aquabacterium*	0	553	Common freshwater bacterium linked to biofilm formation and clean water systems; supports stable aquatic habitats	Kalmbach et al., 1999
5	*Prevotella*	942	534	Indicates organic input and nutrient availability; excessive levels may suggest eutrophication	Palmer, 1969; Omer, 2019
6	*Metamycoplasma*	1066	519	Often associated with organic-rich environments; may indicate moderate ecological stress	Verma et al., 2022
7	*Phocaeicola*	513	436	Organic matter degrader; reflects carbon availability influencing microbial food webs	Omer, 2019
8	*Fuerstia* spp.	169	432	Planctomycetes-associated genus; linked to nutrient cycling and	Fuerst, 1995

				aquatic ecosystem stability	
9	*UBA3015*	48	302	Poorly characterized environmental bacterium; presence suggests diverse and complex microbial assemblages	Hug et al., 2016
10	*Armatimonas*	95	301	Indicator of natural freshwater systems with moderate nutrient levels	Tamaki et al., 2011
11	*Streptococcus*	631	273	Presence may indicate anthropogenic influence or organic contamination; excessive abundance linked to poorer habitat quality	Omer, 2019
12	*Klebsiella*	518	250	Known pollution-associated bacterium; higher abundance suggests reduced water quality	Verma et al., 2022
13	*Escherichia*	526	201	Indicator of fecal or organic contamination; negatively associated with pristine bird habitats	Palmer, 1969; Omer, 2019
14	*Methanobrevibacter smithii*	318	291	Methanogenic archaeon; reflects anaerobic micro-zones and organic matter breakdown	Stumm & Morgan, 1981
15	*Limnohabitans*	1102	166	Beneficial freshwater bacterium linked to well-oxygenated waters and healthy planktonic food webs supporting fish availability	Hahn et al., 2010

The Oriental Darter's clear preference for Site 3 aligns with previous studies highlighting its dependence on clean, well-oxygenated waters and structurally undisturbed habitats (Ali and Ripley, 1978; Surya Babu and Raju Thomas, 2003). From an environmental science perspective, this study underscores the ecological relevance of water biochemical analysis, as parameters such as pH, dissolved oxygen, free CO_2, alkalinity, and hardness not only influence microbial assemblages but also cascade through trophic levels to affect higher vertebrates. Microbial community composition acts as an early-warning signal of ecosystem stress, often responding more rapidly to environmental change than visible fauna (Kovacs, 1992; Lange-Bertalot, 1979). The inclusion of metagenomic analysis therefore strengthens conventional water quality assessment by revealing underlying biological processes that may not be evident through physicochemical parameters alone. Importantly, the GIS-based habitat suitability mapping provided spatial validation of these relationships by integrating water quality data, microbial indicators, land-use patterns, and bird sightings into a single analytical framework. The identification of Site 3 as a high-suitability zone and Site 2 as an unsuitable habitat corroborated field observations and microbial signatures, demonstrating the effectiveness of GIS as a decision-support tool for environmental monitoring and conservation planning (Lillesand et al., 2015; Wu, 2017).

The significance of this study lies in its interdisciplinary approach, which bridges environmental chemistry, microbial ecology, avian biology, and geospatial science at a local scale - an area often overlooked in broader regional assessments. By focusing on a university campus ecosystem, the study highlights how semi-urban landscapes can function as biodiversity refuges while simultaneously remaining vulnerable to subtle ecological degradation. The findings have practical implications for eco-campus management, wetland restoration, and biodiversity conservation, particularly for Near Threatened species such as the Oriental Darter that rely on high-quality freshwater habitats (BirdLife International, 2016). Furthermore, this work opens avenues for future research, including long-term monitoring of microbial functional genes, seasonal trophic interactions between microbes and fish, and predictive habitat modeling under climate and land-use change scenarios. Expanding the framework to include additional bioindicator species and functional metagenomics would further enhance ecosystem-level understanding. In conclusion, the integrated analysis presented here demonstrates that combining water biochemical parameters, microbial community structure, avian habitat preference, and GIS-based spatial analysis provides a robust and replicable approach for assessing freshwater ecosystem health and guiding evidence-based conservation strategies.

5. Conclusion

The present study demonstrates that habitat suitability of the Oriental Darter (*Anhinga melanogaster*) at the ADBU Tapesia Campus is closely governed by the combined influence of water biochemical parameters, microbial community composition, and spatial habitat characteristics. The species showed a clear preference for aquatic habitats exhibiting neutral pH, higher dissolved oxygen, and lower free carbon dioxide, conditions that also supported balanced and ecologically stable microbial assemblages. Metagenomic insights further revealed that preferred habitats were characterized by freshwater-associated and functionally beneficial microbial taxa, whereas sites with poorer water quality harboured microbe's indicative of organic enrichment and ecological stress. The integration of GIS-based habitat mapping effectively translated these physicochemical and biological relationships into spatial patterns, identifying zones of high, moderate, and low suitability across the campus landscape. Collectively, these findings highlight the ADBU campus as a functional semi-urban freshwater ecosystem capable of supporting sensitive avian bioindicators when environmental conditions remain favourable. The study provides valuable baseline ecological data for campus-level wetland conservation and eco-management initiatives and establishes an interdisciplinary, replicable framework for monitoring freshwater ecosystem health in similar semi-urban landscapes

7. References

1. Ali, S. and Ripley, S.D. (1978). *Handbook of the birds of India and Pakistan*, Vol. 1 (2nd Ed.). Oxford University Press, New Delhi, pp. 43–46.
2. APHA (2017). *Standard methods for the examination of water and wastewater* (23rd Ed.). American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC. https://www.standardmethods.org/
3. BirdLife International (2016). *Anhinga melanogaster*. The IUCN Red List of Threatened Species 2016:e.T22696712A93582012. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22696712A93582012.en
4. Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F. and Bai, Y. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, **37**, 852–857. https://doi.org/10.1038/s41587-019-0209-9
5. Fuerst, J.A. (1995). The Planctomycetes: emerging models for microbial ecology, evolution and cell biology. *Microbiology*, 141, 1493–1506. https://doi.org/10.1099/13500872-141-7-1493
6. Hahn, M. W., Scheuerl, T., Jezberová, J., Koll, U., Jezbera, J., Šimek, K., & Vannini, C. (2012). The passive yet successful way of planktonic life: Genomic and experimental analysis of the ecology of a free-living Polynucleobacter population. PLOS One, 7(3), e32772. https://doi.org/10.1371/journal.pone.0032772
7. Inam, U., Wu, Q., Sun, Y., Rajpar, M.N., Khan, T.U., Khan, J.A., Ahmad, T. and Rehman, F.U. (2024). Population dynamics and habitat preferences of waterbirds across wetlands at different geographical scales. *Scientific Reports*, **14**, 1–14. https://doi.org/10.21203/rs.3.rs-4640895/v1
8. Kalmbach, S., Manz, W. and Szewzyk, U. (1999). Dynamics of biofilm formation in drinking water: phylogenetic affiliation of microbial populations. *Water Research*, 33, 3441–3451. https://doi.org/10.1111/j.1574-6941.1997.tb00379.x
9. Kovacs, M. (1992). *Biological indicators in environmental protection*. Ellis Horwood, Chichester, UK. https://doi.org/10.1016/S0176-1617(11)82111-5
10. Lange-Bertalot, D. (1979). Pollution tolerance of diatoms as a criterion for water quality estimation.

Nova Hedwigia, Beiheft, **64**, 285–304.
11. Lillesand, T.M., Kiefer, R.W. and Chipman, J.W. (2015). *Remote sensing and image interpretation* (7th Ed.). John Wiley & Sons, New York.
12. Mishra, A., Kumar, S., Patra, R.N., Kumar, A., Chandra, N., Pande, C.B. and Alshehri, F. (2023). Physicochemical parameters of water and their implications on avifauna and habitat quality. *Sustainability*, **15**, 9494. https://doi.org/10.3390/su15129494
13. Morris, R.M., Rappé, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, C.A. and Giovannoni, S.J. (2002). SAR11 clade dominates ocean surface bacterioplankton communities. *Nature*, 420, 806–810. https://doi.org/10.1038/nature01240
14. Omer, N.H. (2019). Water quality parameters. In: *Water quality – science, assessments and policy* (pp. 1–34). IntechOpen. https://doi.org/10.5772/intechopen.89657
15. Palmer, C.M. (1969). A composite rating of algae tolerating organic pollution. *Journal of Phycology*, 5, 78–82. https://doi.org/10.1111/j.1529-8817.1969.tb02581.x
16. Rotenberry, J.T., Preston, K.L. and Knick, S.T. (2006). GIS-based niche modeling for mapping species' habitat. *Ecology*, **87**(6), 1458–1464. https://doi.org/10.1890/0012-9658(2006)87[1458:GNMFMS]2.0.CO;2
17. Stumm, W. and Morgan, J.J. (1981). *Aquatic chemistry: chemical equilibria and rates in natural waters* (2nd Ed.). John Wiley & Sons, New York.
18. Surya Babu, S., & Raju Thomas, K. (2023). Feeding ecology of Near Threatened Oriental Darter (*Anhinga melanogaster*) in the Pokkali wetlands of Ernakulam district, Kerala, India. International Journal of Creative Research Thoughts, 11(2), b804–b807. https://www.ijcrt.org/IJCRT2302222
19. Tamaki, H., Tanaka, Y., Matsuzawa, H., Muramatsu, M., Meng, X.Y., Hanada, S., Mori, K. and Kamagata, Y. (2011). Armatimonas rosea gen. nov., sp. nov., of a novel bacterial phylum. *International Journal of Systematic and Evolutionary Microbiology*, 61, 1442–1447. https://doi.org/10.1099/ijs.0.025643-0
20. Verma, D.K., Satyaveer, N.K.M., Kumar, P. and Jayaswa, R. (2022). Important water quality parameters in aquaculture: An overview. *Agriculture and Environment*, **3**(3), 24–29. https://www.researchgate.net/publication/368755962
21. Wu, Q. (2017). GIS and remote sensing applications in wetland mapping and monitoring. *Preprints*. https://doi.org/10.20944/preprints201709.0058.v1