Integrated Evaluation of Toxicity and Seasonal Water Productivity in Urban Lakes: A Multimodal Approach to Aquatic Ecosystem Health

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Abstract
This study evaluates the ecological health of three lakes in North Bangalore: Ullal, Herohalli, and Lingadheeranahalli by combining primary productivity metrics with developmental toxicity assays using zebrafish. Primary productivity was measured using the light and dark bottle method and chlorophyll analysis. Herohalli Lake showed the highest gross (10.67±9.50) and net productivity (9.40±13.36) along with elevated chlorophyll levels (46.9±13.1), indicating a healthier aquatic ecosystem. In contrast, Lingadheeranahalli Lake exhibited very low productivity and chlorophyll levels, pointing to anthropogenic stress and eutrophic degradation. Complementing this, zebrafish bioassays revealed notable variations in embryonic heart rate, somite development, and acetylcholinesterase (AChE) activity across the lakes. Herohalli Lake showed minimal toxicity, while Lingadheeranahalli Lake (0.0000263 µmol/min) exhibited pronounced neurotoxic effects correlating with poor productivity. The findings highlight a clear relationship between pollution load, aquatic productivity and biological impact, underscoring the importance of multimodal assessment strategies in urban lake monitoring and management.

Introduction
Urban lakes are ecosystems playing a vital role in groundwater recharge and microclimate regulation.[1] Encroachment of urban lands has led to surface sedimentation, pesticide and untreated sewage disposal into lakes, causing oxygen depletion, eutrophication and biodiversity loss.[2] Globally, lakes in cities like Nairobi, Beijing, Sao Paulo, etc have exhibited drastic anthropogenic stress, impairing aquatic productivity and ecosystem function.[3]

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Urbanization has greatly affected the ecological condition of aquatic systems, particularly in fast-growing cities like Bangalore, India. Urban lakes, which were once crucial for biodiversity and water regulation, now struggle with intense human-induced pressures such as sewage pollution, solid waste accumulation, and eutrophication.[4,5] These issues have led to poorer water quality, decreased aquatic life, and disrupted ecosystems.[6]

Primary productivity, an essential measure of aquatic health, indicates a water body's capacity for autotrophy and is greatly affected by factors like temperature, nutrient levels, light penetration, and pollution.[7,8] Metrics such as chlorophyll-a concentration and oxygen production via photosynthesis offer insights into trophic status and ecological balance.[9] Seasonal changes, especially during the monsoon, affect nutrient cycling and influence productivity dynamics.[10] Besides physicochemical indicators, biological assays with model organisms like zebrafish provide sensitive and comprehensive tools for ecological monitoring.[11] Zebrafish embryos and adults have been used to detect aquatic toxicity, developmental abnormalities, and neurotoxicity through parameters such as heartbeat, somite development, and acetylcholinesterase (AChE) activity.[12,13] AChE inhibition, in particular, is a recognized biomarker for exposure to neurotoxic substances such as organophosphates and heavy metals.[14] Despite the ecological importance of Bangalore's urban lakes, few studies have combined limnological and toxicological methods to evaluate their ecological health. This research aims to assess the seasonal productivity of three urban lakes viz. Ullal, Herohalli, and Lingadheeranahalli—through a combination of seasonal productivity analysis and zebrafish-based toxicity tests, providing a multimodal framework for monitoring urban lake health. Previous studies on these lakes showed varying limnological characteristics.[15]

Materials and Methods
Study Area
The current research was conducted on Ullal Lake (12.9609° N, 77.4807° E), Herohalli Lake (12.9891° N, 77.4896° E), and Lingadheeranahalli Lake (13.0035° N, 77.4827° E) in the northern urban district of Bangalore as depicted in Figure 1. All three lakes are situated in residential areas and have faced considerable human-induced stress due to urbanization.
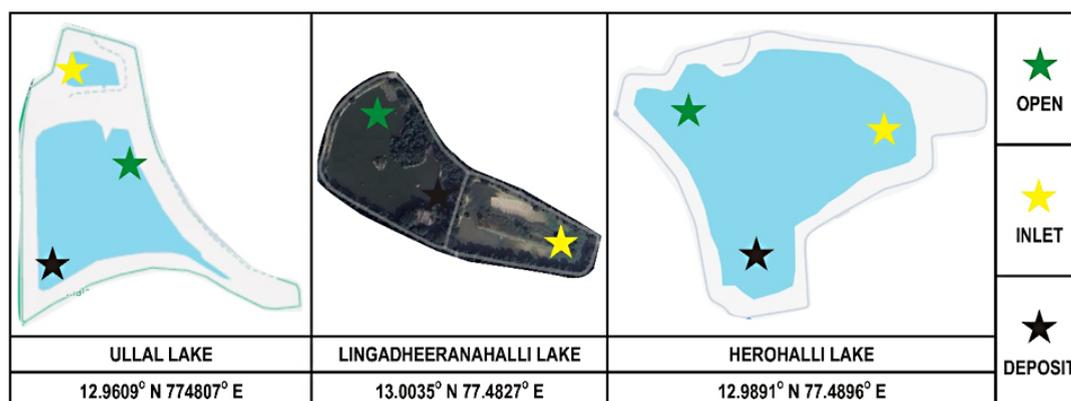

Fig. 1: Sampling stations along with the coordinates for the three lakes

Limnological Assessments
The research examined three sampling stations from each lake: open, deposit, and inlet stations. The assessments concentrated on water productivity by measuring oxygen-dependent parameters, specifically primary productivity and chlorophyll.

Primary Productivity
Light and Dark bottle Method
Water samples collected from three sampling stations were transferred into BOD bottles; one was kept in dark conditions, and the other was exposed to light conditions for 24 hours. Initial and post-incubation

DO was measured using Winkler's method and a calibrated DO sensor (Lutron DO-5509).[16] The net productivity, respiration rate and gross productivity were calculated using the following formulae.

Respiration Rate (R) (mg O_2/L/day)
R = (Initial oxygen Concentration (C_O) – Oxygen concentration dark (C_D) / Time (t)
Gross Primary Productivity (P_G) (mg O_2/L/day)

PG = (Oxygen concentration light (C_L) - Oxygen concentration dark C_D) / Time (t)
Net Primary Productivity (P_N) (mg O_2/L/day)
$P_N = P_G$ - R

Chlorophyll Method
The lake water samples collected were filtered using Whatman filter paper (Millipore grade 1), which was then finely cut into small pieces along with 80% acetone. The samples were centrifuged (4500 rpm, 10 minutes), and the supernatant was collected. Absorbance was measured using a Labman LMSP UV 1200 spectrophotometer at 645 nm, 663 nm, and 652 nm for chlorophyll a, chlorophyll b, and total chlorophyll, respectively, using acetone as a blank solution. The absorbance is then used in Arnon's equation.[17]

Chlorophyll a (mg/L) = 12.7 × A663 - 2.69 × A645
Chlorophyll b (mg/L) = 22.9 × A645 - 4.68 × A663
Total Chlorophyll (a + b, mg/L) = 20.2 × A645 + 8.02 × A663

Zebrafish Culture
Adult *D. rerio* (Hamilton,1822) weighing between 0.85g and 1.50g were grown in well-aerated aquarium tanks (5 liter capacity) regulated at 25°C - 30°C with a 13-hr light and 11-hr dark cycle. For spawning, which was induced during the morning hours, one male fish and three female fish were taken into small breeding tanks fitted with a mesh, as demonstrated by Rahman *et al*.[13]

Somite Stage and Heartbeat Assessment
Harvested zebrafish embryos were transferred into small tanks containing reverse osmosis (RO) water and individual lake water samples. Developmental abnormalities were evaluated through microscopic examination.[12] Heart rate measurements were taken during the torpedo stage.[11] The experimental setup was adopted from Brand and Granato.[18]

Acetylcholine Esterase Activity
Nine adult male zebrafish (three per group) were obtained and exposed to lake water samples under controlled conditions for 24 hours. After exposure, the fish were euthanized, and their brain tissues were dissected and homogenized in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 4°C at 10,000 rpm for 10 minutes, and the resulting supernatant served as an enzyme source for acetylcholinesterase (AChE) using Ellman's method which was slightly modified.[19] Acetylthiocholine iodide (ATCI) acted as the substrate, while Ellman's reagent, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), functioned as the chromogen. A reaction mixture of 300 µL was prepared, consisting of 240 µL of phosphate buffer (0.1 M, pH 7.4), 10 µL of DTNB (0.01 M), 40 µL of tissue supernatant (the enzyme source), and 10 µL of ATCI (0.075 M). The absorbance was measured at 412nm using a UV-Vis spectrophotometer (Labman) over 5 minutes to assess the reaction kinetics. The enzyme activity was calculated using the following formula.

Enzyme Activity [U] = (ΔOD × Vt)/(ε × L × Vs × t)

ΔOD = change in absorbance,
Vt = Total volume of reaction (in ml),
ε = Molar extinction coefficient,
L = Path length (in cm),
Vs = Sample volume used in assay (in ml),
t = Time duration of reaction (in min)

Results
The seasonal dynamics of respiration rate, gross productivity (P_G), and net productivity (P_N) across Herohalli, Ullal, and Lingadheeranahalli lakes exhibited significant spatiotemporal variation influenced by environmental and anthropogenic factors.

**Table 1: Productivity Metrics (Mean ± SD) for Respiration, Gross, and Net Productivity
in Three Urban Lakes**

Sample Stations	Monsoon	Autumn	Winter	Spring	Summer
Lingadheeranahalli Lake – Respiration Rate (mg/L)					
Open	0.47±0.81	-3.3±5.59	-0.45±0.92	2.2±1.13	1.57±2.04
Deposit	-0.60±3.14	-7.0±9.05	0.55±0.07	2.05±0.78	0.80±0.20
Inlet	0.30±0.52	7.7±10.29	1.2±1.41	2.3±0.85	3.10±3.28
Lingadheeranahalli Lake – Net productivity (mg/L)					
Open	8.87±1.45	10.6±2.90	0.25±9.97	2.55±0.49	0.73±3.76
Deposit	14.67±11.72	15.3±0.94	-1±7.07	2.75±1.48	2.83±0.25
Inlet	1.13±1.96	8.4±9.40	6.5±2.40	3.5±0.85	0.63±4.27
Lingadheeranahalli Lake – Gross Productivity (mg/L)					
Open	9.33±0.64	7.3±2.69	3.7±5.37	4.75±1.63	2.30±1.73
Deposit	12.73±6.36	7.7±7.17	2.45±3.04	4.8±0.71	3.63±0.38
Inlet	1.43±2.48	1.1±1.52	7.1±1.84	5.8±1.70	3.73±1.22
Ullal Lake - Respiration Rate (mg/L)					
Open	1.53±1.36	-4.35±9.40	0.85±2.62	2.35±1.63	1.07±0.47
Deposit	-0.33±3.21	-3.67±4.71	-0.4±1.41	1.6±0	1.13±0.67
Inlet	1.37±1.52	-3.37±6.62	0.05±0.78	3.1±0.71	2.03±1.31
Ullal Lake – Net Productivity (mg/L)					
Open	8.67±3.06	11.5±6.36	-0.2±3.39	3.6±1.13	0.90±0.75
Deposit	14.20±8.45	10.6±5.09	1.35±2.47	4.15±1.06	1.10±1.31
Inlet	20.90±13.65	14.55±8.98	4.05±3.75	2.15±1.63	1.97±0.74
Ullal Lake – Gross Productivity (mg/L)					
Open	10.20±1.71	2.15±10.11	3.7±1.70	5.95±0.49	1.97±0.31
Deposit	13.87±6.82	13.93±0.09	3.55±0.21	5.75±1.06	2.10±1.15
Inlet	22.27±12.13	19.23±4.29	5.7±2.26	5.25±2.33	4.00±0.69
Herohalli Lake – Respiration Rate (mg/L)					
Open	1.47±2.19	-2.77±5.99	0.45±0.78	3.95±1.20	3.87±3.88
Deposit	4.00±2.84	-0.80±6.79	1.35±1.34	2.75±1.06	3.50±2.88
Inlet	1.27±3.87	1.13±0.19	0.8±0.14	1.7±2.12	1.53±1.71
Herohalli Lake – Net Productivity (mg/L)					
Open	3.93±4	6.97±4.29	4.35±2.19	-0.95±1.91	-0.57±3.78
Deposit	5.67±5.69	6.93±1.79	2.55±6.15	2.75±3.89	-0.63±4.20
Inlet	9.40±13.36	6.20±4.53	1.9±6.22	6.85±9.26	3.77±4.08
Herohalli Lake – Gross Productivity (mg/L)					
Open	5.40±6.12	1.20±5.94	7.2±0.42	3±0.71	3.30±0.10
Deposit	9.67±8.52	3.53±8.67	6.85±0.64	5.5±2.83	2.83±1.27
Inlet	10.67±9.50	7.33±4.71	5.65±1.91	8.55±7.14	5.30±3.24

Table 2: Chlorophyll Concentrations in Water Samples from Three Urban Lakes

Sample Stations	Monsoon	Autumn	Winter	Spring	Summer
Lingadheeranahalli Lake - Chlorophyll A (mg/L)					
Open	8.63±0.55	9.7±0.07	9.9±1.77	7.75±0.07	6.41±3.72
Deposit	12.20±0.46	10.2±0.5	9.4±0.3	8.5±0.1	13.3±5.8
Inlet	8.07±0.6	9.7±0.1	8.9±0.4	7.8±0.1	17.9±12.7
Lingadheeranahalli Lake - Chlorophyll B (mg/L)					
Open	23.96±2.30	26.4±3.20	28.22±1.45	17.75±1.34	12.46±7.66
Deposit	23.53±0.74	24.1±3.68	27.2±2.12	27.2±0.57	26.93±5.22
Inlet	20.90±5.54	27.7±1.41	26.65±1.34	17.75±1.34	29.97±20.08
Lingadheeranahalli Lake - Total Chlorophyll (mg/L)					
Open	32.59±2.84	36.1±3.27	38.18±3.22	25.5±1.41	18.88±11.12
Deposit	35.73±1.2	34.3±3.2	36.6±2.4	35.7±0.7	40.2±10.2
Inlet	29±6.1	37.4±1.3	37.4±1.3	25.5±1.4	47.9±32.7
Ullal Lake - Chlorophyll A (mg/L)					
	Monsoon	Autumn	Winter	Spring	Summer
Open	8.03±	8.2±0.1	8.3±0.8	7.5±0.4	10.9±5.9
Deposit	8.06±	9.5±0.4	8.9±0.4	7.8±0.1	10.3±2.7
Inlet	7.86±	8.4±1.1	8.5±0.1	8.2±1.4	20.3±12.2
Ullal Lake - Chlorophyll B (mg/L)					
Open	23.33±2.22	27.7±1.41	18.1±5.66	17.75±1.34	10.90±16.19
Deposit	20.73±5.25	22±7.35	27.2±2.12	27.2±0.57	18.92±4/42
Inlet	23.37±5.77	21.4±8.20	17.75±1.34	26.2±0.71	38.92±15.39
Ullal Lake - Total Chlorophyll (mg/L)					
Open	31.4±2	35.9±1.3	26.4±4.8	25.3±0.9	21.8±21.8
Deposit	28.8±5	31.5±7.8	36.1±2.5	35.0±0.5	29.2±7.1
Inlet	31.2±6.1	29.8±9.3	26.3±1.2	34.4±0.7	58.6±26.3
Herohalli Lake - Chlorophyll A (mg/L)					
	Monsoon	Autumn	Winter	Spring	Summer
Open	8.23±	9.2±0.6	8.3±0.3	7.8±0.1	15.4±12.1
Deposit	8.43±	9.4±0.3	8.9±0.4	7.8±0.8	15.7±8.1
Inlet	8.26±	8.3±0.2	8.5±0.7	7.6±0.5	17.0±5.3
Herohalli Lake - Chlorophyll B (mg/L)					
Open	25.50±1.54	18.10±5.66	18.45±6.15	20.4±2.4	24.65±14.57
Deposit	20.90±5.54	27.70±1.41	18.65±6.43	19.45±3.75	27.47±12.04
Inlet	17.67±4.07	18.70±0	22.7±5.66	17.75±1.34	29.87±8.69
Herohalli Lake - Total Chlorophyll (mg/L)					
Open	32.7±1.5	27.3±6.2	26.8±6.4	28.2±2.3	40.1±26.7
Deposit	29.3±5.2	37.1±1.1	27.6±6.9	27.3±4.6	43.1±19.9
Inlet	25.9±4.3	27.0±0.2	31.0±6.4	25.3±0.8	46.9±13.1

Herohalli Lake exhibited the most consistent and elevated productivity, with peak net productivity at the inlet during the monsoon (9.40 ± 13.36 mg O_2/L/day) and the highest gross productivity in spring (8.55 ± 7.14 mg O_2/L/day). Negative P_N values in spring and summer at the open station suggest increased respiration, potentially due to higher organic matter. Ullal Lake exhibited its highest productivity during the monsoon and autumn seasons, especially at the inlet, with gross productivity reaching 22.27 ± 12.13 mg O_2/L/day and net productivity at 20.90 ± 13.65 mg O_2/L/day. Conversely, productivity decreased in winter and summer, with net productivity falling to -0.2 ± 3.39 mg O_2/L/day in winter. Lingadheeranahalli Lake exhibited lower and more erratic productivity, with modest gross productivity in spring (5.8 ± 1.70 mg O_2/L/day) and net productivity fluctuating between -1.0 and 15.3 mg O_2/L/day, indicating varying organic and nutrient loads. Chlorophyll concentrations supported these trends, with inlet stations during summer consistently showing the highest chlorophyll a and total chlorophyll levels across lakes. Ullal Lake recorded a peak total chlorophyll of 58.6 ± 26.3 µg/L at the inlet in summer, while Herohalli and Lingadheeranahalli followed, with inlet values of 46.9 ± 13.1 µg/L and 47.9 ± 32.7 µg/L, respectively. Chlorophyll-b concentrations were consistently higher than chlorophyll-a, with notable summer peaks at both inlet and deposit stations.

Overall, the monsoon and autumn seasons enhanced productivity across lakes. Conversely, despite elevated chlorophyll levels, summer showed lower or negative net productivity. Herohalli Lake ranked highest in overall productivity, followed by Ullal, while Lingadheeranahalli exhibited signs of ecological stress and eutrophic degradation.
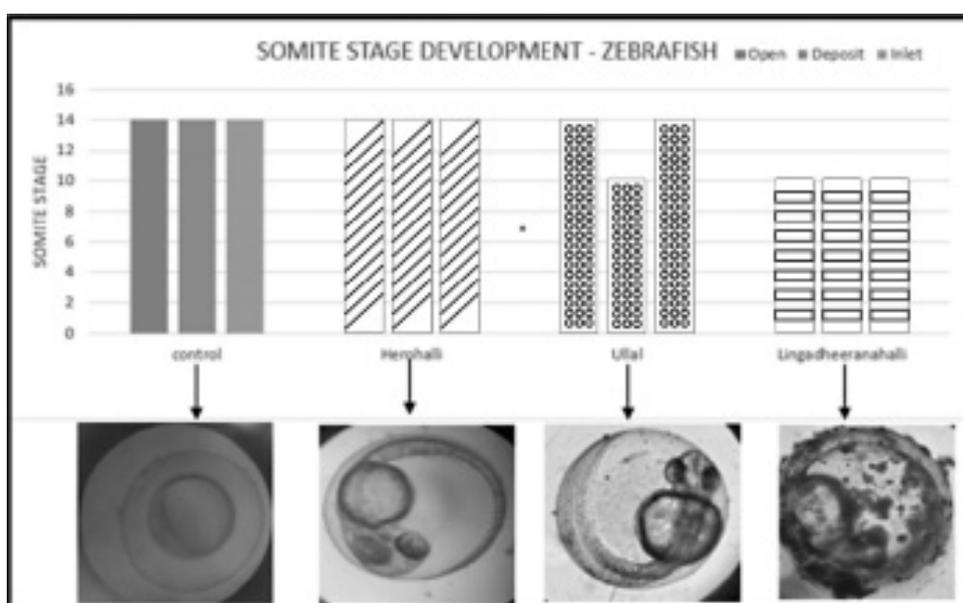

Fig. 2: Somite Formation in Zebrafish Embryos across Three Urban Lake Exposure Groups

Embryos exposed to Herohalli Lake water demonstrated normal somite development (Figure 2). They maintained a healthy heartbeat (Figure 3) of 113 to 108 beats per minute (bpm) during the torpedo stage, similar to the control group, indicating minimal toxicity. In contrast, Ullal Lake-exposed embryos exhibited mild developmental delays in somite segmentation and a reduced heart rate ranging from 89 to 127 bpm. Meanwhile, embryos exposed to Lingadheeranahalli Lake showed the most pronounced toxicological effects, with significant delays in somite formation, morphological abnormalities such as tail curvature, and a substantially lower heart rate, ranging from 21 to 99 bpm.
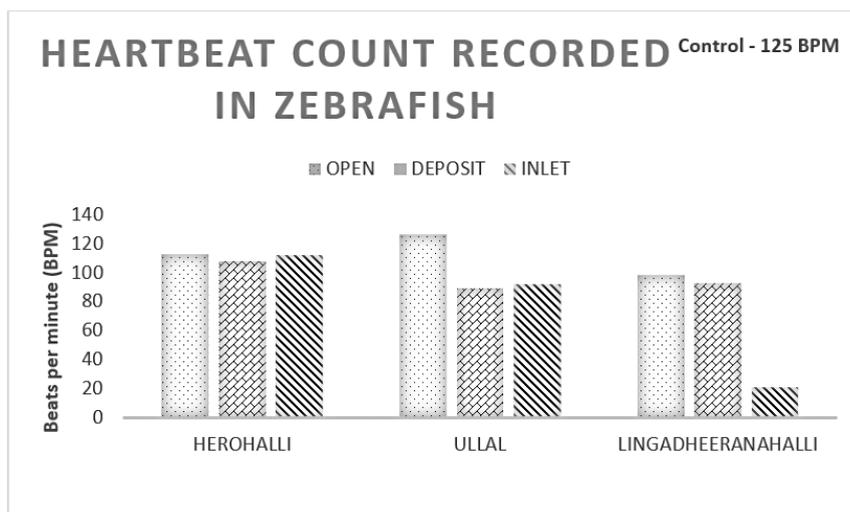
Fig. 3: Heartbeat rate in zebrafish from the three urban lakes

Table 3: Kruskal-Wallis and DSCF Statistical Results for Productivity and Chlorophyll Variables across Lakes, Stations, and Seasons

A. Kruskal-Wallis statistics indicating significance for respiration rate, gross productivity and net productivity between lakes through the light and dark bottle method, **p<0.05

Lakes	Respiration Rate			Gross Productivity			Net Productivity		
	χ^2	df	p	χ^2	df	p	χ^2	df	p
Herohalli Lake	3.64	4	0.457	1.36	4	0.851	3.22	4	0.522
Ullal Lake	5.38	4	0.250	9.83	4	0.043	8.72	4	0.069
Lingadheeranahalli Lake	8.26	4	0.083	4.62	4	0.329	7.83	4	0.098

B. Kruskal-Wallis statistics indicating significance for chlorophyll a, chlorophyll b and total chlorophyll between lakes through the chlorophyll method, **p<0.05

Lakes	Chlorophyll A			Chlorophyll B			Total Chlorophyll		
	χ^2	df	p	χ^2	df	p	χ^2	df	p
Herohalli Lake	8.79	4	0.067	2.97	4	0.562	3.06	4	0.547
Ullal Lake	9.64	4	0.047	2.72	4	0.606	3.01	4	0.556
Lingadheeranahalli Lake	9.39	4	0.052	5.88	4	0.208	4.41	4	0.353

From table 3 and table 4, it is deduced that significant differences exist in total chlorophyll content between the lakes and also during various seasons, but no significant differences between the sampling stations, that is, open, deposit and inlet.

The acetylcholinesterase (AChE) activity measured in zebrafish exposed to lake water samples (Figure 4) varied significantly among the three lakes. The control group exhibited a baseline AChE activity of 0.0000345 µmol/min, serving as a reference

for neurophysiological normalcy. Exposure to Herohalli Lake water markedly increased AChE activity to 0.0000822 µmol/min, indicating a possible stimulatory or compensatory neural response. Conversely, zebrafish exposed to Lingadheeranahalli Lake showed the lowest AChE activity of 0.0000263 µmol/min, suggesting neuro-inhibition. Fish exposed to Ullal Lake demonstrated AChE activity of 0.0000347 µmol/min, similar to the control, reflecting a relatively moderate impact on neurotoxicity.

Table 4: Kruskal-Wallis and DSCF Pairwise Comparisons between Lakes, Stations, and Seasons

| Category / Comparison C. Kruskal-Wallis Test	Statistic / Pair	p
Between Lakes	$\chi^2 = 6.44$	0.0399
Between Stations	$\chi^2 = 3.10$	0.2123
Between Seasons	$\chi^2 = 14.21$	0.0027
D. DSCF Pairwise Comparisons – Lakes	Herohalli vs Ullal	0.3909
	Herohalli vs Lingadheeranahalli	0.0535
	Ullal vs Lingadheeranahalli	0.0124
E. DSCF Pairwise Comparisons – Seasons	Monsoon vs Summer	0.0053
	Autumn vs Summer	0.0049
	Winter vs Summer	0.0053
	Spring vs Summer	0.0053
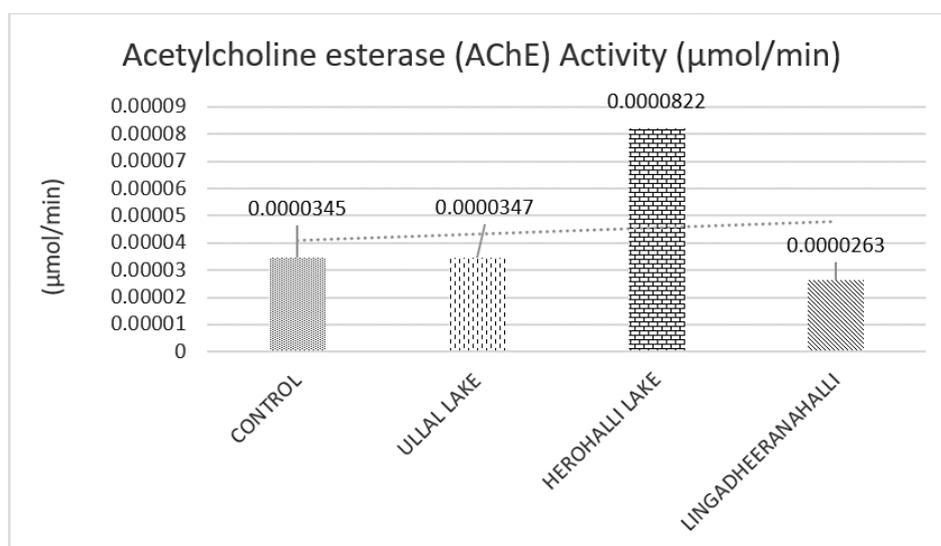
Fig. 4: Acetylcholine esterase activity (AChE) from the three urban lakes

Discussion
The present study employed a multimodal approach—combining seasonal limnological parameters with zebrafish bioassays—to assess ecological and neurotoxic stress in three urban lakes in North Bangalore. Our findings reveal a significant interplay between productivity parameters (gross and net productivity, chlorophyll levels) and biological indicators (heartbeat, somite formation, and AChE activity) across various seasonal cycles and lake environments. Primary Productivity and Chlorophyll Trends: Herohalli Lake consistently exhibited the highest productivity values, particularly during the monsoon and spring, as evidenced by

peak gross productivity (10.67 ± 9.50 mg O_2/L/day) and elevated chlorophyll concentrations at the inlet station (chlorophyll-a: 17.0 ± 5.3 µg/L; total chlorophyll: 46.9 ± 13.1 µg/L in summer). These findings align with Jones *et al*, who reported monsoonal peaks in nutrient influx and autotrophic activity in semi-urban water bodies.[20] In contrast, Lingadheeranahalli Lake displayed highly variable and generally reduced productivity and chlorophyll levels, with gross productivity often falling below 6 mg O^2/L/day and net productivity showing negative values during winter (-1.0 ± 7.07 mg O_2/L/day). These trends suggest intense anthropogenic stress, nutrient overload, and ecological imbalance, reflecting the eutrophic degradation patterns.[4,21] Reduced chlorophyll and productivity levels suggest ecological imbalance leading to algal blooms.[22] The seasonal variations in productivity metrics highlight climate-induced changes such as shifts in rainfall, evaporation and stratification, which are increasingly affecting urban lakes.[23,24]

Zebrafish embryo toxicity test (ZEFT) remains economical with a high output compared to traditional toxicity tests.[25] Zebrafish embryos exposed to Herohalli Lake exhibited standard developmental patterns and heartbeat values (113–108 bpm), corroborating the lake's relatively healthier water quality, which aligns with ecological studies conducted on Herohalli Lake.[26] In contrast, embryos exposed to Lingadheeranahalli showed developmental deformities, with heartbeats dropping as low as 21 bpm and delayed somite segmentation. These findings strongly indicate toxicity linked to pollutant accumulation and align with studies on Ibalur Lake.[27]

Acetylcholinesterase (AChE) activity is an active biomarker for the assessment of neurological toxicity to aquatic health.[14,28] Zebrafish exposed to Herohalli water showed increased AChE activity (0.0000822 µmol/min), potentially reflecting cholinergic system compensation. In stark contrast, AChE activity was lowest in Lingadheeranahalli-exposed fish (0.0000263 µmol/min), revealing substantial neuro-inhibition, possibly due to pesticide or heavy metal contamination.[11,13] Ullal Lake samples induced moderate physiological stress with AChE values near the control. These integrated findings underscore the importance of coupling physicochemical water quality monitoring with sensitive biological endpoints, such as zebrafish bioassays, for a more comprehensive understanding of aquatic health, particularly in urban ecosystems vulnerable to rapid anthropogenic encroachment.[29,30]

Conclusion

The integrated evaluation of three urban lakes through limnological assessments and zebrafish-based bioassays revealed insights into the seasonal productivity and ecological health of urban aquatic systems. Herohalli Lake exhibited good productivity and minimal biological toxicity, reflecting its relative ecological stability. Ullal Lake demonstrated moderate productivity and toxicity, suggesting transitional stress. However, Lingadheeranahalli Lake showed significant impairment, erratic productivity, low chlorophyll levels, and considerable neurotoxic effects on zebrafish embryos. The findings underscore the importance of combining traditional water quality assessments with biological markers such as AChE activity and developmental endpoints in zebrafish. This multimodal approach facilitates early detection of ecological degradation and aids in formulating targeted lake restoration and pollution management strategies.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from other Sources

Not Applicable

Author Contributions

•   **Mathews P Raj:** Conceptualization, Data collection, Methodology, Writing, and Statistical interpretation.

•   **Reena Susan Philip:** Writing – Review & Editing.

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