

Ecological status of streams in Zambales riverscape inferred from benthic macroinvertebrates as biological indicators

Carlos Miguel G. Orale^{*1,2}, Eliza Rose Y. Aquino¹, Lilian N. Dela Cruz¹, Miguel L. Estrada¹, Hannah Lorraine C. Frias¹, Aleck Andrei R. De Guzman¹, Allyzxia Venisse H. Navarro³, Ma. Angela Klarizze H. Solomon³, Allan S. Gilles Jr.^{1,3}, Justine de Leon^{1,3,4}, Dino T. Tordesillas^{1,2,3}, Rey Donne S. Papa^{1,2,3}, and Elfritzson M. Peralta^{1,2,3}

¹Department of Biological Sciences, College of Science, University of Santo Tomas, España Boulevard, Manila, 1008, Philippines

²The Graduate School, University of Santo Tomas, España Boulevard, Manila, 1008, Philippines

³Research Center for the Natural and Applied Sciences, University of Santo Tomas, España Boulevard, Manila, 1008, Philippines

⁴Department of Science and Technology, Science Education Institute, Taguig, 1631, Philippines

ABSTRACT

Lotic ecosystems, such as rivers and streams, play vital roles in sustaining biodiversity, regulating nutrient cycles, and providing key ecosystem services. However, these systems are increasingly threatened by anthropogenic pressures, particularly in tropical regions like the Philippines. This study assessed the ecological integrity of lotic ecosystems in Zambales, Philippines, by integrating benthic macroinvertebrate community structure with physicochemical parameters across six stream sites. Sampling was conducted during the transitional season of October 2023. Physicochemical parameters, including dissolved oxygen (DO), conductivity, total dissolved solids, salinity, pH, and nutrient concentrations (ammonia, nitrite, nitrate, phosphate), were measured and analyzed using principal component analysis to determine environmental gradients and site clustering. Benthic macroinvertebrates were collected and identified to family and genus levels. Diversity metrics (Shannon Index, evenness), EPTC indices (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera), and taxon richness and density were computed to evaluate biological responses. Canonical correspondence analysis revealed strong correlations between environmental stressors and macroinvertebrate assemblage structure. Results indicated that streams traversing forested and less disturbed areas supported higher diversity and

more pollution-sensitive taxa, particularly EPTC, whereas streams in residential and industrial zones exhibited reduced diversity and dominance of tolerant taxa such as Diptera. Notably, sites 1 (agricultural land) and 6 (residential area) lacked detectable EPTC taxa and displayed signs of poor ecological health, evidenced by high conductivity, nutrient enrichment, and low DO concentrations. These findings underscore the impact of land use on stream conditions and highlight the value of integrating biological and physicochemical assessments for effective monitoring and conservation of freshwater ecosystems.

INTRODUCTION

Freshwater ecosystems are critically important habitats that support biodiversity, regulate nutrient cycles, and sustain human livelihoods (Apostolaki 2020; Encarnacion 2024). Among these, lotic systems, like rivers and streams, are particularly significant as they serve as ecological corridors that connect terrestrial and aquatic environments (Lin et al. 2022). These systems are vital for maintaining ecological balance, sustaining diverse species, and delivering key ecosystem functions (Bautista et al. 2024; Isla et al. 2024). However, these freshwater ecosystems are facing increasing threats from human activities, especially in tropical nations such as the Philippines (Rodriguez et al. 2023; Encarnacion 2024). Rapid urbanization, expanding agriculture, infrastructure development, and resource extraction have all

*Corresponding author

Email Address: cmgorale@gmail.com

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contributed to the degradation of watersheds (Nilsson and Renöfält 2008; Liang et al. 2025; Ma and You 2025).

In the province of Zambales, located in Central Luzon, freshwater systems traverse a mosaic of forested uplands, agricultural lowlands, and expanding residential and industrial zones. Despite their ecological and socio-economic significance, many of these rivers and streams remain unprotected and under-monitored. Activities like sand mining, road construction that causes sedimentation, and nutrient pollution from farming practices are disrupting natural flow patterns, degrading water quality, and threatening aquatic biodiversity (Meng 2021; Farooq et al. 2024). The resulting decline in ecological integrity poses serious risks to native biota and ecosystem services, yet scientific evaluations of these systems remain limited.

Although the Department of Environment and Natural Resources–Environmental Management Bureau (DENR–EMB) conducts routine water quality monitoring of select river systems in the Philippines, such information for the Zambales river basins is limited or unavailable, particularly regarding the ecological health of its riverine systems. Likewise, published studies that provide comprehensive physicochemical, land use and land cover (LULC), and ecological assessments for the province are scarce. This gap reflects broader patterns in watershed research, where authors emphasize that effective riverine assessment requires the combined evaluation of water quality, LULC patterns, and biotic responses (De Mello et al. 2023). Thus, establishing integrated baseline information for Zambales is essential for accurately characterizing ecological conditions and informing conservation planning.

While traditional water quality assessments provide insight into the chemical condition of aquatic environments, they often fail to detect cumulative or chronic disturbances (Armijos-Arcos et al. 2025). In contrast, benthic macroinvertebrates are widely recognized as reliable biological indicators of stream health (Costa et al. 2024; França et al. 2025), owing to a distinct set of advantages (Deborde et al. 2016). These taxa exhibit varying levels of tolerance to pollutants, are relatively sedentary, and reflect site-specific environmental conditions over time (Sumudumali and Jayawardana 2021; Kadim et al. 2025). Their community structure and diversity can reveal subtle shifts in ecosystem integrity that might be overlooked by snapshot physicochemical measurements alone (Peralta et al. 2019). Instead of relying solely on standard water chemistry assessments, stream health evaluation would benefit from an integrated approach that incorporates physical, chemical, and biological metrics. Such a holistic framework not only strengthens diagnostic power but also supports more informed conservation and management strategies. Biological monitoring using macroinvertebrate indices, including diversity metrics and functional group composition, has been successfully applied in many stream ecosystems worldwide (Peralta et al. 2020; Doong et al. 2021). However, such approaches have yet to be widely adopted in the Philippine context, particularly in regional landscapes like Zambales, where ecological baselines are scarce and environmental management remains fragmented.

This study aims to assess the ecological integrity of lotic ecosystems in Zambales by evaluating how water quality influences benthic macroinvertebrate assemblages. Specifically, we intend to understand the environmental gradients of streams in Zambales based on select physicochemical parameters and nutrient levels, assess the response of benthic macroinvertebrate communities using diversity and biotic indices (e.g., Shannon Index, species richness and evenness, EPTC richness and density, and %EPTC composition), and identify how these environmental variables influence benthic macroinvertebrate

biodiversity using multivariate analyses. Based on previous studies, forested and upstream sites will exhibit better water quality and support greater macroinvertebrate diversity (i.e. pollution-sensitive taxa), whereas agricultural or residential sites will exhibit nutrient enrichment and poor water quality, resulting in reduced diversity and evenness, EPTC richness, and dominance of pollution-tolerant taxa. Furthermore, the gradients in dissolved oxygen, conductivity, and nutrient concentrations are expected to emerge as primary drivers structuring macroinvertebrate community composition. By integrating physicochemical measurements with biological indicators, this research provides a more holistic understanding of stream health in Zambales. Establishing the ecological status of these streams can inform future local and national monitoring, management, and conservation strategies for lotic ecosystems.

MATERIALS AND METHODS

Study area

Six sampling sites were purposively selected across Zambales to represent different stream conditions and surrounding land use types rather than through random selection. This stratified approach allowed us to capture ecological variability across gradients of disturbance. The study sites chosen were: site 1 (Namatacan), site 2 (Yangil), site 3 (Sagpat Falls), site 4 (Santo Tomas River), and sites 5 (midstream) and 6 (downstream) along Alusiis River. The classification of each site according to dominant land use was also considered (Figure 1). In San Narciso, the Santo Tomas, Alusiis, and Namatacan Rivers are located at 15°01'34.2"N, 120°06'34.6"E; 15°01'29.3"N, 120°04'27.9"E; and 15°00'22.6"N, 120°08'39.2"E, respectively. San Felipe, located north of San Narciso, includes the Yangil and Maloma Rivers, with coordinates 15°06'08.3"N, 120°06'20.2"E; and 15°05'10.3"N, 120°06'11.4"E, respectively. The study site map was generated using QGIS software, with waterway delineation aided by ESRI Sentinel-2 10-Meter Land Use/Land Cover data from 2023. The selected sites are characterized by pollution from wastewater, erosion, and stormwater runoff. Elevated oil and grease concentrations, primarily due to domestic activities, were observed during field sampling, along with signs of erosion driven by both natural events and anthropogenic disturbances. According to the Philippine Statistics Authority (2021) and PhilAtlas (2021), the populations of San Felipe and San Narciso were reported as 3,896 and 30,759, respectively, in the 2020 census.

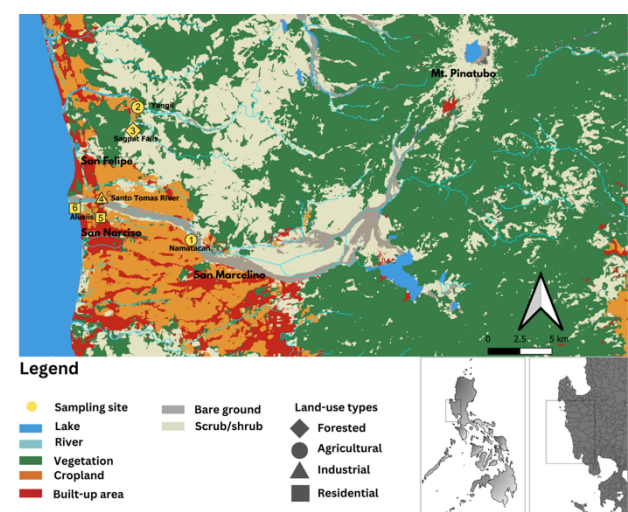


Figure 1: Land use and land cover map of San Narciso, and San Felipe, Zambales, showing the six sampling sites.

Environmental variables

Environmental variables, specifically physicochemical parameters, were measured at each stream sampling site with three replicate measurements per site. Dissolved oxygen (DO), conductivity, total dissolved solids (TDS), salinity, and pH were measured using a CTD profiler (YSI EXO2 Sonde) and an API Freshwater Master Test Kit was used to measure nutrient concentrations (i.e., ammonia, nitrite, nitrate, and phosphate). After collecting and treating water samples with standard solutions, the resulting color of the solutions was compared to the reference color concentration range to determine the nutrient levels. Data collection was conducted from 1–3 October 2023, an ideal transition period between the wet and dry seasons based on Climate Type 1 of Zambales (Corporal-Lodangco and Leslie 2017). Sites were numbered 1–6 from upstream to downstream, with site 1 at Namatacan and site 6 at Alusiis.

Collection and identification of benthic macroinvertebrates

Benthic macroinvertebrates were collected at each sampling point using a 30 cm × 30 cm Surber sampler with a 500 µm mesh for water depths <30 cm. The streambed was disturbed using a screwdriver, allowing sediments to flow into the net. Each replicate sample was pooled into a labeled ziplock bag, sorted within 24–48 hours post-collection, transferred to vials with 95% ethanol within 48 hours for preservation, and stored in a cooler at 5°C (Peralta et al. 2020). In the laboratory, the collected samples were further identified, classified, and counted based on the family and genus of the benthic macroinvertebrates. Observation and identification of the samples were carried out using stereo microscopes and taxonomic keys of Pescador et al. (1995), Epler (1996), Dudgeon (1999), Yule and Yong (2004), Mekong River Commission (2006), Chapman (2007), Merritt et al. (2008), Sartori et al. (2008), Madden (2010), and Bae (2010).

Data analysis

Mean values of the physicochemical parameters were run through a principal component analysis (PCA) to statistically illustrate the differences among the sites based on selected water quality parameters. This revealed clustering among the sites, allowing assessment of parameter variation across sites and evaluation of the impact of land use types on water quality. Prior to PCA, temperature, salinity, total dissolved solids, pH, ammonia, and nitrate were excluded from the analysis to reduce multicollinearity among explanatory variables. A correlation matrix was first computed to identify highly correlated variables ($|r| > 0.70$), and this was further evaluated using the variance inflation factor (VIF).

Diversity indices such as the Shannon Diversity Index (H) and Evenness (E) were computed to assess the macroinvertebrate species diversity within the sampling sites. The H' value, which indicates species diversity, was interpreted following the categories proposed by Baliton et al. (2020). Evenness was interpreted as approaching 0 when a few species dominate the river community and as approaching 1 when species are equally abundant species (Wilsey and Stirling 2007). EPT and EPTC indices were also calculated from the percentages of the insect orders Ephemeroptera, Plecoptera, Trichoptera, and Coleoptera. This index helped determine the taxa richness within the insect groups, serving as the primary bioindicators sensitive to pollution (Compin and Céréghino 2003; Selvanayagam and Abril 2015).

The statistical difference across sites based on environmental parameters and community measures was evaluated using the Kruskal-Wallis test and the corresponding Dunn's post-hoc test for applicable variables. These tests were employed to detect possible site-specific differences in stream habitat conditions and macroinvertebrate community structure. Appropriate tests

for normality and homoscedasticity of datasets were carried out prior to these univariate and inferential statistics.

Lastly, multivariate analysis was used to determine the relationships between environmental variables and benthic macroinvertebrates, understanding how each physicochemical parameter influences the response of these organisms. For this, detrended correspondence analysis (DCA) was used to determine whether a unimodal or linear ordination technique is appropriate based on the macroinvertebrate matrix. A canonical correspondence analysis (CCA) was subsequently conducted to generate a unimodal ordination triplot (DCA Axis 1 = 4.7) and illustrate the relationships between environmental variables and macroinvertebrate assemblages across study sites (Herman and Nejadhashemi 2015; Peralta 2020). All statistical analyses were conducted using RStudio (RStudio, Inc., Boston, MA), and the vegan (Oksanen et al. 2025) and ggplot2 (Wickham 2016) packages.

RESULTS AND DISCUSSION

Environmental variables

The PCA bi-plot (Figure 2) illustrates how dominant land use types may influence stream physicochemical parameters across six sampling sites. Principal components (PCs) with eigenvalues greater than 1 were retained for interpretation, and loadings $>|0.50|$ were regarded as qualitatively high. PC1 and PC2 explained a substantial portion of the total variance (84.76%), revealing distinct gradients of environmental variables and their associations with different land uses. The observed clustering indicates clear differences in water quality parameters among sites, reflecting the role of land use in shaping stream conditions (Haidari et al. 2013). Notably, site 3, a forested area, formed a distinct cluster associated with better water quality compared to sites characterized by agricultural and residential activities (Baillie and Neary 2015; Peralta et al. 2019; Peralta et al. 2020). In contrast, sites 4 and 6, which represent industrial and residential areas, grouped closely together and showed clear separation from the forested site. This divergence highlights the degraded water quality typically found in more disturbed landscapes (Neddeau 2003; Panda 2009; Jolejole et al. 2021). Interestingly, the two agricultural sites (sites 1 and 2) were distributed across clusters, aligning with better or poorer water quality conditions. This variation likely reflects differences in the intensity of agricultural practices, with more intensive activities contributing to poorer water quality through runoff. Site 2, in particular, clustered closely with the forested site, suggesting that agricultural activities at this location may be managed in a way that minimizes their impact on water quality. In contrast, site 1 showed a closer association with the industrial and residential cluster. Interestingly, site 2, which represents an agricultural land use, was closely aligned with elevated dissolved oxygen (DO) levels, suggesting that moderate agricultural practices in this watershed may have a lesser impact on water quality compared to more intensive land uses. However, its proximity to the DO vector should be interpreted cautiously, as seasonal fluctuations in agricultural runoff or differences in land management practices could influence the site's long-term ecological status. The sustainability of agricultural land use depends on the extent and management of activities, as highlighted by previous studies (Mehdi et al. 2015). The clustering of site 2 with the forested site supports the interpretation that water quality at this location is relatively high, contrasting with site 1 (also agricultural), which aligns more closely with areas of degraded water quality.

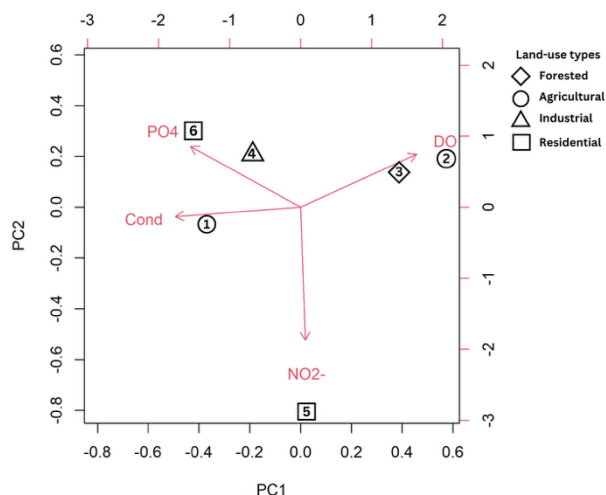


Figure 2: Principal component analysis bi-plot based on two major principal component scores across sites in Zambales.

Dissolved oxygen (DO) serves as a key indicator of water quality in Zambales streams, where low DO concentrations typically reflect degraded conditions (Morosanu et al. 2016). Lower DO levels, especially in residential stream areas, may indicate heightened microbial activity likely fueled by elevated nutrient inputs from domestic pollution (Peralta et al. 2020; Ramachandra et al. 2020). Conversely, higher DO levels recorded in upstream sections of Zambales streams reflect better water quality, characterized by adequate oxygen availability and non-corrosive conditions that sustain aquatic life (EPA 2023; EPA 2024). Notably, elevated DO levels observed at sites 2 and 3 were accompanied by lower conductivity values (Table 1), highlighting an inverse relationship between these parameters

along the water quality gradient. This pattern aligns with findings that human disturbances, such as urban runoff, elevate conductivity through increased nutrient and dissolved solid inputs (Arimoro et al. 2015). Salinity levels (Table 1) were notably lowest at site 3 compared to other sampling locations, reflecting its relationship with DO dynamics (Manasrah 2006). Salinity reflects the concentration of dissolved ions in water, and its measurement is essential in freshwater systems, as elevated salinity can be toxic or disruptive to aquatic ecosystems. The strong factor loadings of total dissolved solids (TDS), conductivity, and salinity likely reflect the collective influence of ions on freshwater conductivity, indicating a positive correlation among these parameters (Zinabu et al. 2002; Rusydi 2018). Elevated levels of these parameters suggest substantial nutrient inputs from anthropogenic sources, such as domestic and industrial discharges (Rusydi 2018; Peralta et al. 2020; Jolejole et al. 2021). Conductivity levels showed significant variations among specific sites while remaining relatively stable at others (sites 1, 3, and 4). These discrepancies in conductivity suggest potential differences in ion concentrations or pollutant levels across the sampling sites, given their mixed residential and industrial nature. Such variations could have profound implications for water quality, including concerns about the discharge of untreated wastewater into streams and rivers, posing risks to human health, safety, and the environment. Discharges from industrial or municipal facilities may persist over extended periods and may occur due to incorrect connections of sanitary sewers to drainage systems or damage to pipes. Additionally, pollutants may accidentally spill from ships, oil pipelines, or other sources, sometimes undetected, further exacerbating the environmental impact (Chusov et al. 2014).

Table 1: Mean (\pm standard deviation) values of the chosen stream parameters.

| Parameters | 1 | 2 | 3 | 4 | 5 | 6 |
|----------------------------|--------------------|---------------------|--------------------|--------------------|--------------------|---------------------|
| Dissolved oxygen (mg/L) | 5.505 (0.092) | 8.035 (0.573) | 7.620 (0.028) | 7.243 (0.005) | 6.350 (0.028) | 6.285 (0.276) |
| TDS (ppt) | 270.200 (0.707) | 109.000 - | 144.000 - | 292.000 - | 209.850 (1.202) | 226.850 (28.072) |
| Salinity (%) | 0.200 - | 0.800 - | 0.100 - | 0.210 - | 0.150 - | 0.165 (0.021) |
| Conductivity (μ S/cm) | 451.800 (1.272) | 172.150 (15.486) | 236.300 (0.283) | 498.333 (0.577) | 354.400 (2.404) | 394.350 (41.931) |
| pH | 7.200 - | 7.850 (0.071) | 8.500 | 8.047 (0.010) | 7.085 (0.007) | 7.330 (0.099) |
| Ammonia | 1.125 (1.237) | UD | UD | UD | 1.000 (1.414) | UD |
| Nitrite | UD | UD | UD | UD | 0.125 (0.177) | UD |
| Nitrate | UD | UD | UD | UD | UD | UD |
| Phosphate | 0.750 (0.354) | 0.125 (0.177) | 0.250 (0.354) | 1.000 | 0.250 | 2.000 |

UD (undetectable)= Concentration is below detectable limits

A substantial proportion of nutrients enter streams and rivers via runoff or urban wastewater effluents, largely stemming from domestic, industrial, and precipitation-driven outflows (Zamparas 2021). Elevated nutrient concentrations in lotic

systems enhance autotrophic biomass and primary production, often altering species composition (Weijters et al 2009). While nitrogen (N) and phosphorus (P) are essential nutrients for aquatic ecosystem health, excessive concentrations can lead to

eutrophication and the proliferation of algae and noxious aquatic plants. This process ultimately depletes dissolved oxygen levels, potentially causing fish kills (Mishra 2023). Recent data from the Philippine Statistics Authority (2021) indicate that the country's urbanization rate increased markedly from 51.73% in 2015 to 54.00% in 2020. This trend, combined with ongoing industrialization, elevates the risk of water quality deterioration and disrupts biodiversity by altering the physical and chemical conditions essential for aquatic life (Peralta et al. 2019). Ammonia, a pollutant that occurs naturally in aquatic environments, can also be augmented by anthropogenic sources such as industrial effluents, agricultural runoff, and sewage discharges, contributing to elevated levels observed in agricultural (site 1) and residential (site 5) areas (Soler et al. 2021; Xue et al. 2025). At low concentrations, ammonia is an essential nutrient for freshwater ecosystems; however, excessive levels can induce toxicity and eutrophication, reducing dissolved oxygen and creating unsuitable conditions for aquatic organisms (Richardson et al. 2021). Elevated ammonia concentrations at site 1 suggest influence from nitrogenous waste generated by livestock and runoff from agricultural lands (EPA 2023). Phosphate concentrations were consistently high across all sampling sites, with particularly elevated levels downstream of Alusiis (2 mg/L). These findings suggest contributions from industrial discharges and agricultural runoff, as indicated by the land-use types of the sampling sites (Badamasi et al. 2019; Peralta et al. 2020; Jolejole et al. 2021). In contrast, nitrate levels were undetectable across sites, possibly reflecting a slow rate of nitrification influenced by site-specific environmental conditions such as low dissolved oxygen, which limits the activity of nitrifying bacteria, and the potential for denitrification processes wherein facultative anaerobic bacteria reduce nitrates to nitrogen gas under oxygen-poor conditions

(Ward 2008; Burghate and Ingole 2014). These interacting factors suggest that the combination of low DO and the absence of measurable nitrates reflects an underlying biogeochemical dynamic shaping nutrient availability in the studied streams. The elevated phosphate concentrations align with previous observations that residential developments contribute significant nutrient loads through direct domestic wastewater discharges (Peralta et al. 2020).

Table 2 summarizes site-specific differences ($p < 0.001$) in DO, TDS, salinity, conductivity, and pH, indicating variability in physicochemical conditions among sites, with Dunn's post-hoc test further identifying the specific site contrasts that drive these patterns. In contrast, ammonia, nitrite, nitrate, and phosphate did not differ significantly across sites. For biological metrics, none of the indices (e.g., H' , H_{max} , evenness, richness, density) exhibited statistically significant site-specific differences. This pattern suggests that despite differences in land use and localized disturbances such as agricultural runoff, residential effluents, and industrial inputs, the overall level of biodiversity remains relatively stable because tolerant and opportunistic taxa compensate for declines in sensitive groups. Similar stability in diversity indices despite shifts in community structure has been documented in other disturbed tropical streams, where functional redundancy and the dominance of pollution-tolerant taxa buffer diversity measures against significant changes (Bae and Kim 2025; Enns et al. 2025). This compensatory dynamic occurs alongside pronounced site-to-site variation in physicochemical parameters, indicating differing levels of environmental degradation across the riverscape. Thus, while habitat conditions and macroinvertebrate assemblages vary among sites, the overall diversity patterns remain consistent.

Table 2: Comparison of environmental parameters and biological metrics across sites using the Kruskal-Wallis test and the corresponding post-hoc test.

| Parameters | H | DF | p-value | Dunn's Post-Hoc |
|----------------------------|--------|----|---------|---|
| Dissolved oxygen (mg/L) | 27.813 | 5 | <0.001 | Site 1 < Sites 2, 3 |
| TDS (ppt) | 30.419 | 5 | <0.001 | Site 1 > Sites 2 and 3; Sites 2 and 3 < Site 4 |
| Salinity (%) | 31.383 | 5 | <0.001 | Site 3 > Sites 1, 2, and 4; Sites 5 and 6 > Site 2 |
| Conductivity (μ S/cm) | 31.089 | 5 | <0.001 | Site 1 > Sites 2 and 3; Sites 2 and 3 < Site 4; Site 2 < Site 6 |
| pH | 29.259 | 5 | <0.001 | Site 3 > Sites 1 and 5; Site 4 > Site 5 |
| Ammonia | 7.167 | 5 | 0.209 | - |
| Nitrite | 4.500 | 5 | 0.479 | - |
| Phosphate | 8.337 | 5 | 0.139 | - |
| Diversity index (H') | 6.867 | 5 | 0.231 | - |
| H_{max} | 6.936 | 5 | 0.225 | - |
| Evenness (HE) | 3.499 | 5 | 0.623 | - |
| Taxon richness | 6.798 | 5 | 0.236 | - |
| Taxon density | 4.804 | 5 | 0.440 | - |
| EPTC richness | 10.628 | 5 | 0.059 | - |

Collectively, the results highlight the critical role of land-use patterns in shaping stream environmental conditions. Forested sites exhibit favorable physicochemical profiles, while urbanized streams are characterized by high nutrient concentrations and low oxygen levels. These findings underscore the importance of preserving riparian forest cover and implementing effective wastewater management strategies to mitigate eutrophication and sustain stream biodiversity in the country (Peralta et al. 2019; Peralta et al. 2020). Seasonal shifts in rainfall, runoff, and land-use activities, unaccounted for in this study, may also influence water quality parameters and macroinvertebrate responses. Future studies that incorporate multi-seasonal sampling will be essential for a more comprehensive assessment of stream ecological status.

Benthic macroinvertebrate assemblage

A total of 474 individuals were identified from six sites in Zambales with the Order Diptera (33%) being the dominant order, followed by Trichoptera (25%), Ephemeroptera (15%), Decapoda (9%), Coleoptera (5%), Hemiptera (5%), Subclass

Caenogastropoda (5%), and Lepidoptera (3%). Among the six sampling sites, site 3 exhibited notably higher macroinvertebrate diversity than the others. It recorded the highest values for almost all indices, including the diversity index ($H' = 2.17$), Hmax (2.803), and evenness ($HE = 0.54$), which, although relatively higher than those of all the other sites, still indicates low diversity and an unstable community structure (Baliton et al. 2020; Krebs 1989). This site also showed the greatest taxon richness (16.5) and taxon density (118.5 individuals/m² or appropriate unit), suggesting a more complex and abundant macroinvertebrate assemblage. Furthermore, it had the highest EPTC richness (67.50), typically reflecting favorable water quality conditions. These results indicate that site 3 supports a more diverse and abundant community of macroinvertebrates, potentially influenced by localized environmental factors or habitat conditions not immediately evident in other sites. For EPTC abundance, site 5 had the highest value, 77.50, while sites 1 and 6 presented undetected EPTC (Table 3).

Table 3: Mean (\pm standard error) values of macroinvertebrate diversity indices in Zambales.

| Indices | Zambales | | | | | | Grand Mean |
|--------------------------|--------------------|--------------------|---------------------|--------------------|-------------------|-------------------|--------------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Diversity index (H') | 0.564 | 1.817 | 2.169 | 0.710 | 2.149 | 1.351 | 1.460 |
| Hmax | 1.099 | 2.351 | 2.803 | 1.540 | 1.792 | 1.609 | 1.866 |
| Evenness (HE) | 0.141 | 0.454 | 0.541 | 0.177 | 0.537 | 0.337 | 0.364 |
| Taxon richness | 3.000 (2.000) | 10.500 (0.500) | 16.500 (3.500) | 4.670 (4.180) | 6.000 (1.000) | 5.000 (1.000) | 7.610 (0.610) |
| Taxon density | 12.000 (11.000) | 63.500 (30.500) | 118.500 (68.500) | 10.666 (7.446) | 18.000 (6.500) | 14.500 (9.500) | 39.611 (17.768) |
| EPTC richness | - | 19.000 (6.000) | 67.500 (31.500) | 4.667 (2.906) | 14.500 (5.500) | - | 17.611 (10.470) |
| EPTC abundance | - | 24.839 (1.203) | 60.433 (7.492) | 32.381 (16.930) | 77.500 (2.500) | - | 32.525 (12.879) |

– No computed values and/or standard error

Forested and upstream areas of the sampling sites allow more species to thrive, especially pollution-sensitive taxa, due to the availability of better water quality (Farukuzzaman et al. 2023; Peralta et al. 2019). This is because factors such as riparian shade and tree cover help create suitable conditions for these organisms to thrive (Magbanua et al. 2023). This finding is also corroborated by other studies revealing a higher ecological integrity in forested streams compared to agricultural and residential areas (Song et al. 2009; Corbi et al. 2013). Meanwhile, residential and agricultural streams still had an anthropogenic impact on water quality, resulting in undetectable EPTC species that were strongly influenced by pollution and disturbances posed to the streams (Compín and Céréghino 2003; Peralta et al. 2020). The results show low macroinvertebrate diversity among sites, and two sites (1 and 6) have undetectable EPTC, indicating poor water quality. This is supported by the composition of Diptera, including species in the family Simuliidae, which are considered very sensitive to organic pollution (Lock et al. 2014). The low diversity indices and

undetectable EPTC at some sites indicate that the streams are prone to, and have undergone, disturbances. This reflects poor urban stream habitat conditions and benthic biodiversity. As such, rehabilitation and mitigation measures must be prioritized in these areas prior to any development that may further result in biodiversity loss and habitat destruction compromising ecosystem services.

Benthic community response

Utilizing the species abundance and the environmental parameters across sampling sites, a CCA ordination triplot was generated (Figure 3) with 97.08% constrained variance (inertia = 2.22) and unconstrained variance 2.92% (inertia = 0.07). The first two axes of the CCA triplot explained 69.92% of the variation. Specifically, axis 1 accounted for 37.36% and axis 2 for 32.56% of the variation. Axis 1 is mainly associated with DO and PO₄ concentrations. Site 6 has high phosphate levels, which coincide with the presence of taxa such as *Aphelocheirus* sp., *Copelatus* sp., *Arctopsyche* sp.,

Cheumatopsyche sp., *Hydropsyche* sp., Hydrophilidae, *Heleocoris* sp., *Chimarra* sp., *Wormaldia* sp., *Cernotina* sp., *Polycentropus* sp., *Simulium* sp. Sites 2 and 4 are associated with high DO levels and contain taxa such as *Helophorus* sp., *Neritina* sp., *Brotia* sp., Parathelphusidae, *Macrobrachium* sp., *Melanoides* sp., *Tarebia* sp. Meanwhile, axis 2 is mainly characterized by DO and nutrient levels (i.e., NO₂ and PO₄). Site 5 has moderate phosphate levels and detectable nitrites, which are associated with taxa such as *Baetis* sp., *Microcylloepus* sp., *Larsia* sp., *Teloganodes* sp., *Sparsorythus* sp.

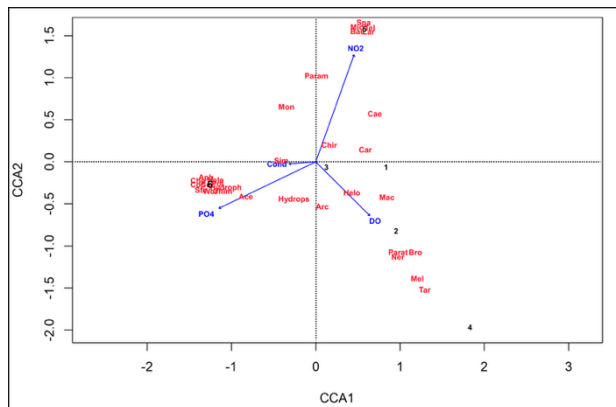


Figure 3: Triplot of the first and second CCA axes of macroinvertebrate taxa, environmental variables (dissolved oxygen, conductivity, NO₂, and PO₄) and sampling sites in Zambales. Taxa from Apheloceridae: *Aphelocheirus* sp., Atyidae: *Caridina* sp., Baetidae: *Acentrella* sp., *Baetis* sp., Caenidae: *Caenis* sp., Chironomidae: *Chironomus* sp., Crambidae: *Paraponyx* sp., Dytiscidae: *Copelatus* sp., Elmidae: *Microcylloepus* sp., Stenelmis sp., Hydropsychidae: *Arctopsyche* sp., *Cheumatopsyche* sp., *Hydropsyche* sp., Hydrophilidae: *Helophorus* sp., Naucoridae: *Heleocoris* sp., Neritidae: *Neritina* sp., Pachychilidae: *Brotia* sp., Parathelphusidae, Palaemonidae: *Macrobrachium* sp., Philopotamidae: *Chimarra* sp., *Wormaldia* sp., Polycentropodidae: *Cernotina* sp., *Polycentropus* sp., Simuliidae: *Simulium* sp., Tanypodinae: *Larsia* sp., *Monopelopia* sp., *Paramerina* sp., Teloganonidae: *Teloganodes* sp., Thiaridae: *Melanoides* sp., *Tarebia* sp., and Tricorythidae: *Sparsorythus* sp.

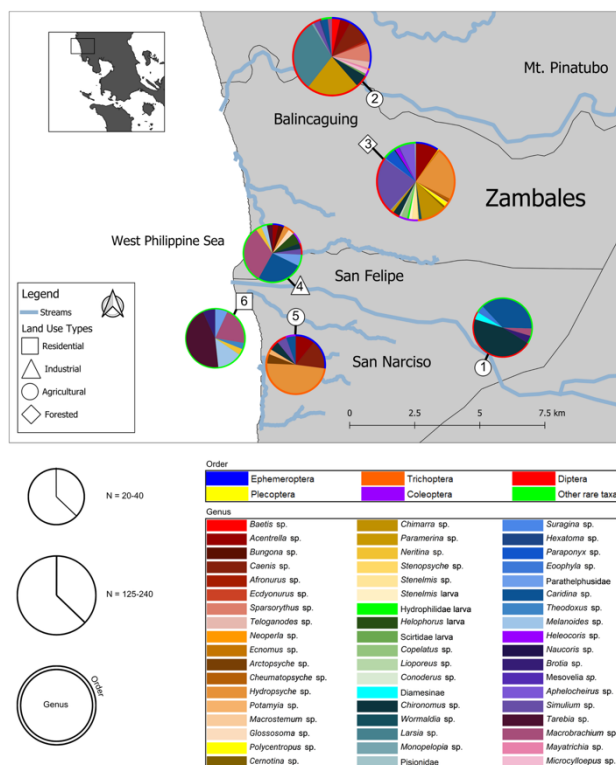


Figure 4: Benthic macroinvertebrate taxa distribution and population map per sampling site in Zambales.

Some species of the Hydropsychidae family, such as *Hydropsyche* sp., are influenced by phosphate inputs and organic matter in the environment (Gallardo-Mayenco and Ruiz 2007). This pattern can be seen at site 6 as it is characterized as a residential area. Residential areas are known as one of the contributors of increasing nutrient loads and organic pollution, hence affecting aquatic species biodiversity (Atasoy et al. 2006). *Copelatus* sp. from the Family Dytiscidae was also present in the site, this family of Coleopterans are known to be able to widely adapt to various freshwater habitats (Ghalley et al. 2021). Thiaridae (i.e., *Melanoides* sp., *Tarebia* sp.) were observed at sites associated with high DO levels. Even at low DO levels, this family of gastropods is known to be abundant in polluted waters (Makumbe et al. 2022). Despite detected high levels of nutrient concentrations in site 5, which is a residential area, pollution-sensitive taxa from Order Ephemeroptera (i.e., *Baetis* sp., *Teloganodes* sp., and *Sparsorythus* sp.) were among the organisms found in these conditions. This may be because some families, such as Baetidae, can survive in environments with moderately low oxygen and are often used as bioindicators of water quality (Alhejoj et al. 2014). Although it is associated with the presence of nutrients, the minimum oxygen requirement of some Ephemeroptera species is 4.6 mg/L, indicating that the DO in this site (6.35 mg/L) is more than enough for the species to thrive (Gaufin et al. 1974). Elmidae are known to primarily thrive in clean, oxygen-rich streams and rivers; however, the presence of *Microcylloepus* sp. suggests that some members of this family can tolerate changes in water quality (Braun et al. 2018; Braun et al. 2019).

CONCLUSION

This study provides a comprehensive evaluation of the ecological status of streams in the Zambales riverscape, relating physicochemical parameters with benthic macroinvertebrate assemblages. The findings demonstrate a clear relationship between land use and stream ecological integrity. Streams in forested areas exhibited relatively higher water quality and macroinvertebrate diversity, including the presence of pollution-sensitive EPTC taxa, while streams impacted by residential, agricultural, and industrial activities showed degraded water quality and diminished biological diversity. Specifically, sites 1 and 6 revealed signs of severe ecological stress, with undetectable EPTC species and a predominance of tolerant Diptera taxa, highlighting the consequences of anthropogenic disturbances such as nutrient enrichment, sedimentation, and organic pollution. PCA and CCA confirmed that physicochemical gradients, particularly dissolved oxygen, conductivity, and nutrient levels, are key drivers of macroinvertebrate community structure.

The integration of biological and chemical assessments offers a more holistic understanding of stream health than either approach alone. The results underscore the urgent need for enhanced monitoring, conservation planning, and implementation of best management practices, particularly in vulnerable areas where land use changes threaten aquatic biodiversity and ecosystem services. Conservation strategies should prioritize the protection and restoration of riparian forest cover, the reduction of point-source and diffuse pollution, and the implementation of sustainable land management practices. This study establishes baseline information for future ecological assessments in Zambales and contributes valuable insights toward freshwater ecosystem management and policy development in tropical riverine environments.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Carlos Miguel G. Orale: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Eliza Rose Y. Aquino, Lilian N. Dela Cruz, Miguel L. Estrada, Hannah Lorraine C. Frias:** Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - editing, Visualization. **Aleck Andrei R. De Guzman:** Methodology, Investigation, Writing - original draft. **Allyzxia Venisse H. Navarro, Ma. Angela Klarizze H. Solomon:** Methodology, Formal analysis, Investigation, Data curation. **Allan S. Gilles Jr.:** Supervision, Writing - review & editing. **Justine de Leon:** Methodology, Formal analysis, Investigation, Writing - review & editing. **Dino T. Tordesillas:** Supervision, Writing - review & editing. **Rey Donne S. Papa:** Project Leader, Investigation, Supervision, Writing - review & editing. **Elfritzon M. Peralta:** Conceptualization, Methodology, Formal analysis, Supervision, Project administration. Writing - review & editing.

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