International Journal of Medical Toxicology & Forensic Medicine
2026, Volume 16, E52454

Short Communication

Aquatic Plant–Associated Microbiomes as Biological Clocks for Postmortem Submersion Interval Estimation in Tropical Freshwater: A Proposed Framework

Riya Raj CA[1*], Don Caeiro[1], Nandini Katare[1], Mayank David Raiborde[1], Swetha Thomas[2]

1. *Department of Forensic Science, School of Biological and Forensic Sciences, Kristu Jayanti Deemed-to-be University, Bengaluru, Karnataka, India.*

Citation Raj CA, Caeiro D, Katare N, Raiborde MD, Thomas S. Aquatic Plant–Associated Microbiomes as Biological Clocks for Postmortem Submersion Interval Estimation in Tropical Freshwater: A Proposed Framework. International Journal of Medical Toxicology and Forensic Medicine. 2026;16:E52454.

https://doi.org/10.22037/ijmtfm.v16.52454

Article info:

Received: 10 June, 2026

Accepted: 25 June, 2026

Published: 29 June, 2026

Keywords:

Postmortem submersion interval, Forensic microbiology, Necrobiome, Aquatic macrophytes, 16S rRNA sequencing, Microbial succession

ABSTRACT

Background: Estimating the postmortem submersion interval (PMSI) is a persistent challenge in the forensic examination of bodies recovered from water. Established approaches like diatom analysis, aquatic entomology, and gross decomposition staging to lose reliability once remains are skeletonized or when seasonal and environmental conditions limit insect and algal evidence. The microbial "necrobiome" provides a complementary line of evidence: during decomposition, microbial communities undergo a time-ordered, reproducible sequence of changes.

Methods: We hypothesize that a decomposing body submerged in freshwater alters the microbial communities associated with the biofilms of nearby aquatic macrophytes, notably water hyacinth (Eichhornia crassipes), in a time-dependent manner usable for PMSI estimation. We outline a controlled mesocosm framework that compares three macrophytes, utilising destructive sampling across a 28-day interval, 16S rRNA amplicon sequencing, and supervised machine-learning models. These models are validated by a leave-one-tank-out strategy and an independent run.

Results: No data have yet been collected; this communication presents the hypothesis, rationale, and study design. If supported, plant-associated microbiomes could provide an independent PMSI estimator for tropical freshwater settings, a context currently absent from the necrobiome literature.

Conclusion: This study presents a hypothesis-driven framework for PMSI estimation based on microbial succession associated with aquatic macrophytes. Experimental validation may establish this approach as a complementary forensic tool for aquatic death investigations in tropical freshwater ecosystems.

*** Corresponding Author:**
Riya Raj CA, Assistant Professor
Department of Forensic Science, Kristu Jayanti (deemed-to-be) University, Bengaluru, Karnataka, India.
E-mail: *riyaraj.ca@kristujayanti.com*

Introduction

Bodies recovered from freshwater present some of the most difficult timelines to reconstruct in forensic practice. Estimation of the postmortem submersion interval (PMSI) currently relies on diatom analysis, aquatic insect colonization, water chemistry, and gross decomposition staging [1, 2]. Each degrades in reliability under precisely the conditions investigators most often encounter: advanced decomposition or skeletonisation, turbid water, and seasonal absence of colonizing insects.

The microbial necrobiome offers a promising, independent line of evidence. Microbial communities associated with decomposing remains undergo a reproducible, time-ordered succession, and this "microbial clock" has been used to estimate the postmortem interval in terrestrial settings [3, 4]. Aquatic decomposition involves analogous microbial dynamics [5, 6], yet the great majority of necrobiome research has been conducted in temperate terrestrial environments, and very little attention has been paid to microbial communities living on aquatic plants near submerged remains.

Aquatic macrophytes particularly water hyacinth (Eichhornia crassipes) that carry extensive root-associated biofilms with high surface area and strong nutrient-trapping capacity, allowing them to act as passive integrators of local chemical and microbial disturbance [7, 8]. We propose that these plant-associated communities may "record" the influence of a nearby decomposing body more stable than transient free-water samples, and may do so in a time-dependent way that can be read as a PMSI estimator. To our knowledge, plant-associated microbiomes have not been systematically evaluated as PMSI indicators, and not at all in tropical Indian freshwater, where decomposition rates, native microbiota, and macrophyte species differ substantially from temperate systems.

Hypothesis and Proposed Approach

Central hypothesis

A decomposing body alters the microbial communities associated with nearby aquatic plants, and these changes occur in a time-dependent, reproducible manner that can be used to estimate PMSI. The corresponding null hypothesis is that plant-associated community structure near a carcass does not differ from control beyond background environmental drift.

We outline a controlled mesocosm study under semi-controlled outdoor conditions to retain tropical realism. For each of three macrophytes (water hyacinth, duckweed [Lemna sp.], and hydrilla [Hydrilla verticillata]), three treatment groups are maintained: plant with carcass, plant without carcass (control), and a plant-free carcass blank to separate plant-associated from free-water communities. A standardized animal-tissue model (pig, Sus scrofa, as the human decomposition proxy) is used at a fixed mass-to-volume ratio, contingent on institutional animal-ethics approval.

Tanks are sampled destructively (independent replicate tanks per timepoint, rather than repeated sampling of the same tank, which would disturb the biofilm) at Days 0, 3, 7, 14, 21, and 28. Root biofilm, leaf biofilm, and filtered water are collected in triplicate, with physicochemical parameters (temperature, pH, dissolved oxygen, ammonia, nitrate, phosphate) logged as covariates. Microbial community composition is characterized by 16S rRNA V3–V4 amplicon sequencing (Illumina), with mock-community and negative controls on every batch; amplicon sequence variants are inferred and classified against a reference database, and succession analyzed by alpha/beta diversity and differential-abundance methods.

Microbial features and physicochemical covariates are then assembled into a per-sample matrix with PMSI (days) as the target, and supervised regression models (e.g., random forest) are trained and evaluated using nested cross-validation and a leave-one-tank-out strategy, followed by an independent validation run in fresh tanks. Feature-importance analysis identifies candidate biomarker taxa and indicates which macrophyte yields the most accurate estimator.

Anticipated Outcomes and Significance

We anticipate a reproducible, time-resolved shift in plant-associated communities in carcass tanks relative to controls, with characteristic taxa emerging at successive intervals. If realized, the framework would yield three outputs of forensic value: a tropical-freshwater reference dataset of microbial succession; a validated PMSI prediction model with error reported in days; and a plant-specific biomarker panel.

The principal significance is the provision of an independent PMSI estimator that does not depend on soft tissue or insect evidence surviving, addressing exactly the cases where current methods fail. In casework, plants growing near a recovered body could be sampled and their microbiome profile compared

against the reference database to back-calculate submersion time. The comparative design additionally identifies which macrophyte functions as the most reliable biological clock, moving beyond observation of a pattern toward a usable, biomarker-defined tool.

Conclusion

This communication proposes aquatic plant–associated microbiomes as a novel, independent biological clock for PMSI estimation in tropical freshwater. The hypothesis is testable through the controlled mesocosm framework outlined here. We present the concept and design at this stage to invite methodological discussion; empirical validation through the proposed experiments, including pilot work, is the necessary next step. Integration of volatile organic compound profiling is identified as a valuable future extension once analytical instrumentation is available.

Acknowledgment

None.

Funding

This study was not funded by any organization.

Conflicts of Interest

The authors report there are no competing interests to declare.

References

[1] Haskell NH, McShaffrey DG, Hawley DA, Williams RE, Pless JE. Use of aquatic insects in determining submersion interval. J Forensic Sci. 1989;34(3):622-32. [Link]

[2] Zimmerman KA, Wallace JR. The potential to determine a postmortem submersion interval based on algal/diatom diversity on decomposing mammalian carcasses in brackish ponds in Delaware. J Forensic Sci. 2008;53(4):935-41. [DOI: 10.1111/j.1556-4029.2008.00748.x]

[3] Metcalf JL, Wegener Parfrey L, Gonzalez A, Lauber CL, Knights D, Ackermann G, et al. A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. eLife. 2013;2:e01104. [DOI: 10.7554/eLife.01104]

[4] Pechal JL, Crippen TL, Benbow ME, Tarone AM, Dowd SE, Tomberlin JK. The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing. Int J Legal Med. 2014;128(1):193-205. [DOI: 10.1007/s00414-013-0872-1]

[5] Benbow ME, Pechal JL, Lang JM, Erb R, Wallace JR. The potential of high-throughput metagenomic sequencing of aquatic bacterial communities to estimate the postmortem submersion interval. J Forensic Sci. 2015;60(6):1500-10. [DOI: 10.1111/1556-4029.12859]

[6] Dickson GC, Poulter RTM, Maas EW, Probert PK, Kieser JA. Marine bacterial succession as a potential indicator of postmortem submersion interval. Forensic Sci Int. 2011;209(1-3):1-10. [DOI: 10.1016/j.forsciint.2010.10.016]

[7] Ávila MP, Oliveira-Júnior ES, Reis MP, Hester ER, Diamantino C, Veraart AJ, et al. The water hyacinth microbiome: link between carbon turnover and nutrient cycling. Microb Ecol. 2019;78(3):575-88. [DOI: 10.1007/s00248-019-01331-9]

[8] Yadav R, Rajput V, Dharne M. Insights into the community structure and environmental functions of water hyacinth rhizobiome in an urban river ecosystem. Environ Sci Pollut Res Int. 2024;31(49):59571-82. [DOI: 10.1007/s11356-024-35187-5]