



Beyond Exact Replication: Reflections on Ecological Reproducibility from Allozyme and Microsatellite Studies of *Perna viridis* in Malaysia

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Abstract

Scientific reproducibility has become a major concern across multiple research disciplines, particularly following reports that many published findings in biomedical sciences could not be independently replicated. However, the universal application of strict reproducibility standards across all scientific disciplines may overlook the inherent complexity and variability of ecological and biological systems. This commentary reflects upon two population genetic studies of the green-lipped mussel *Perna viridis* conducted in Peninsular Malaysia using fundamentally different molecular approaches: starch gel electrophoretic allozymes and microsatellite DNA markers. Although the studies differed in marker systems, sampling years, analytical sensitivity, and geographical coverage, both revealed remarkably similar large scale population structuring patterns. Both studies independently demonstrated low to moderate genetic differentiation among Malaysian mussel populations, geographical clustering between northern and southern populations, and sufficient genetic similarity supporting the use of *P. viridis* as a biomonitoring organism for heavy metal contamination. These findings provide an important example of conceptual reproducibility in ecology, where broad biological interpretations remain consistent despite methodological and environmental variability. This paper argues that ecological sciences should not be evaluated solely based on exact numerical duplication but rather through converging lines of evidence, ecological consistency, and robustness across independent methodologies. Variability in ecological studies should not automatically be interpreted as scientific weakness because natural systems are dynamic across space and time. Instead, ecological variability itself often represents meaningful biological and environmental information.

Keywords: Biomonitoring; Conceptual Reproducibility; Ecological Variability; Allozymes; Microsatellites; *Perna viridis*

Introduction

In recent decades, reproducibility has become one of the central discussions in modern science. Concerns intensified following reports that many published findings, particularly in biomedical and pharmaceutical sciences, could not be independently replicated [1, 2]. These concerns generated debates regarding research quality, publication pressure, scientific integrity, and the reliability of scientific discoveries.

However, while reproducibility is unquestionably important, the universal application of rigid reproducibility standards across all scientific disciplines deserves careful reconsideration. Ecology, ecotoxicology, biomonitoring, and population genetics differ fundamentally from tightly controlled laboratory sciences because they involve dynamic natural systems continuously influenced by environmental variability, climatic fluctuations, hydrodynamic processes, evolutionary mechanisms, and spatial heterogeneity [3, 4].

As a long-term ecotoxicologist and biomonitoring researcher working with the green-lipped mussel *Perna viridis* since the late 1990s, I have often reflected upon the meaning of reproducibility in ecological studies. This reflection became particularly meaningful when comparing two independent population genetic studies conducted on *P. viridis* populations from Peninsular Malaysia. The earlier study employed starch gel electrophoretic allozyme markers [5], while a later study conducted by my student and collaborators used microsatellite DNA markers [6]. Although the two studies differed substantially in molecular methodologies, analytical sensitivities, sampling

locations, and periods of investigation, their overall biological conclusions were remarkably similar.

This commentary reflects upon the significance of these similarities and argues that ecological reproducibility should not be interpreted solely as exact numerical duplication. Rather, ecology often demonstrates conceptual reproducibility, where independent approaches consistently support similar biological interpretations despite environmental and methodological variation.

The 2002 Allozyme Study: An Early Population Genetic Perspective

The 2002 study by Yap *et al.*, [5] investigated genetic variation among eight populations of *Perna viridis* collected from the west coast of Peninsular Malaysia using horizontal starch gel electrophoresis. Fourteen polymorphic loci were successfully analysed across geographically separated populations including Penang, Pulau Aman, Bagan Lalang, Telok Emas, Sungai Muar, Tanjung Kupang, Pantai Lido, and Kampung Pasir Puteh.

At that time, allozyme electrophoresis represented one of the major population genetic tools available for ecological studies. Allozymes measure electrophoretically detectable variants of enzymes encoded by different alleles. Because enzymes are products of gene expression, allozyme patterns may be influenced not only by genetic differences but also by physiological and environmental conditions.

Despite these limitations, the study demonstrated several important findings. First, the populations displayed moderate genetic differentiation with a mean F_{ST} value of 0.149. Second, the UPGMA dendrogram revealed two major geographical groupings: northern populations clustered together while central and southern populations formed another cluster. Third, the genetic distance values ranged from 0.004 to 0.091, indicating that all populations belonged to the same species while maintaining regional differentiation.

Most importantly, the study concluded that *P. viridis* populations from Peninsular Malaysia were genetically similar enough to support their use as biomonitoring organisms for heavy metal contamination studies.

At that stage, the findings reflected a balance between regional population structuring and larval mediated gene flow. Although *P. viridis* possesses a pelagic larval stage lasting approximately three weeks, the study suggested that larval dispersal alone was insufficient to completely homogenize populations across the Straits of Malacca.

The 2009 Microsatellite Study: Independent Validation Using DNA Markers

Several years later, Ong *et al.*, [6] revisited the genetic structure of *Perna viridis* populations in Peninsular Malaysia using microsatellite DNA markers. Microsatellites are highly polymorphic, selectively neutral, codominant molecular markers generally considered more sensitive and informative than allozymes for population genetic analyses.

The microsatellite study expanded the geographical scope to include ten populations distributed across northern, central, southern, and eastern Peninsular Malaysia. Nineteen polymorphic microsatellite loci were analysed. Interestingly, despite employing a fundamentally different molecular approach, the overall biological conclusions were remarkably similar to those obtained in the earlier allozyme study.

The microsatellite study reported: a) low to moderate genetic differentiation with a mean F_{ST} value of 0.098, b) high overall genetic similarity among populations, c) and geographical clustering separating northern populations from central and southern populations, d) Furthermore, the study explicitly acknowledged the earlier allozyme findings and stated that the microsatellite analyses were conducted partly to validate previous conclusions obtained using allozymes.

The consistency between these two studies is scientifically important because exact replication was never realistically expected. The studies differed in: a) molecular marker systems, b) sampling years, c) loci numbers, d) analytical sensitivities, e) geographical coverage, and f) environmental conditions. Yet, despite these differences, the major biological interpretations remained highly consistent.

Conceptual Reproducibility in Ecology

The similarities between the allozyme and microsatellite studies provide an excellent example of conceptual reproducibility rather than exact reproducibility. Exact reproducibility refers to obtaining nearly identical numerical outcomes when repeating experiments under highly controlled conditions. This concept is highly relevant in many laboratories based biomedical sciences. However, ecology and environmental biology operate within fundamentally different realities because ecosystems are dynamic systems continuously changing across time and space [7].

Conceptual reproducibility occurs when independent studies using different methods arrive at similar scientific conclusions [8]. In the present case, both allozyme and microsatellite studies independently demonstrated: a) moderate population differentiation, b) regional geographical structuring, c) high overall genetic similarity, d) and suitability of *P. viridis* as a biomonitoring species. These converging findings strongly suggest that the observed patterns reflect real biological processes rather than methodological artifacts.

The consistency is especially meaningful because allozymes and microsatellites differ fundamentally in their biological properties. Allozymes measure expressed proteins that may be influenced by environmental conditions and selective pressures, while microsatellites measure selectively neutral DNA polymorphisms. Therefore, identical numerical outputs between the two studies were neither expected nor necessary.

Instead, the similarity of broad biological conclusions strengthens confidence in the ecological interpretations.

Ecological Variability as Scientific Information

One of the major lessons emerging from long term biomonitoring research is that variability itself often represents meaningful ecological information. Natural ecosystems are not static laboratory systems. Coastal hydrodynamics, sediment transport, salinity gradients, pollution inputs, larval dispersal patterns, reproductive cycles, and local environmental conditions continuously shape biological populations.

Consequently, expecting ecological studies to produce perfectly identical outcomes across different years and methodologies may overlook the very nature of ecological systems.

The differences observed between the allozyme and microsatellite

studies are therefore scientifically expected. Yet the persistence of similar large scale geographical structuring patterns despite these differences demonstrates ecological robustness.

In ecotoxicology and biomonitoring, the challenge is not to eliminate variability but to interpret variability meaningfully. Temporal fluctuations in genetic structure, contaminant accumulation, and biological responses may themselves reflect ecological adaptation, environmental stress, or changing ecosystem conditions.

Thus, ecological reproducibility should be evaluated through: a) robustness of interpretation, b) transparency of methodology, c) convergence across independent evidence, d) and ecological consistency across scales.

Reflections from Long-Term Biomonitoring Research

Reflecting upon these two studies after many years, I increasingly appreciate that ecological science differs profoundly from mechanistic laboratory sciences. Nature is dynamic. Mussel populations sampled twenty years apart experience different environmental histories, pollution exposures, climatic conditions, and ecological pressures.

Even researchers themselves change through time in terms of experience, interpretation, analytical approaches, and scientific understanding. Yet, despite this complexity, certain ecological patterns persist. The repeated detection of northern *versus* southern population structuring in Malaysian *Perna viridis* populations across independent molecular approaches suggests that ecological reality can still emerge clearly from within natural variability.

This experience has reinforced my belief that ecological sciences require a more nuanced understanding of reproducibility. Scientific rigor remains essential, but ecological rigor must recognize that living systems are inherently dynamic rather than static. The true strength of ecological science may therefore lie not in producing identical numerical duplication, but in revealing consistent biological understanding through multiple independent perspectives.

Conclusion

The comparison between the 2002 allozyme study and the 2009 microsatellite study of *Perna viridis* populations in Peninsular Malaysia demonstrates the importance of conceptual reproducibility in ecological sciences. Although the studies differed substantially in methodology, sampling design, and analytical sensitivity, both independently supported similar biological conclusions regarding

population structure and biomonitoring suitability. These findings suggest that ecological reproducibility should not be interpreted solely through exact numerical duplication. Instead, ecological robustness often emerges through converging lines of evidence obtained across different methodologies, time periods, and environmental contexts. The natural world is dynamic and continuously changing. Ecological variability therefore represents not scientific weakness but ecological reality itself. Future discussions regarding reproducibility should recognize the disciplinary differences between highly controlled laboratory sciences and environmentally dynamic ecological systems.

Declaration on the Use of Artificial Intelligence

The author declares that generative artificial intelligence tools were used in a supportive capacity during the preparation of this manuscript. These tools assisted with language refinement, structural organisation, and stylistic clarity of the text. All ideas, interpretations, reflections, and conclusions presented in this paper are the author's own and are grounded in personal experience and scholarly judgment. The author retains full responsibility for the content, accuracy, originality, and integrity of the manuscript.

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