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Evaluating the Reliability of Biological Trait Indicators for Detecting Subtle Environmental Changes

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Abstract: Biological characteristics are increasingly recognized as early indicators of environmental changes, although their performance for capturing subtle environmental stress remains uncertain. The potential for five stream macroinvertebrate traits to detect a 0.15 change in a standardized 0-1 environmental stress index was assessed. A simulated freshwater monitoring program was constructed with 12 sites over five years, measuring 60 individuals each year. The traits analyzed included body size, emergence date, activity rate, fluctuating asymmetry and gill-to-body ratio. Canopy cover was treated as a confounding variable and survey year as a blocking factor. Trait performance was quantified using standardized gradient effects, repeatability, temporal consistency, statistical power of detection, and minimum detectable change to produce a composite measure of trait reliability. To avoid pseudo replication, site-year mean responses were used for all calculations and were presented with confidence limits to demonstrate uncertainty. Emergence date had weak overall performance and fluctuating asymmetry was highly unreliable; however, body size and gill-to-body ratio showed the best results (respectively 0.92 and 0.83 reliability), with high repeatability (>0.93) and consistent responses in all five years. Detection power reached 98% for body size and 77% for gill-to-body ratio. Activity rate was unreliable, while body size and gill-to-body ratio were both suitable early indicators of subtle environmental change. Results illustrate the importance of temporal stability, high replication, and consistent environmental responses when monitoring the gradual deterioration of freshwater systems.

Keywords: Biological Traits; Bioindicators; Environmental Monitoring; Detection Power; Repeatability; Stream Macroinvertebrates.

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I. Introduction

1.1 Traits as Indicators of Environmental Response

Traits are characterizations of what organisms are and do, for example, size, phenology, behavior, or body shape and proportions (Sotomayor et al., 2025). As traits are directly associated with key ecological processes, they will change as organisms respond to warming, pollution, or habitat degradation, in many cases even before the species itself is lost. Consequently, trait-based methods are providing a way to assess environmental change mechanistically that is comparable among systems with very different species composition, which is why traits are increasingly being used for biological monitoring, where feedback to management requires interpretable and transferable indicators among locations and years (Lima et al., 2017).

1.2 Detecting Subtle Change Amid Natural Variability

Environmental change may be hard to pick up because trait values fluctuate among individual organisms, sites, seasons, and years (Kitaka et al., 2024). If this background variation is high, then a slight change in the environment results in a response smaller than the variation around it, so either the change goes unnoticed, or the monitoring program detects a false change that is really just weather or sampling effort (Mureithi et al., 2025). Other factors, such as shading, flow, and habitat structure, also cause this lack of independence and increase uncertainty. As a result, robust indicators need to show a response to the environment coupled with low variation and relative stasis over time.

1.3 Research Gap and Objectives

While most trait studies document whether a trait is related to an environmental gradient, few document whether such a relationship is repeatable, the same across years, and sufficiently strong to detect small effects. Filled this knowledge gap by comparing five traits in the same study system. The goals were to measure the strength of a gradient response with standard methods, while controlling for canopy cover and year, to calculate the repeatability and constancy of temporal responses, and to calculate the minimum detectable change for each trait and the powers of the tests. Predicted that morphology would outperform behavior and phenology because morphology integrates over longer periods and varies less across years, but that prediction is a crude test of the measurement system.

The rest of this document is organized as follows. Section 2 provides background on trait indicators in biology, research gaps, and aims. Section 3 outlines the theoretical framework. Section 4 explains the setup of the simulated system, measurement of biological trait indicators, and evaluation of reliability. Section 5 displays the results of the experiment. Section 6 discusses the results, comparisons, and challenges. Section 7 wraps up with monitoring advice and future work.

II. Background and Conceptual Framework

2.1 Trait Indicators versus Species-Based Indicators

A trait indicator is a quantifiable attribute of an organism where there is a known response of the trait value to a stressor (Firmiano et al., 2017). This relationship allows the quantifiable trait to be used as a descriptor of the stressor. Biological trait indicators are different from species-based indicators in that species presence, absence, or abundance relies on regional species pools and taxonomic expertise, while traits describe function, not identity, allowing a given trait to be compared between communities with few common species. This functional focus leads to higher transferability, but requires standard measurement protocols and knowledge of trait-stressor relationships (Teresa & Casatti, 2017).

2.2 Traits that are Sensitive to Environmental Change

Size is commonly assumed to decrease with warming and enrichment, owing to greater survival and faster development of smaller bodies, while phenotype (emergence date) is known to change with temperature across a broad spectrum of taxonomic groups. Behavioral traits (activity rate) may change within hours but vary with light and food. Fluctuating asymmetry shows how instability during development increases under stress, and gill-to-body ratio indicates oxygen-uptake investment (Menezes et al., 2010). The five traits include a morphological trait, a phenological trait, a behavioral trait, a measure of developmental stability, and a physiological trait covering response speed, and can be measured quantitatively so that reliability can be directly compared (Correa et al., 2022).

2.3 Variability among Species, Sites, and Temporal Scales

Trait responses are not constant. Taxa vary in physiological tolerance such that the same driver may increase trait values in some taxa and reduce them in others. Sites vary in mean conditions, so differences in the baseline trait value at each location can obscure patterns along spatial gradients if the sites aren't well-represented or replicated (Mueller et al., 2013). The same applies over time: behavioral and phenological traits are sensitive to conditions in a single year and fluctuate strongly across years, while morphological traits reflect the gradient's integrated effect on exposure over a longer window, across several years, with slower rates of change. So, performance in one place or time does not necessarily guarantee performance elsewhere: efficacy should be explicitly tested (Van den Brink et al., 2011).

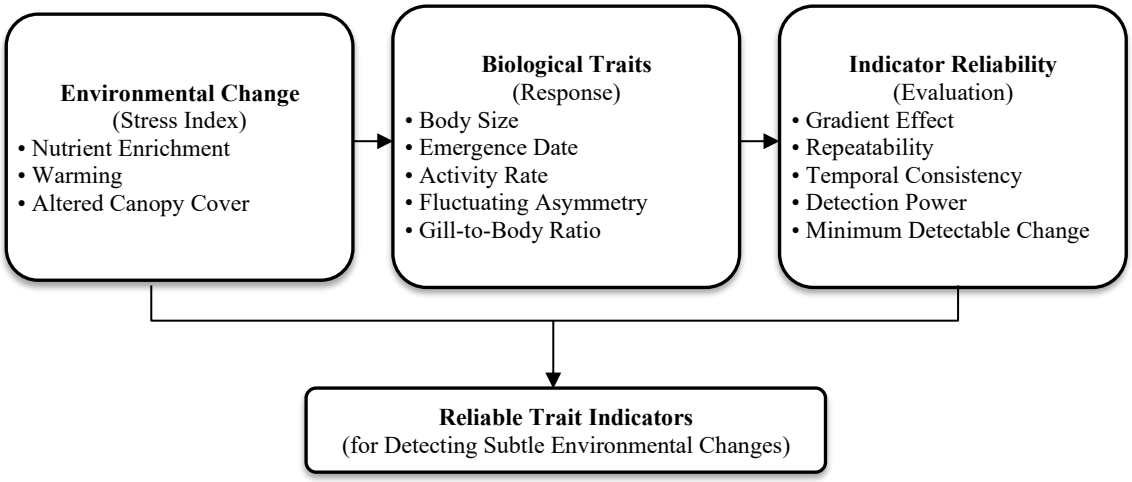


Figure 1: Conceptual Framework for Evaluating Biological Trait Indicator Reliability

Figure 1 shows the Conceptual model of the five biological traits influenced by environmental stress assessed in this study. Trait responses are assessed using gradient sensitivity, reproducibility, temporal stability, ability to detect change and minimum detectable change. This assessment identifies suitable biological indicators for detecting low levels of environmental changes.

III. Methods

3.1 Study Sites, Organisms, and Environmental Conditions

They simulated a monitoring program based on 12 stream sites covering the entire range of the standardized stress index, E, of from 0.05 to 0.95, where E combines nutrient enrichment and temperature load. Sampling occurred over five years. The six most stressed and vulnerable sites were assumed to be impacted by an additional increase of 0.15 in E for all five years, meaning there was a slight deterioration in stress load, while the six least impacted sites remained stable. Canopy cover, C, varied among sites but was only weakly related to E (r= 0.32). 60 individuals of each macroinvertebrate were sampled for each site each year, amounting to 3,600 individual trait measurements.

3.2 Attributes and Data Accumulation

For each individual, generated five traits: body size, emergence date, activity rate, fluctuating asymmetry, and gill-to-body ratio. Means, gradient slopes, canopy effects, and variance components of these five traits are summarized in table 1. Individual trait values were sampled according to equation (1), where site and year effects were nested random effects and residual was individual effect and measurement error. Effect sizes were chosen to include strong to null responses so that reliability could be estimated across a wide range of effect sizes. All data were generated from a fixed seed for complete reproducibility, and means per site and year were used in analyses so that individuals were not assumed to be independent replicates of the environment.

Table 1: Generating Parameters for the Five Traits

Trait	μ	β_E	β_C	σ_s	σ_y	σ_ε
Body Size (mm)	8.00	−1.60	0.80	0.30	0.20	1.10
Emergence Date (Day of Year)	150.0	−9.0	−6.0	2.5	3.2	11.0
Activity Rate (Moves min^{-1})	12.0	2.0	0.0	0.7	0.6	3.2
Fluctuating Asymmetry (Index)	0.040	0.012	0.004	0.004	0.003	0.018
Gill-to-Body Ratio	0.220	0.040	0.010	0.012	0.007	0.045

3.3 Evaluating Indicator Reliability

Reliability was composed of five indices. Standard sensitivity, S (Equation 2), is the gradient of a regression of site-year means, with S being the standardized slope. E was used as a predictor, with canopy cover and survey year as covariates to eliminate confounding effects. The order of sites over years, is measured by the repeatability, R (Equation 3). Temporal consistency was the number of years a significant Spearman correlation in the predicted direction, was detected. Power to detect small changes, (Equations 4 and 5), and Minimum Detectable Change, (Equation 6), quantify sensitivity to subtle change, while the Standardised Index of Reliability, (Equation 7), combines sensitivity, R, and power. Two-sided significance tests were used, with a significance level of 0.05.

$$y_{ijk} = \mu + \beta_E E_{ij} + \beta_C(C_i - \bar{C}) + u_i + v_j + \varepsilon_{ijk} \quad (1)$$

$$S = \frac{|\hat{\beta}_E|\sigma_E}{\sigma_{\bar{y}}} \quad (2)$$

$$R = \frac{\sigma_s^2}{\sigma_s^2 + \sigma_w^2} \quad (3)$$

$$\Pi(\Delta E) = \Pr\left(|T'| > t_{1-\alpha/2,k-1}\right) \quad (4)$$

$$\lambda = \frac{\sqrt{k}|\hat{\beta}_E|\Delta E}{\sigma_w} \quad (5)$$

$$\Delta E_{min} = \min\{\Delta E : \Pi(\Delta E) \geq 0.80\} \quad (6)$$

$$RI = \sqrt[3]{SR\Pi} \quad (7)$$

IV. Results

4.1 Trait Responses along the Stress Gradient

Body size showed a negative trend across the stress gradient (slope = 1.63 mm / stress gradient, 95% confidence interval 1.95 to 1.32, $p < 0.001$), as did gill to body ratio (0.055), fluctuating asymmetry (0.0085), and emergence date (8.1 days; all $p < 0.001$; Table 2), while activity rate was unaffected (slope = 0.43, 95% confidence interval 0.42 to 1.27, $p = 0.32$). Partial R2-values accounting for variation in canopy cover and year, respectively, were 0.67 for body size and 0.64 for gill to body ratio. The effect of body size was most strongly standardised. In figure 2(a-f), the site-year means of all four traits are presented; for all traits, effects standardised by the effect of slope in the full model with confidence intervals.

Table 2: Environmental Stress Gradient Responses of Biological Traits with Statistical Significance and Sensitivity Measures

Trait	Slope	95% CI	p	Partial R^2	ρ	S
Body Size (mm)	−1.63	[−1.95, −1.32]	< 0.001	0.67	−0.78	0.83
Emergence Date (Day of Year)	−8.11	[−11.35, −4.88]	< 0.001	0.32	−0.52	0.55
Activity Rate (Moves min^{-1})	0.43	[−0.42, 1.27]	0.32	0.02	0.04	0.13
Fluctuating Asymmetry	0.0085	[0.0053, 0.0117]	< 0.001	0.35	0.54	0.59
Gill-to-Body Ratio	0.0547	[0.0433, 0.0661]	< 0.001	0.64	0.79	0.78

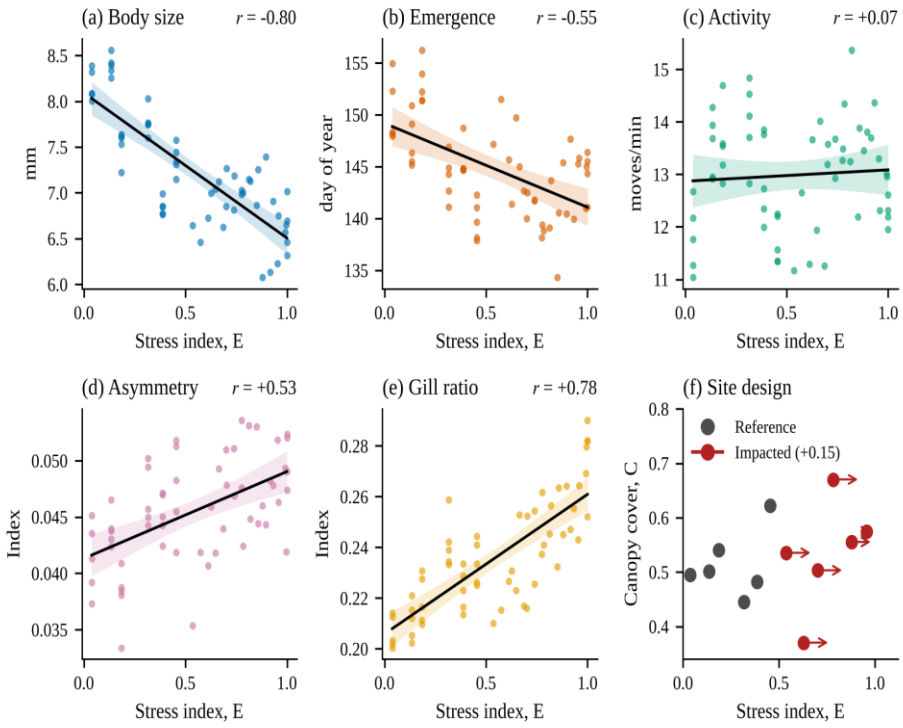


Figure 2(a-f) : Biological Trait Responses to Environmental Stress Gradients and Study Site Distribution

4.2 Sensitivity and Consistency of Individual Traits

Figure 3(a-c) shows that Change in body size, emergence date and gill-to-body ratio for reference and impacted sites across the five survey years. Size is consistently lower in impacted sites, although patterns of change over years suggest a response to human impacts. Emergence date varied among years, but impacted sites showed a tendency for earlier emergence. Gill-to-body ratio was consistently higher in impacted sites and significantly different in temporal change between impacted and reference sites ($p = 0.007$). Shaded regions encompass site means ± standard error.

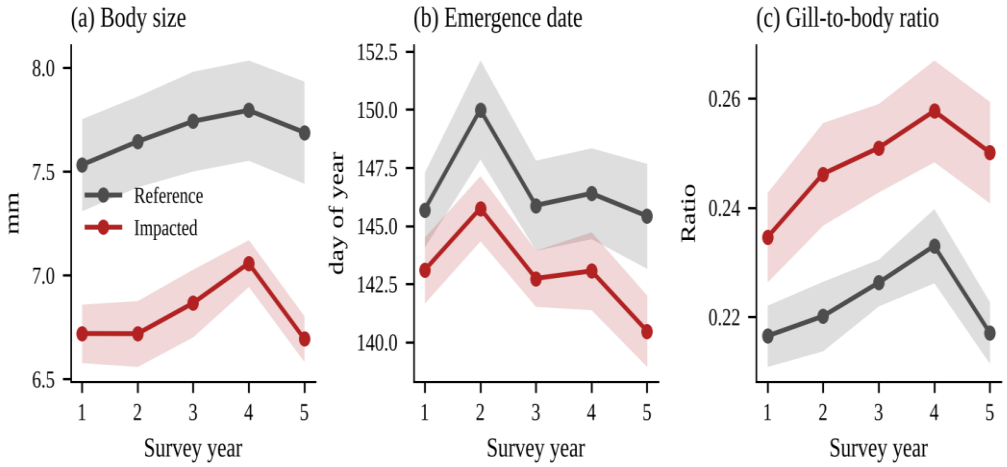


Figure 3(a-c): Annual Trajectories (Mean ± Standard Error Across Sites) of Body Size, Emergence Date, And Gill-To-Body Ratio in Reference and Impacted Sites

4.3 Statistical Evidence, Uncertainty, and Group Differences

Detection power at the benchmark change of 0.15 was 0.98 for body size, 0.77 for gill-to-body ratio, 0.33 for emergence date, 0.20 for fluctuating asymmetry, and 0.06 for activity rate. Minimum detectable changes were 0.10, 0.16, 0.28, and 0.38 for the four responsive traits and exceeded the index range for activity rate. The reliability index ranked body size first (0.92), followed by gill-to-body ratio (0.83), emergence date (0.54), fluctuating asymmetry (0.45), and activity rate (0.19). In year 5, the standardised differences between impacted and reference sites reached 2.13 in magnitude for body size and 1.76 for gill-to-body ratio (Table 3).

Table 3: Comparative Reliability Assessment of Biological Trait Indicators Based on Signal-to-Noise Ratio, Repeatability, Detection Power, and Minimum Detectable Change

Trait	SNR	R	Years	Π	MDC	RI
BODY Size	2.09	0.97	5	0.98	0.10	0.92
Emergence Date	0.76	0.88	2	0.33	0.28	0.54
Activity Rate	0.16	0.85	0	0.06	> 1.00	0.19
Fluctuating Asymmetry	0.56	0.76	2	0.20	0.38	0.45
Gill-to-Body Ratio	1.39	0.93	5	0.77	0.16	0.83

V. Discussion

5.1 Which Traits Detect Subtle Change, and When

Two items isolated reliable from unreliable responses: the magnitude of a signal compared to temporal variation, and the consistency of site rankings across years. Body size and gill-to-body ratio met both, allowing them to detect change of about 0.10 and 0.16 index units using six sites. Emergence date was sensitive to the gradient, but variation in the timing of emergence across years had a too-low ratio of 0.76, necessitating longer series or more sites. Fluctuating asymmetry experienced a real but noisy signal, and activity rate failed as temporally-variable site effects contributed an estimated 0.43 when the slope that generated it was 2.0. A trait is a window or a mirror!

5.2 Comparison with Other Studies and Other Explanations

The strong performance of body size aligns with the evidence of a strong and predictable response of size to warming. The poorer performance of phenology stands in contrast to its prominent role in climate studies (where long records downweight the importance of annual variation) and the small magnitude of fluctuating asymmetry (which has been ascribed to measurement noise for some time). Canopy cover could also have confounded results and differences in the variance assigned to each trait could have biased some traits in its favour. The rankings thus reflect the structure of variance as much as being representative of any one river.

5.3 Limitations

There are some limitations. Measurement error was incorporated as an additional component of individual variance, not a second model factor, so consistency across field measurements depends on protocol detail; lower precision protocols would reduce the reliability, especially where those traits display fluctuating asymmetry. Sampling was restricted to 12 sites so estimates of the gradient are less precise; activity rate has a very wide confidence interval. Trait error was modelled with zero skewness and a normal distribution; real populations may have more variable trait distribution, which could vary with species. Validity could also be improved by measuring multiple stress indices; how their combined effects interact is not understood. Several validation steps are required before operational use of these reliability indices.

VI. Conclusion

Many varieties of indicator reliability emerged from these five traits. Body size and gill-to-body ratio indicated strong gradient responses, had reliability of > 0.93, and were detected in all five years. Emergence date and fluctuating asymmetry were sensitive to the gradient but were not detected in three of five years and provided limited power for detecting subtle change. Activity rate showed no consistent response. Consequently, indicator reliability was less related to whether the trait was correlated with environment than to the magnitude of its response relative to temporal noise, a distinction that simple correlations do not capture. Therefore, reliability must be tested before a trait is used for monitoring. Body size is the best indicator, detecting changes of about 0.10 index units with six sites and two survey years per period. Gill-to-body ratio is nearly as good and was the only trait for which within-site changes were significantly different in impact and reference sites. It is suitable for long-term monitoring of thermal and nutrient stress in streams with similar sampling effort. Emergence date may complement these traits in long-term records and fluctuating asymmetry can be used as a secondary screening tool when the measurement protocols are standardised. A monitoring programme could start with body size and gill-to-body ratio, then incorporate other traits as resources allow. Research should validate these rankings in long-term field datasets covering multiple taxa and locations, incorporate repeated measurements to estimate error, and test non-linear and threshold responses. Monitoring should prioritise morphological traits, use at least five sites with two years of sampling, record canopy cover as a covariate, and report effect sizes and power in addition to trait correlations. Behavioural traits should only be used after demonstrable higher local repeatability. Standardised trait collection protocols would enable comparison of reliability estimates among studies. Raw data should be stored for future recalculations of reliability as methodology advances.

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