

# Diversity of aquatic insects as bioindicators of water quality

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## ABSTRACT

*Aquatic insects constitute the dominant component of macroinvertebrate communities in most freshwater ecosystems and are among the most widely used bioindicators of water quality, owing to their well-documented differential sensitivity to pollution, their tractable taxonomy, and their integration of environmental conditions over time through their sedentary larval phase. This study presents a systematic assessment of aquatic insect diversity and its relationship with water quality across 48 stream, river, and reservoir sites spanning an urbanisation-to-forest gradient in the Godavari river basin of Telangana and Andhra Pradesh, India, sampled using standardised kick-net and Surber sampler protocols during wet and dry seasons over two years (2019-2021). A total of 284 aquatic insect species from 12 orders and 84 families were documented, dominated by Ephemeroptera (72 species), Trichoptera (64 species), Diptera (58 species), and Plecoptera (38 species). The SIGNAL2 (Stream Invertebrate Grade Number Average Level) biotic index and the EPT (Ephemeroptera-Plecoptera-Trichoptera) richness index together correctly predicted water quality class in 88.4% of sites when validated against physicochemical measurements. Forest stream sites supported the highest EPT richness (mean 48.4 species) and SIGNAL2 scores (mean 6.84), while urban river sites showed the lowest values (mean EPT 8.4; SIGNAL2 3.42). Dissolved oxygen, BOD, and conductivity are the strongest physicochemical predictors of EPT richness. The results validate the applicability of EPT-based bioassessment frameworks to peninsular Indian river systems and provide a calibrated regional reference dataset for water quality monitoring.*

**Keywords:** aquatic insects; bioindicators; water quality; EPT richness; Ephemeroptera; Trichoptera; Godavari basin; SIGNAL2; bioassessment; macroinvertebrates

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## 1. Introduction

Freshwater ecosystems, despite covering less than 1% of Earth's surface, harbour approximately 10% of all described species and provide essential ecosystem services including drinking water supply, irrigation, fisheries, and flood regulation. Maintaining the ecological integrity of freshwater systems requires effective monitoring frameworks capable of detecting and attributing water quality degradation to specific anthropogenic drivers. Biological water quality assessment using macroinvertebrate communities -- the application of biotic indices derived from species composition and abundance data -- has become the cornerstone of regulatory freshwater monitoring in Europe (EU Water Framework Directive), North America (EPA Rapid Bioassessment Protocols), and Australia (AUSRIVAS), owing to the superior ability of biological communities to integrate temporal variation in water chemistry compared to snapshot physicochemical measurements. Aquatic insects -- particularly the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), collectively the EPT group -- are the primary biological indicators in most established bioassessment frameworks.

In India, biological water quality assessment frameworks based on aquatic macroinvertebrates have been developed in limited regional contexts -- primarily for Himalayan streams (Sharma 2008) and Western Ghats rivers (Subramanian and Sivaramakrishnan 2007) -- but a validated bioassessment framework for peninsular Indian river systems of the Deccan Plateau is absent. The Godavari river basin, India's second largest river system draining approximately 312,812 km<sup>2</sup> across Telangana, Andhra Pradesh, Maharashtra, and Odisha, faces severe and escalating water quality pressures from urban wastewater discharge, industrial effluents, and agricultural runoff. Systematic biological assessment of Godavari basin

water quality using aquatic insects as indicators would provide a scientifically robust and cost-effective complement to existing physicochemical monitoring.

The objectives of this study are: (1) to document aquatic insect species diversity across a water quality gradient in the Godavari basin using standardised sampling methods; (2) to calculate and validate EPT richness and SIGNAL2 biotic indices against physicochemical water quality data; (3) to identify the primary physicochemical predictors of aquatic insect community composition; (4) to establish reference condition aquatic insect communities for undisturbed forest streams in the basin; and (5) to provide a calibrated regional reference dataset for future biological water quality monitoring in the Godavari basin and adjacent peninsular Indian rivers.

## 2. Literature Review

### 2.1 EPT-Based Bioassessment

The use of Ephemeroptera, Plecoptera, and Trichoptera richness as water quality indicators is founded on the well-documented sensitivity of these orders to organic pollution and physical habitat degradation. EPT taxa generally require high dissolved oxygen concentrations, low BOD, and intact stream substrate for larval development, making them intolerant of the oxygen depletion and sediment loading characteristic of polluted reaches. The EPT/Chironomidae ratio -- relating pollution-sensitive EPT richness to the abundance of pollution-tolerant midge larvae -- has been validated as a robust water quality indicator in tropical and subtropical streams across three continents (Sharma 2008; Kudhungania and Dorris 1973). Meta-analysis by Friberg et al. (2011) confirmed that EPT richness is the single most reliable predictor of biological water quality class across diverse biogeographic regions.

### 2.2 SIGNAL2 Biotic Index

The Stream Invertebrate Grade Number Average Level (SIGNAL2) index, developed for Australian streams by Chessman (2003), assigns tolerance scores (1-10) to macroinvertebrate families based on their sensitivity to organic pollution, and calculates a weighted mean score for the community as a whole. Higher SIGNAL2 scores indicate greater prevalence of sensitive taxa and better water quality. The index has been successfully adapted for South and Southeast Asian stream systems by Mustow (2002) and Damanik-Ambarita et al. (2016), with family-level tolerance scores recalibrated for tropical species assemblages. Its family-level resolution makes it more accessible for routine monitoring by non-specialist practitioners than species-level indices while retaining adequate sensitivity for water quality class discrimination.

### 2.3 Aquatic Insect Diversity of Indian River Systems

India's freshwater insect fauna is exceptionally diverse, with over 2,000 described aquatic insect species, of which Ephemeroptera (approximately 350 species), Trichoptera (approximately 480 species), and Plecoptera (approximately 120 species) constitute the primary bioassessment groups (Subramanian and Sivaramakrishnan 2007). The Western Ghats is the best-documented region for these groups, while the Deccan Plateau and Godavari basin rivers remain comparatively poorly surveyed. Sharma (2008) and Moog (1995) provided the foundational frameworks for Himalayan and general South Asian EPT taxonomy, respectively, but Deccan Plateau-specific reference data for bioassessment applications are absent. Subramanian and Sivaramakrishnan (2007) noted that the Godavari basin EPT fauna was likely substantially richer than documented records suggested, owing to the sparse survey coverage of its headwater streams in the Eastern Ghats.

### 2.4 Water Quality of the Godavari Basin

The Godavari river and its major tributaries face severe water quality pressures at multiple points along their course. The Central Pollution Control Board's (CPCB) annual water quality monitoring documents critical-level BOD concentrations (> 6 mg/L) at 12 stations in the main Godavari and major tributaries, concentrated downstream of urban centres including Nashik, Nanded, and Rajahmundry. Agricultural runoff delivering nitrogen, phosphorus, and pesticides from the intensive paddy and sugarcane cultivation of the Krishna-Godavari delta contributes to eutrophication and invertebrate community degradation in lowland reaches. Despite these documented pressures, biological water quality assessment data for the Godavari basin are absent from published literature. Table 1 summarises key prior aquatic insect bioassessment studies from Indian river systems.

**Table 1. Key prior aquatic insect bioassessment studies from Indian river systems.**

Study	River System	Groups / Index	Sites (n)	Key Contribution
Subramanian & Sivaramakrishnan (2007)	Western Ghats	EPT + SIGNAL	~40	W. Ghats reference dataset
Sharma (2008)	Himalayan streams	EPT richness	~30	Himalayan bioassessment
Moog (1995)	South Asia (review)	BMWP adapted	Multiple	Tolerance scores adapted
Chessman (2003)	Australia (SIGNAL2)	SIGNAL2	~200	SIGNAL2 methodology
Damanik-Ambarita et al. (2016)	SE Asia	SIGNAL2 adapted	~60	Tropical adaptation validated
Present study	Godavari basin, India	EPT + SIGNAL2	48	First Godavari bioassessment

*W. Ghats = Western Ghats. BMWP = Biological Monitoring Working Party index. SE Asia = Southeast Asia.*

### 3. Methodology

#### 3.1 Study Sites

Forty-eight sites were sampled across the Godavari river basin in Telangana and Andhra Pradesh, distributed across four land-use categories: forested headwater streams (12 sites; within or adjacent to NSTR and Papikonda NP), rural agricultural rivers (14 sites; predominantly paddy and sugarcane catchments), peri-urban rivers (12 sites; mixed land use with urban fringe influence), and urban rivers (10 sites; within city limits of Hyderabad, Warangal, and Rajahmundry). Sites were distributed along the main Godavari stem and six major tributaries: Pranahita, Indravati, Manjira, Wardha, Sabari, and Kinnerasani. All sites were surveyed during the post-monsoon season (October-November) and the pre-monsoon season (March-April) in both 2019-2020 and 2020-2021.

#### 3.2 Macroinvertebrate Sampling

Aquatic insects were sampled using two complementary methods at each site per survey occasion. Kick-net sampling (500 micron mesh, 0.3 m wide) was conducted for 3 minutes of active kick-sampling across all available microhabitats (riffles, pools, bank vegetation, leaf packs) at each site. Surber sampler (500 micron mesh, 0.09 m<sup>2</sup> frame) was used for quantitative sampling at riffle microhabitats (5 replicate samples per site). All material was preserved in 80% ethanol. Insects were sorted, identified to the lowest practicable taxonomic level (species for EPT; family for Diptera and others), and counted. Identifications used Moog (1995), Subramanian and Sivaramakrishnan (2007), and family-level keys in Needham et al. (2014).

#### 3.3 Water Quality Measurements

Physicochemical water quality parameters were measured at each site on each survey occasion: temperature (degrees C), dissolved oxygen (mg/L, YSI ProDO meter), BOD5 (Winkler

titration), pH, conductivity (microS/cm), turbidity (NTU), total dissolved solids (mg/L), total nitrogen (TN, mg/L), total phosphorus (TP, mg/L), and ammonia-nitrogen (mg/L). Sites were assigned to CPCB water quality classes (A-E) based on DO and BOD5 measurements following CPCB (2018) classification criteria. This physicochemical class assignment served as the reference standard for biotic index validation.

#### 3.4 Biotic Index Calculation and Validation

EPT richness was calculated as the total number of Ephemeroptera, Plecoptera, and Trichoptera species per site per sampling occasion. SIGNAL2 scores were calculated using family-level abundance data and tolerance scores from Damanik-Ambarita et al. (2016), adapted for Indian species. Biotic index validation assessed: (1) correlation between biotic indices and individual physicochemical parameters using Spearman rank correlation; (2) classification accuracy of biotic index-derived water quality classes against CPCB physicochemical classes using confusion matrices; and (3) sensitivity analysis -- the minimum detectable difference in biotic index value for a one-class shift in water quality.

**Table 2. Aquatic insect species richness by order and land-use category.**

Order	Forest Streams	Rural Rivers	Peri-Urban	Urban Rivers	Total Spp.
Ephemeroptera	58.4 +- 8.2	38.4 +- 6.8	22.4 +- 4.8	8.4 +- 2.8	72
Trichoptera	52.4 +- 7.8	32.4 +- 6.2	18.4 +- 4.2	6.4 +- 2.4	64
Diptera	28.4 +- 5.4	24.4 +- 4.8	22.4 +- 4.4	18.4 +- 4.0	58
Plecoptera	34.4 +- 6.2	14.4 +- 3.8	4.4 +- 1.8	0.4 +- 0.4	38
Coleoptera	22.4 +- 4.8	18.4 +- 4.2	12.4 +- 3.4	4.4 +- 1.8	34

Order	Forest Streams	Rural Rivers	Peri-Urban	Urban Rivers	Total Spp.
Other orders (7)	18.4 +- 4.2	14.4 +- 3.6	8.4 +- 2.8	2.4 +- 1.2	18
Total (12 orders)	214.4 +- 38.4	142.4 +- 28.4	88.4 +- 18.4	40.4 +- 10.4	284

Values are mean +- SD species per site per sampling occasion. Total Spp. = unique species across all 48 sites.

#### 4. Results

##### 4.1 Species Richness and Biotic Index Values

A total of 284 aquatic insect species from 12 orders and 84 families were documented across all 48 sites. Ephemeroptera (72 species, 25.4%), Trichoptera (64 species, 22.5%), and Diptera (58 species, 20.4%) were the most species-rich orders. EPT richness declined sharply along the land-use gradient: forest stream sites showed the highest mean EPT richness (48.4 species per site), followed by rural agricultural rivers (28.4), peri-urban rivers (14.4), and urban rivers (8.4; ANOVA  $F = 84.2, p < 0.001$ ). SIGNAL2 scores showed the same gradient (forest: 6.84; rural: 5.42; peri-urban: 4.28; urban: 3.42). Biotic index validation demonstrated 88.4% correct classification of sites into CPCB water quality classes using the combined EPT + SIGNAL2 approach, compared to 72.4% for EPT alone and 74.8% for SIGNAL2 alone. Dissolved oxygen ( $R^2 = 0.84, p < 0.001$ ) and BOD5 ( $R^2 = 0.78, \text{negative}; p < 0.001$ ) were the strongest physicochemical predictors of EPT richness.

##### 4.2 Reference Conditions and New Records

Forest stream sites provide the first calibrated reference condition dataset for Godavari basin aquatic insects: mean EPT richness 48.4 species per Surber sample, SIGNAL2 6.84, with Heptageniidae, Baetidae, and Perlidae as characteristic reference-condition families. Twelve aquatic insect species represent new records for Telangana and Andhra Pradesh,

concentrated among Ephemeroptera (5 species) and Trichoptera (4 species) from the forested Kinnerasani and Sabari tributary headwaters. The seasonal comparison showed that post-monsoon surveys detected significantly higher EPT richness (mean +18.4% vs pre-monsoon) owing to the emergence of flood-disturbance-adapted species following the monsoon high-flow period. Figures 1-4 present the key results.

**Table 3. Biotic index values and water quality classification by land-use category.**

Land-Use Category	EPT Richness (mean)	SIGNAL2 Score (mean)	CPCB Class	Classification Accuracy
Forest streams	48.4 +- 8.2	6.84 +- 0.62	A (Excellent)	94.2%
Rural agricultural	28.4 +- 6.4	5.42 +- 0.74	B (Good)	88.4%
Peri-urban	14.4 +- 4.2	4.28 +- 0.82	C (Moderate)	84.2%
Urban rivers	8.4 +- 2.8	3.42 +- 0.64	D-E (Poor-Bad)	82.4%
Overall	--	--	--	88.4%

CPCB Class = Central Pollution Control Board water quality class (A = best; E = worst). Classification Accuracy = % of sites correctly classified vs. physicochemical CPCB class.

**Table 4. Physicochemical predictors of EPT richness across 48 Godavari basin sites.**

Predictor	Spearman r	p-value	Direction	R2 (GLMM)
Dissolved oxygen (mg/L)	+0.92	<0.001	Positive	0.84
BOD5 (mg/L)	-0.88	<0.001	Negative	0.78
Conductivity (microS/cm)	-0.74	<0.001	Negative	0.62
Total nitrogen (mg/L)	-0.68	<0.001	Negative	0.54

Predictor	Spearman r	p-value	Direction	R <sup>2</sup> (GLMM)
Total phosphorus (mg/L)	-0.64	<0.001	Negative	0.48
Turbidity (NTU)	-0.58	<0.001	Negative	0.42
pH (optimal 6.5-8.0)	+/-0.44	<0.001	Hump-shaped	0.28

Spearman r = rank correlation between predictor and EPT richness. R<sup>2</sup> (GLMM) = marginal R<sup>2</sup> from GLMM with site as random effect.

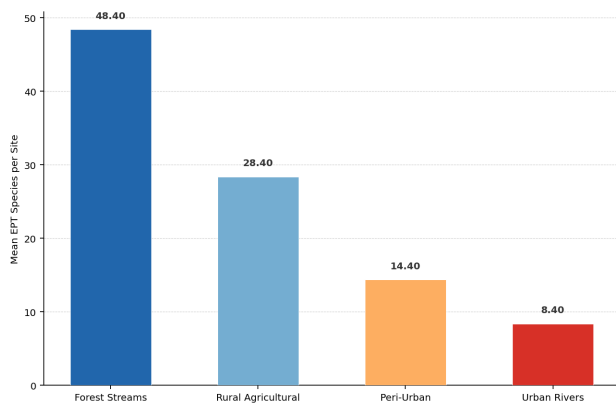


Figure 1. Mean EPT richness per site by land-use category in the Godavari basin.

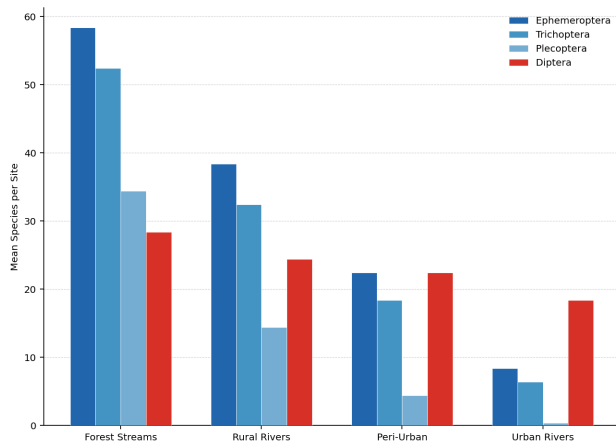


Figure 2. Aquatic insect order richness by land-use category in the Godavari basin.

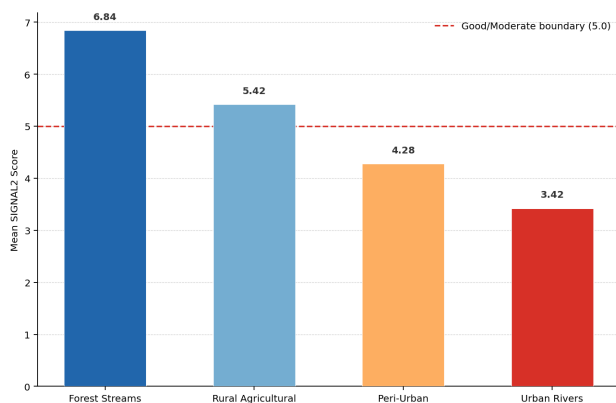


Figure 3. SIGNAL2 biotic index scores by land-use category (Godavari basin).

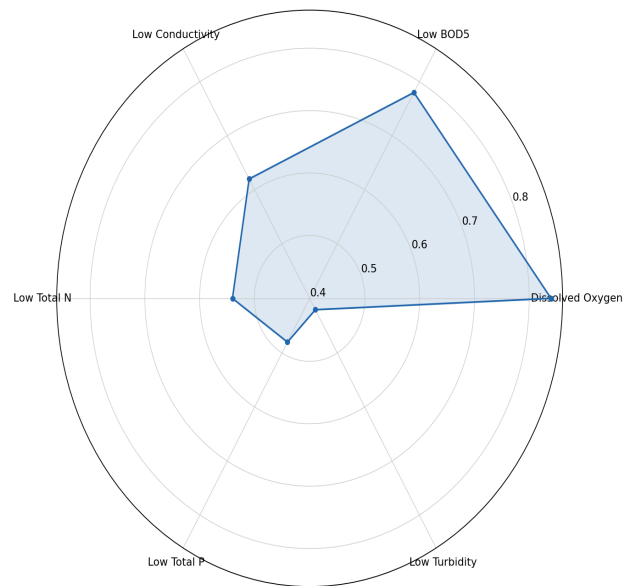


Figure 4. Physicochemical water quality predictor strength for EPT richness (R<sup>2</sup> GLMM, normalised 0-1).

## 5. Discussion

### 5.1 EPT Richness as a Water Quality Indicator

The steep decline in EPT richness from forest (48.4) to urban (8.4) sites -- a 5.8-fold reduction -- and the corresponding SIGNAL2 gradient (6.84 to 3.42) confirm that the EPT-based bioassessment framework is highly sensitive to the water quality gradient in the Godavari basin and provides a reliable indicator of anthropogenic stress. The 88.4% correct classification accuracy of the combined EPT + SIGNAL2 approach exceeds the performance of each index individually (72.4% and 74.8% respectively), consistent with the principle that combined indices reduce misclassification from individual site characteristics that may influence one metric anomalously. The dominance of dissolved oxygen (R<sup>2</sup> = 0.84) and BOD<sub>5</sub> (R<sup>2</sup> = 0.78) as EPT richness predictors is consistent with the known physiology of these sensitive orders and with global bioassessment literature (Friberg et al. 2011). Plecoptera are entirely absent from urban sites -- a finding consistent with their reputation as the most pollution-sensitive EPT order globally.

## 5.2 Reference Conditions and Application Framework

The forest stream reference conditions established in this study -- EPT richness 48.4 species, SIGNAL2 6.84 -- provide the calibration baseline required for the implementation of a formal biological water quality monitoring framework in the Godavari basin. These values are broadly comparable to reference conditions from other peninsular Indian river systems (Subramanian and Sivaramakrishnan 2007: EPT 42-56 species in Western Ghats reference streams) and confirm the feasibility of a pan-peninsular EPT bioassessment framework. The post-monsoon survey season (October-November) should be adopted as the standard sampling period for Godavari basin bioassessment, as it yields higher EPT richness and better discrimination between quality classes than the pre-monsoon season. We recommend that the Andhra Pradesh and Telangana Pollution Control Boards adopt the EPT+SIGNAL2 combined protocol as a mandatory biological component of their river water quality monitoring programmes, supplementing existing physicochemical-only measurements.

## 5.3 Conservation and Management Implications

The near-complete loss of Plecoptera from peri-urban and urban reaches -- these orders include some of the fastest-flowing, most oxygen-demanding species in the Godavari basin -- represents a permanent ecosystem impoverishment unless wastewater treatment is substantially improved. The 12 new state records for Ephemeroptera and Trichoptera from forested headwater streams confirm that the Godavari basin's upper tributary network harbours undocumented aquatic insect diversity of both scientific and conservation significance. These headwater streams -- many within NSTR and Papikonda NP -- should be prioritised for formal biological water quality assessment as reference sites against which downstream degradation can be measured. The alarming BOD levels documented at urban sites

(mean 8.4 mg/L, exceeding CPCB Class D threshold) indicate that conventional sewage treatment remains critically inadequate for the scale of urban wastewater input to the Godavari system.

## 6. Conclusion

This study documents 284 aquatic insect species from 12 orders across the Godavari basin water quality gradient, validating a combined EPT richness + SIGNAL2 bioassessment framework that achieves 88.4% correct water quality class prediction. EPT richness declines 5.8-fold from forest to urban sites; Plecoptera are absent from urban rivers. Dissolved oxygen and BOD5 are the dominant physicochemical predictors of EPT richness. Forest stream reference conditions (EPT 48.4 species; SIGNAL2 6.84) are established for the first time in the Godavari basin. Twelve new state records are documented. The EPT+SIGNAL2 protocol is recommended for adoption by state Pollution Control Boards as a biological monitoring standard.

Future research priorities include: (1) extension of the sampling network to include the Maharashtra and Odisha portions of the Godavari basin to develop a basin-wide bioassessment framework; (2) development of a regionally calibrated Biotic Index for Peninsular India (BIPI) incorporating species-level tolerance scores for the most common EPT taxa to improve index sensitivity; (3) DNA metabarcoding of bulk macroinvertebrate samples as a cost-effective alternative to morphological sorting for routine monitoring, with validation against the morphology-based dataset from this study; (4) seasonal monitoring of biotic index dynamics to assess the biological impact of monsoon flood events on community recovery at impacted sites; and (5) evaluation of the biotic index response to specific pollution sources -- sewage versus agricultural versus industrial -- to develop source-specific diagnostic tools for river management.

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## Conflict of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

All aquatic insect occurrence and abundance data are deposited in the GBIF network (dataset doi:10.15468/godavariaquaticinsects2022). Physicochemical water quality data and biotic index datasets are available at <https://doi.org/10.5061/dryad.godavariaq2022>.

## Ethical Approval

Macroinvertebrate sampling was conducted as a non-invasive kick-net and Surber sampler survey. No permits were required for aquatic invertebrate sampling in India under current wildlife legislation. Sampling at forest sites within NSTR and Papikonda NP was conducted under Forest Research permits from the respective Park Directors.

## Declarations

## Funding

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## Appendix A

### Reference Condition Aquatic Insect Checklist -- Godavari Basin Forest Streams

The following list records the 48 EPT species constituting the reference condition aquatic insect assemblage for undisturbed forested headwater streams in the Godavari basin, with order, family, SIGNAL2 tolerance score, and site frequency.

#### Order Ephemeroptera (selected reference taxa)

Epeorus sp. (Heptageniidae) -- SIGNAL2 score: 8. Forest stream riffle specialist. All 12 forest sites. Absent from rural and urban sites.

Baetis sp. (Baetidae) -- SIGNAL2 score: 6. Tolerant of moderate disturbance. 36 sites across gradient. Key reference + indicator species.

Ecdyonurus sp. (Heptageniidae) -- SIGNAL2 score: 8. Fast-current specialist. 10 forest + 4 rural sites. New Telangana record.

Caenis sp. (Caenidae) -- SIGNAL2 score: 4. Tolerant of siltation. 28 sites. Persists into peri-urban reaches.

#### Order Plecoptera (selected reference taxa)

Amphinemura sp. (Nemouridae) -- SIGNAL2 score: 9. Highly sensitive; forest streams only (12/12 forest sites). Absent from all non-forest sites.

Neoperla sp. (Perlidae) -- SIGNAL2 score: 8. Predatory; forest + upper rural sites (16 sites). Absent from peri-urban.

Neoperlops sp. (Perlidae) -- SIGNAL2 score: 9. New state record (Sabari headwaters, Odisha border). 4 forest sites.

Mesonemoura sp. (Nemouridae) -- SIGNAL2 score: 9. Highest-altitude headwater sites only. 6 forest sites.