

1 **Predicting macroinvertebrate average score per taxon (ASPT) at water quality**
2 **monitoring sites in Japanese rivers**

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16 **Abstract**

17 Freshwater ecosystems provide essential services for human well-being but are impacted
18 by multiple anthropogenic stressors. Biomonitoring with bioindicators such as river
19 macroinvertebrates is fundamental for assessing the status of freshwater systems. In Japan,
20 water quality and biomonitoring surveys are conducted separately, leading to a lack of
21 nationwide information on the biological status of water quality monitoring (WQM) sites.
22 In this study, we examined the co-occurrence of 983 biomonitoring sites with WQM sites
23 to obtain a set of 237 “aligned” sites. Then, we developed a multiple linear regression
24 model to estimate the average score per taxon (ASPT) from river macroinvertebrate data
25 surveyed at these sites. The best model (i.e., with the smallest corrected Akaike

26 information criterion) included eight predictors: elevation, catchment area, biological
27 oxygen demand, suspended solids, minimum pH, the proportions of paddy fields and
28 urban areas in the catchment, and the proportion of urban areas within a 3-km radius. The
29 best multiple linear regression model could predict ASPT with reasonable accuracy, i.e.,
30 with an error of ± 1 for 96% of the aligned data ($R^2 = 0.69$; root mean squared error =
31 0.47) and 84% of the external validation dataset ($R^2 = 0.55$; root mean squared error =
32 0.75). Using the best multiple linear regression model, we estimated ASPT values at 2925
33 WQM sites in rivers nationwide. Although caution should be exercised because of
34 uncertainties in the estimation, the WQM sites were categorized into four levels of river
35 environment quality by estimated ASPT values: “very good” (29% of WQM sites), “good”
36 (50%), “fairly good” (14%), and “not good” (8%). Furthermore, we observed statistically
37 significant correlations ($p < 0.05$; $0.4 \leq r \leq 0.7$) between ASPT and all eight
38 macroinvertebrate metrics examined, such as mayfly (Ephemeroptera) and stonefly
39 (Plecoptera) richness, providing valuable information on the ecological implications of
40 changes in ASPT. Our study provides a valuable statistical model for estimating ASPT
41 and contributes to further understanding of the biological status of rivers across Japan.

42

43 **Keywords**

44 Aquatic insect, Invertebrate, Water pollution, Land use, Bioindicator, Biomonitoring

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46

47 **Introduction**

48 Freshwater ecosystems are essential for human well-being and provide vital material,
49 non-material, and regulating services such as food, recreation, and water purification

50 (Lynch et al. 2023). However, these ecosystems are facing various anthropogenic
51 stressors such as climate change, land-use change, and water pollution (Birk et al. 2020,
52 IPBES 2019, Persson et al. 2022, Reid et al. 2019, Waite et al. 2021). In this context,
53 biomonitoring with bioindicators such as algae, macroinvertebrates, and fish has a
54 fundamental role in capturing the biological status of streams, rivers, and other
55 freshwater systems (Aroviita et al. 2010, Barbour et al. 1999, Birk et al. 2012, Buss et
56 al. 2014, Namba et al. 2020, Niemi & McDonald 2004, Wright 2000). Biomonitoring
57 results can be used to assess and identify any adverse ecosystem impacts, contributing
58 to conservation and sustainable management. This is particularly important for rivers
59 because they are more often affected by multiple stressors than lakes (Birk et al. 2020).
60 Consequently, bespoke management solutions are generally required to address the
61 specific challenges faced by river ecosystems (Birk et al. 2020, Iwasaki et al. 2018).

62 Water pollution is a critical stressor affecting aquatic ecosystems, and streams
63 and rivers are particularly susceptible to high pollution levels because of their limited
64 capacity for dilution (Büttner et al. 2022, Johnson et al. 2020). In Japan, water quality
65 monitoring has been conducted at approximately 6000 river sites nationwide to assess
66 water quality variables including suspended solids (SS) and biochemical oxygen
67 demand (BOD) (Iwasaki et al. 2022). In addition, the Ministry of Land, Infrastructure,
68 Transport and Tourism (MLIT) has initiated a nationwide biomonitoring program called
69 the National Census on the River Environment (NCRE) to assess the biological and
70 ecological status of rivers. The biomonitoring program involves the sampling of various
71 taxa, including fish, benthic invertebrates, plants, birds, terrestrial insects, amphibians,
72 reptiles, and mammals, from over 240 rivers across Japan at 5- or 10-year intervals
73 (Feio et al. 2021). However, because water-quality and biomonitoring surveys are not

74 necessarily conducted at the same locations, the biological status of water quality
75 monitoring sites (hereafter, WQM sites) is largely unknown across Japan. This kind of
76 large-scale comprehensive data on biological status is crucial for informing effective
77 bespoke management strategies aimed at mitigating water pollution and safeguarding
78 aquatic ecosystems (Abell et al. 2008).

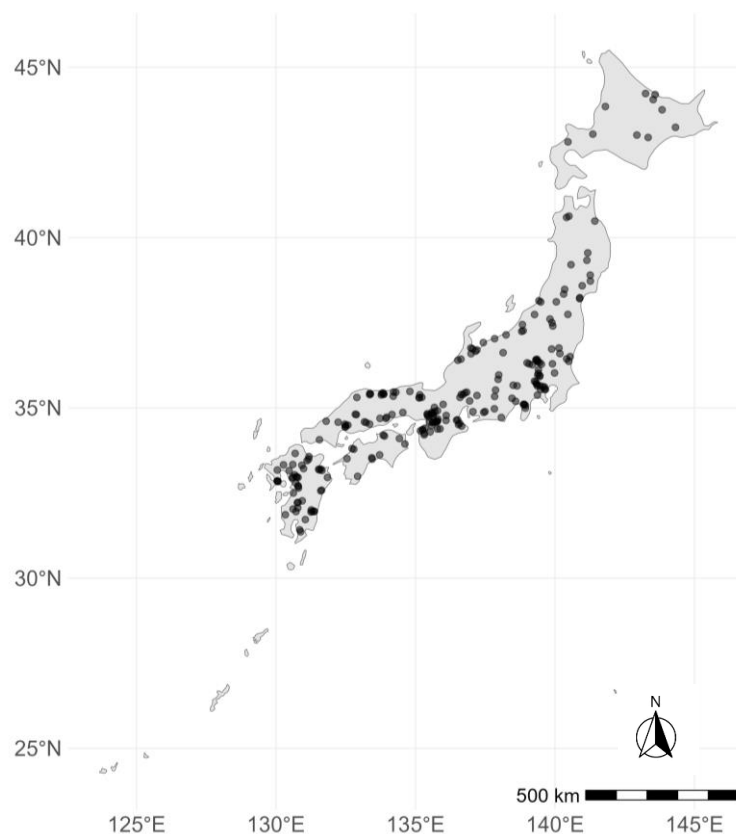
79 Numerous biological metrics have been developed for biomonitoring and
80 bioassessment (Birk et al. 2012, Eriksen et al. 2021). For Japanese rivers, the average
81 score per taxon (ASPT) based on the occurrence of 71 macroinvertebrate taxa (mainly,
82 families) is the only biological metric developed at the national level for assessing river
83 health (MoE 2017, Nozaki 2012). The ASPT is calculated as follows:

$$84 \text{ ASPT} = \frac{\sum \text{Score}_i}{\text{Total number of scoring taxa present}}, \quad \text{Equation (1)}$$

85 where Score_i is the score assigned to macroinvertebrate taxon i , which is expected to
86 represent the value inversely proportional to the taxon's perceived tolerance to water
87 pollution (Yamasaki et al. 1996). ASPT was originally designed as a biological indicator
88 of water quality (specifically, organic pollution) but is also correlated with the impacts
89 of other anthropogenic pressures such as land-use change (Eriksen et al. 2021, Yamasaki
90 et al. 1996). However, the relationships between ASPT and commonly used
91 macroinvertebrate metrics, such as EPT richness (the total number of taxa in
92 Ephemeroptera, Plecoptera, and Trichoptera), remain unexplored in Japanese rivers
93 nationwide. Investigating these relationships could yield valuable insights into the
94 implications of changes in ASPT and their ecological significance.

95 Thus, we first aimed to predict ASPT at 2925 WQM sites, which serve as
96 environmental reference points that are officially used to assess the
97 compliance/exceedance of environmental water quality standards in Japan. To achieve

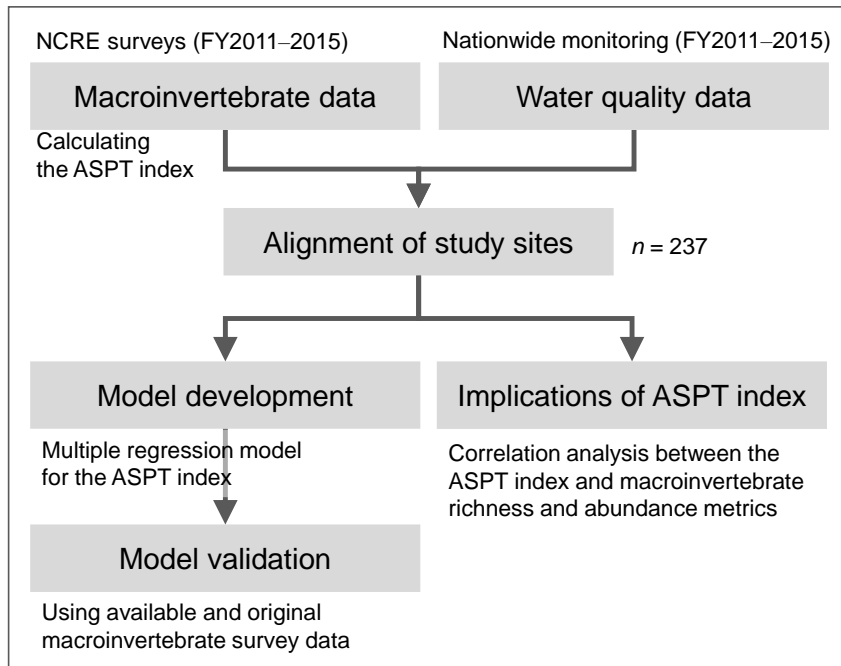
108 this, we examined the co-occurrence of NCRE biomonitoring sites with WQM sites to
109 obtain a total of 237 “aligned” sites (i.e., those at which both macroinvertebrate and
110 water quality monitoring data were available; Fig. 1; see Fig. 2 for our data analysis
111 flowchart). We then developed a multiple regression model with physicochemical
112 variables representing water quality, land use, and other factors to predict ASPT. The
113 application of this model to all 2925 WQM sites would offer a more comprehensive
114 understanding of biological status at streams and rivers across Japan. Furthermore, we
115 investigated the relationships between variations in ASPT and variations in
116 macroinvertebrate metrics such as EPT richness and mayfly richness at the aligned
117 study sites.



108

109

Fig. 1. Map of the 237 aligned study sites (filled circles).



110

111

Fig. 2. Schematic diagram of the methodology used.

112 NCRE, National Census on the River Environment; ASPT, average score per taxon.

113

114 **Materials and Methods**

115 *Alignment of study sites*

116 We first extracted “aligned” study sites at which both macroinvertebrate and water

117 quality monitoring data were available. The geographic coordinates (latitude and

118 longitude) for a total of 983 river sites where quantitative macroinvertebrate sampling

119 was conducted between April 2011 and March 2016 (fiscal years 2011–2015) were

120 compiled by obtaining the original information, including results of macroinvertebrate

121 monitoring from MLIT. Similarly, the geographic coordinates of a total of 5855 WQM

122 sites where sampling was performed during fiscal years 2011–2015 were obtained from

123 the Comprehensive Information Website for Water Environment (<https://water->

124 [pub.env.go.jp/water-pub/mizu-site/](https://water-pub.env.go.jp/water-pub/mizu-site/), accessed November 21, 2023).

125 We then identified suitable pairs of biomonitoring and WQM sites to be
126 matched for later analysis. Initially, we linked each biomonitoring site to the closest
127 WQM site within a Euclidean distance of 2 km. We then examined the suitability of
128 each linked site pair by using Google Earth Pro version 7.3
129 (<https://www.google.com/earth/about/>, accessed November 21, 2023). If the two sites
130 were not identical, their suitability was assessed by considering whether the two sites
131 were located within the same river, the absence of inflow of major tributaries and
132 changes in land use between the sites, and the availability of other more suitable WQM
133 sites. While we carefully matched the biomonitoring and WQM sites, it is impossible to
134 guarantee the absence of any significant changes in physicochemical characteristics,
135 such as pollution from an unknown point source, between the two sites. However, the
136 inclusion of these few cases should not have been materially affected our findings.
137 During this assessment process, we identified multiple sites with inaccurate geographic
138 coordinates and made necessary corrections based on the available information such as
139 river and site names. From this process, 409 pairs of biomonitoring and WQM sites
140 were selected.

141 The 409 pairs of biomonitoring and WQM sites were further winnowed based
142 on the following criteria: (1) quantitative sampling of macroinvertebrates was conducted
143 using a Surber sampler with a 25 × 25 cm quadrat at each biomonitoring site during
144 cold seasons (October to March), (2) macroinvertebrates were collected from
145 cobble/gravel-dominated lotic environments (i.e., runs or riffles), (3) no
146 macroinvertebrate species typical of estuarine environments were collected (to exclude
147 sites influenced by salinity), and (4) all water quality variables used in the multiple

148 regression model (see below) were available. In total, 237 pairs of biomonitoring and
149 WQM sites were selected for model development (Fig. 1).

150

151 *Macroinvertebrates and ASPT*

152 All field sampling and laboratory analysis (including sorting and identification) of river
153 benthic macroinvertebrates were conducted by following the NCRE's Basic Survey
154 Manual (MLIT 2016). Each of the three macroinvertebrate samples collected from
155 riffles or runs at individual biomonitoring sites was washed through a 0.5-mm mesh
156 sieve, and macroinvertebrates remaining on the sieve were sorted and identified
157 generally to species or genus level. We calculated site averages of ASPT based on the
158 presence of individual macroinvertebrate taxa and corresponding scores (Equation 1;
159 see Table S1 for the scores). The scores used in the present study were initially
160 developed by Yamasaki et al. (1996) and revised by the committee launched by the
161 Ministry of the Environment, Japan (MoE 2017).

162 Sites are categorized into four groups of relative river environmental quality
163 based on ASPT values (MoE 2017): "very good" (7.5 and above), "good" (6.0–7.5),
164 "fairly good" (5.0–6.0), and "not good" (below 5.0). It should be noted that the
165 calculation of ASPT (MoE 2017) involves 3 min of kick sampling (1 min of kick
166 sampling at three locations per site) using a D-frame net in riffles or/and runs as well as
167 the suggested use of a sieve with a mesh size of about 1 mm to filter macroinvertebrate
168 samples. These methods differ from those employed in NCRE biomonitoring (MLIT
169 (2016); see also above). However, we adopted the four categories described above
170 despite these differences because, in general, the coarse taxonomic level (i.e., the family
171 level) used to calculate ASPT likely mitigates any influence of methodological

172 differences in the diagnostic evaluation (Armitage et al. 1983, Eriksen et al. 2021,
173 Hawkes 1998; see also the section “*Model for predicting ASPT*”).

174 Furthermore, to investigate relationships between APST and macroinvertebrate
175 metrics, we calculated total taxon richness and total abundance as well as the taxon
176 richness and abundance of three major insect groups (Ephemeroptera, Plecoptera, and
177 Trichoptera). Correlations were examined by calculating Pearson product-moment
178 correlation coefficients (r).

179

180 *Physicochemical characteristics of sites*

181 For the 237 aligned study sites, we compiled a total of 11 physicochemical
182 characteristics, which were used as predictors in the multiple regression model. These
183 characteristics had been previously estimated for most of the aligned study sites by
184 Iwasaki et al. (2022). However, 67 WQM sites corresponding to biomonitoring sites
185 were not included in the 2925 WQM sites analyzed by Iwasaki et al. (2022). Thus, we
186 obtained the physicochemical characteristics of these sites by using the methods
187 described in Iwasaki et al. (2022). For the water quality variables included in the
188 multiple regression model, we calculated 5-year averages of minimum pH, 5-day
189 biochemical oxygen demand (BOD; mg/L), and suspended solids (SS; mg/L) at each
190 WQM site. These averages were derived from measurements taken during fiscal years
191 2011–2015, using the available data in the Comprehensive Information Website for
192 Water Environment. For pH, only minimum and maximum values for each fiscal year
193 were available in the database, so we used the minimum pH as an indicator of river
194 acidity. The 5-year averages of the water quality variables were used as representative
195 values reflecting the general water quality conditions at individual WQM sites during

196 the period that included the timing of macroinvertebrate sampling, although they may
197 not accurately reflect the water quality at the specific time of the macroinvertebrate
198 sampling (see the section “*Model development and validation*” for more discussion
199 about temporal changes).

200 The catchment area (km²) of each WQM site was estimated by delineating each
201 catchment based on 30 × 30 m raster data of hydrologically adjusted elevations (Japan
202 Flow Direction Map version 1.0; Yamazaki et al. 2018) using ArcGIS Pro (ESRI,
203 version 2.6.0). In addition, the land uses both in the catchment areas and within a 3-km
204 radius were estimated. Specifically, the proportions of urban areas, paddy fields, and dry
205 fields (cropland) were derived as indicators of anthropogenic disturbance based on the
206 High-Resolution Land Use and Land Cover Map (2014–2016; version 18.03) provided
207 by the Japan Aerospace Exploration Agency
208 (https://www.eorc.jaxa.jp/ALOS/en/dataset/lulc_e.htm, accessed November 21, 2023).
209 The land uses within a 3-km radius were included as an indicator of land use patterns in
210 the immediate vicinity of WQM sites, although downstream land uses are unlikely to
211 directly affect water quality or the biological status at WQM sites given natural flow
212 patterns. Additionally, the average elevation (m) within a 100-m radius was calculated
213 for each WQM site. Because of the high correlation between the proportions of forest
214 and urban areas ($r = -0.85$), we chose not to include the proportion of forest as a
215 predictor in the multiple regression model (see below).

216

217 *Model development and validation*

218 To predict ASPT, we employed multiple linear regression models with a normal error
219 distribution and a total of 11 predictors, including the three water quality variables

220 (minimum pH, BOD, and SS), catchment area, elevation, and six land use variables.
221 Catchment area, elevation, BOD, and SS were \log_{10} -transformed to reduce skewness
222 before analysis. Model selection was performed by using the Akaike information
223 criterion corrected for small sample size (AICc; Burnham and Anderson 2004, Burnham
224 et al. 2011), and the model with the lowest AICc was selected as the best among all
225 possible models (i.e., 2048 models) considered. The coefficient of determination (R^2)
226 and root mean square error (RMSE) were calculated to evaluate goodness of fit for the
227 best model selected. All data processing and statistical analyses were performed in R
228 version 4.2.0 (R Core Team 2022), and the model selection was carried out by using the
229 function “dredge” in the “MuMIn” library (Bartoń 2022). We also employed a random
230 forest, which is a machine learning algorithm (Ryo & Rillig 2017), as a preliminary
231 analysis to model ASPT. However, during external validation, the best multiple linear
232 regression model outperformed the random forest model, likely because of the limited
233 coverage of the model development data.

234 To perform external validation for the best model using an entirely new dataset,
235 we examined macroinvertebrate survey data collected at 75 river sites that were at or
236 near WQM sites (environmental reference points) across Japan (Table S2; Fig. S1).
237 Most of these surveys were conducted using a D-frame net following the sampling
238 method described in MoE (2017) (see Table S2 for more details). Similarly, we
239 conducted our own macroinvertebrate surveys at 28 WQM sites in different regions.
240 Specifically, we surveyed 10 WQM sites in the Tohoku area (Iwate, Miyagi, and
241 Fukushima prefectures) in January 2021, 9 WQM sites in Aichi prefecture in January
242 2022, and 9 WQM sites in Hokkaido (the city of Sapporo) in December 2022 (see Fig.
243 S1 for map). These surveys were performed by following the NCRE’s Basic Survey

244 Manual (MLIT 2016). We then compared ASPT values predicted from the best model to
245 those calculated from the macroinvertebrate survey data by assessing two diagnostic
246 metrics, R^2 and RMSE. The timing of the surveys used for model validation (especially
247 those conducted for this study) did not coincide with the data period (2011–2015) used
248 for the multiple regression model. However, we assumed that marked changes in land
249 use and water quality variables during these periods were relatively unlikely for the
250 majority of WQM sites as compared to past changes observed between the 1980s and
251 2010s (see, e.g., Ye and Kameyama 2020) for temporal changes in SS and BOD). Given
252 these variations in sampling methods and timing, validating the best model with the
253 external datasets would be valuable to test the robustness of the best model as well as
254 use of ASPT for the prediction. All data and R code used are available from a GitHub
255 repository at https://github.com/yuichiwsk/predict_ASPT_Japan.

256

257 **Results and Discussion**

258 *Model for predicting ASPT*

259 The best multiple linear regression model included 8 predictors: elevation, catchment
260 area, BOD, SS, minimum pH, the proportions of paddy fields (%Paddy) and urban areas
261 (%Urban) in the catchment, and the proportion of urban areas within a 3-km radius
262 (%Urban-3km) (Table 1). All predictors except catchment area and minimum pH were
263 included in all of the top 10 models (see Table S3). The negative regression coefficients
264 of BOD, %Urban, SS, %Paddy, and %Urban-3km in the best model, along with the
265 positive coefficient of minimum pH, are all consistent with the expected adverse
266 impacts of these factors on macroinvertebrates as reported in previous studies (Iwasaki
267 et al. 2018, Larsen et al. 2009, Ormerod & Durance 2009, Roy et al. 2003, Schmidt et al.

268 2019, Waite et al. 2019), although inferring causal relationships is beyond the scope of
269 the present study (Takeshita et al. 2022). The positive regression coefficient of elevation
270 aligns with the general expectation that upland river sites at higher elevation would have
271 lower water temperature (resulting in higher dissolved oxygen essential for aquatic
272 organisms) and be less impacted by anthropogenic factors and disturbances. However,
273 correlations between elevation and other predictor variables included in the present
274 study were not evident ($|r| \leq 0.29$). Additionally, the positive yet nonsignificant
275 coefficient of catchment area (an indicator of the magnitude of river discharge) might be
276 associated with dilution capacity (Büttner et al. 2022, Johnson et al. 2020) for water
277 pollution other than BOD and SS, although other factors, such as natural longitudinal
278 changes in benthic macroinvertebrate communities in response to environmental
279 conditions (Vannote et al., 1980), cannot be excluded.

280 Table 1. Estimated intercepts and coefficients of the best multiple linear regression
 281 model

Predictors	Estimates (SE)	Standardized Coefficients	<i>p</i> value
Intercept	5.65 (0.77)	NA	<0.001
Elevation	0.21 (0.06)	0.15	<0.001
Catchment area	0.09 (0.06)	0.07	0.110
BOD	-1.73 (0.21)	-0.41	<0.001
SS	-0.36 (0.11)	-0.14	0.002
Minimum pH	0.19 (0.11)	0.07	0.073
%Paddy	-0.021 (0.006)	-0.14	0.001
%Urban	-0.016 (0.003)	-0.27	<0.001
%Urban-3km	-0.004 (0.002)	-0.12	0.011

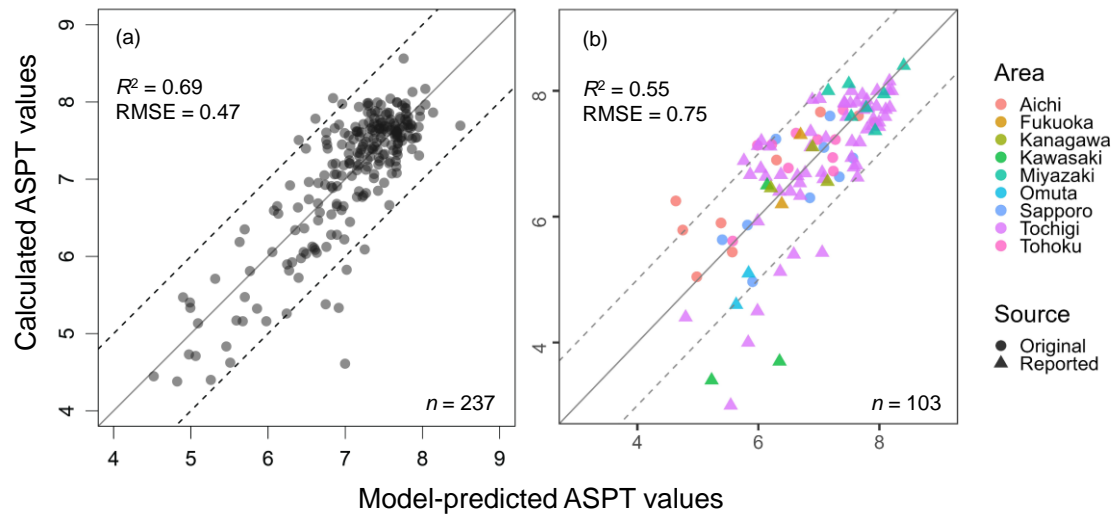
282 SE, standard error; NA, not available; BOD, biochemical oxygen demand; SS,
 283 suspended solids; %Paddy, proportion of paddy fields in the catchment; %Urban,
 284 proportion of urban areas in the catchment; %Urban-3km, proportion of urban areas
 285 within a 3-km radius. See the text for more details about predictors.

286

287 The best multiple linear regression model estimated ASPT values with an error
 288 of ± 0.5 for 76% of the aligned data and an error of ± 1 for 96% of the aligned data ($R^2 =$
 289 0.69, RMSE = 0.47; Fig. 3a). There were two WQM sites where the observed ASPT
 290 value was lower than the predicted value by 1.5 or more. These two sites had
 291 catchments larger than 1500 km² that were predominantly covered by forest (>70%),
 292 and the proportions of urban areas, paddy fields, and dry fields were limited (<10%).
 293 These land use characteristics suggest that the two sites were only weakly affected by
 294 anthropogenic disturbances. However, the specific reasons for the deviation between
 295 observed and predicted ASPT values at these sites remain uncertain.

296

297



298

299 Fig. 3. Relationships between model-predicted average score per taxon (ASPT) and
300 ASPT values calculated from macroinvertebrate survey data during model development
301 (a) and validation (b). Solid lines indicate 1:1 lines, and dashed lines show ± 1 of the 1:1
302 lines. Some ASPT values were calculated from macroinvertebrate surveys conducted as
303 part of the present study (i.e., “original” surveys); these values are indicated by filled
304 circles in panel (b).

305 R^2 , coefficient of determination; RMSE, root mean square error

306

307 In the external validation, the best multiple linear regression model predicted
308 ASPT values with an error of ± 0.5 for 57% of the data and an error of ± 1 for 84% of the
309 data ($R^2 = 0.55$, RMSE = 0.75; Fig. 3b). These performance evaluation metrics for the
310 validation data were somewhat worse than those obtained with the model development
311 data. Although this is to be expected, this drop-off in performance can be attributed, at
312 least partly, to the presence of several sites where the predicted ASPT value markedly

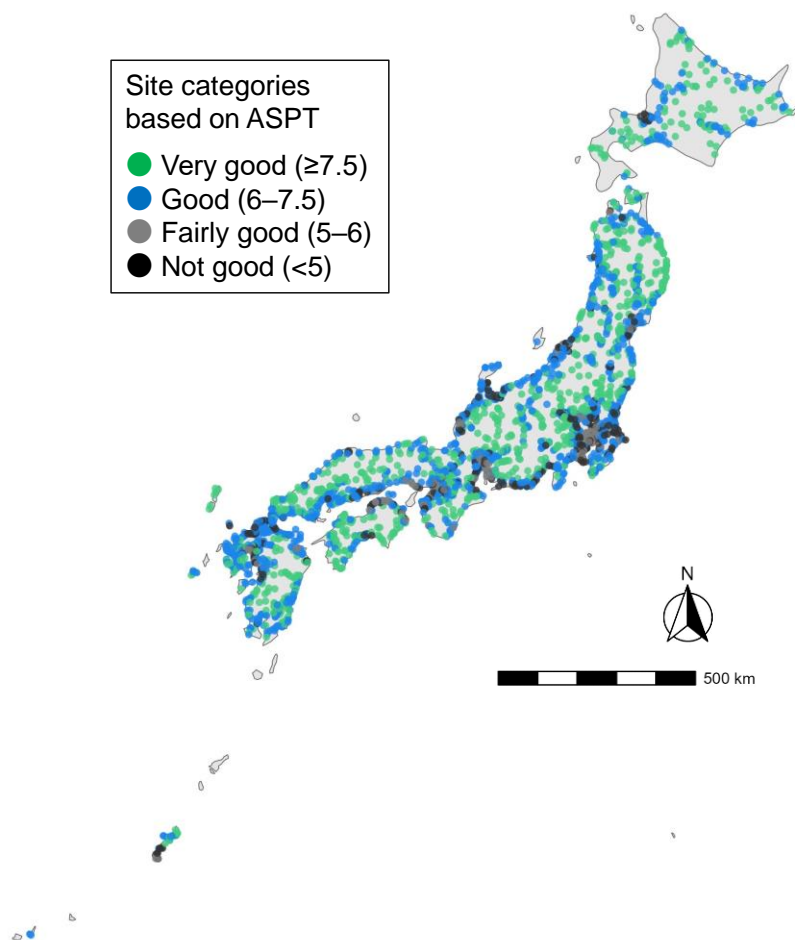
313 deviated from the ASPT value calculated from macroinvertebrate survey data. For
314 instance, there were six sites where the predicted ASPT value was >1.5 greater than that
315 calculated from the macroinvertebrate data. Based on an examination of aerial
316 photographs, we suspect that suitable habitats for macroinvertebrate surveys (i.e., riffles
317 and runs) might have been absent at these sites, possibly due to factors such as
318 straightened and channelized watercourses. Considering the deviations observed with
319 the model development data as well, it is likely that other unmodeled factors, including
320 the morphological alternation and episodic changes in water quality, contributed to these
321 deviations. Importantly, despite the inconsistency in macroinvertebrate sampling
322 methods (kick and quadrat sampling; see Table S2), no systematic deviations (i.e.,
323 under/overestimation) were observed for the validation dataset (Fig. 3b). This is
324 consistent with our initial assumption that differences in macroinvertebrate sampling
325 technique do not materially affect the calculation of ASPT.

326

327 *ASPT values at water quality monitoring sites across Japan*

328 Based on the predictor values obtained from our published database (Iwasaki et al.
329 2022), the ASPT values for all 2925 WQM sites (i.e., environmental reference points)
330 were estimated by using the best multiple linear regression model (Fig. 4). These ASPT
331 values indicated that 29% of the WQM sites should be classified as “very good,” 50%
332 as “good,” 14% as “fairly good,” and 8% as “not good.” Iwasaki et al. (2022) classified
333 all 2925 WQM sites into four groups based on physicochemical characteristics such as
334 those used in our modeling. As expected, the majority (84%) of the WQM sites
335 categorized as “fairly good” and “not good” were characterized by a high prevalence of
336 paddy and dry fields or urban land uses associated with poor water quality (Iwasaki et

337 al. 2022). Although our categorizations based on ASPT could provide valuable
338 information for screening-level assessments of WQM sites, caution is required when
339 interpreting these results given the relatively large 95% prediction intervals of ASPT
340 values (approximately 2), as well as the reliance on some extrapolation in the estimation
341 process (see Fig. S2 for the distributions of predictor variables in different datasets).
342



343
344 Fig. 4. Categorization of 2925 water quality monitoring sites (environmental reference
345 points) into four river environmental quality categories based on average score per
346 taxon (ASPT) as estimated by using the best multiple linear regression model.

347

348

349 *Relationships between ASPT and macroinvertebrate metrics*

350 Statistically significant correlations were observed between ASPT and all eight
351 macroinvertebrate metrics examined for taxon richness and abundance, although the
352 correlation coefficients varied between 0.17 and 0.70 (Fig. 5). Richness metrics such as
353 stonefly (Plecoptera) and mayfly (Ephemeroptera) richness had especially high
354 correlation coefficients with ASPT ($r > 0.6$). Compared to mayflies and caddisflies
355 (Trichoptera), stoneflies were rarely found even at the “good” status WQM sites with
356 ASPT values of 6–7 (Fig. 5). Regarding responses of mayflies, the richness and
357 abundance of Baetidae, which is relatively tolerant to water pollution (assigned score =
358 6; Table S1), had remarkably weak correlations with ASPT ($r = 0.22$ and 0.17 ,
359 respectively), and stronger correlations were observed between ASPT and the richness
360 abundance of mayflies except Baetidae ($r > 0.7$; Fig. S2). Despite some variations in
361 different richness and abundance metrics, these results indicate strong associations
362 between variations in ASPT and variations in macroinvertebrate richness metrics that
363 are commonly used for biological assessments in rivers (Carlisle & Clements 1999,
364 Namba et al. 2020). Together with the observed considerable correlations between
365 ASPT and other stressor-specific indices designed to detect the impacts of e.g., fine
366 sediment, pesticides, and low flow (Jones et al. 2023), our results support the idea that
367 ASPT should not be used as the sole indicator of water pollution.

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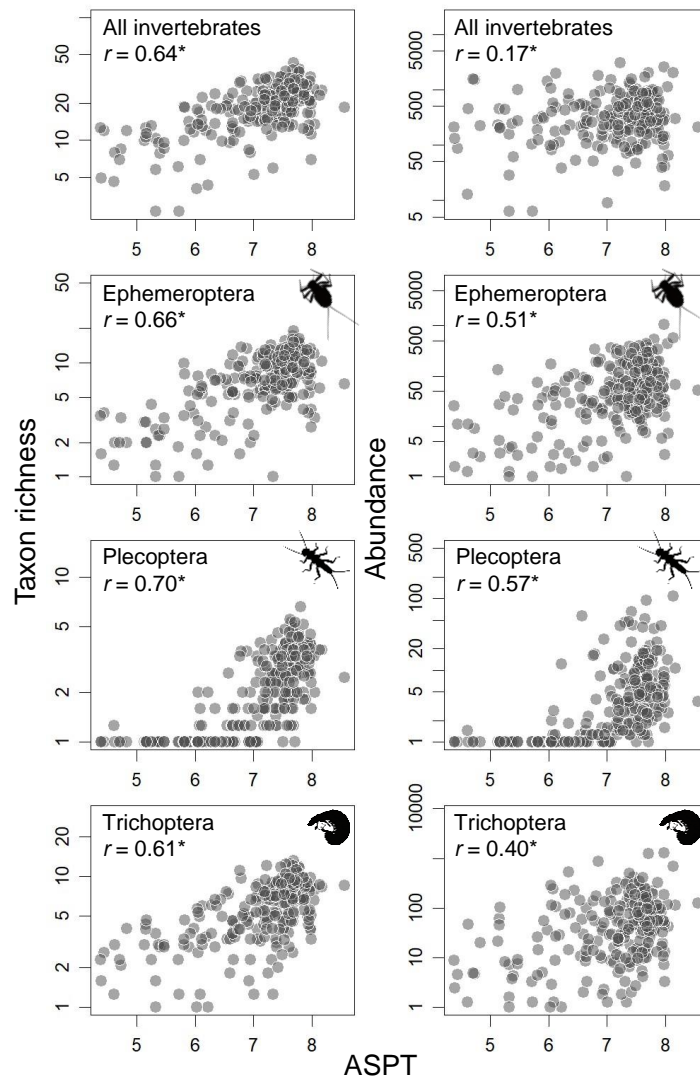
369 **Conclusions**

370 In the present study, we developed a multiple linear regression model based on model
371 selection with AICc to estimate ASPT at 2925 WQM sites. The best model included
372 elevation, catchment area, three land use variables (%Paddy, %Urban, and %Urban-

373 3km), BOD, SS, and minimum pH. The model performed well and was able to estimate
374 ASPT values with a reasonable level of accuracy (i.e., an error of ± 1 for most sites). Use
375 of the multiple linear regression model to estimate ASPT values for all 2925 WQM sites
376 enabled the categorization of these sites into four groups (“very good,” “good,” “fairly
377 good,” and “not good”), providing the first nationwide categorization of WQM sites in
378 terms of relative river environmental quality. However, these site categorizations should
379 be interpreted with caution because of the uncertainties in the estimation process as well
380 as observed overestimation at certain sites. In addition, there may be some river sites
381 that have naturally poor benthic communities, resulting in lower ASPT values, thereby
382 leading to, for example, a “not good” status. Our approach does not take account such
383 reference/historical conditions, as is done in systems like the River InVertebrate
384 Prediction and Classification System (RIVPACS; Wright (2000) and see Aroviita et al.
385 2009 for the example application in Finland). To address this issue, it is fundamental to
386 develop a RIVPACS-type predictive model that can predict the presence/absence of
387 macroinvertebrate taxa, preferably using data from least impacted reference sites (see
388 Torii et al. 2023 for a similar modeling attempt in Japan).

389 The ASPT showed significant correlations with macroinvertebrate metrics
390 frequently used for assessing the biological status of river sites. Therefore, despite the
391 caveats mentioned above, the categorization based on ASPT provides initial but
392 valuable information to capture the biological status of rivers across Japan and can
393 inform effective river management strategies. Particularly in Japan, the compliance of
394 environmental water quality standards in freshwater is assessed at environmental
395 reference points, which correspond to the WQM sites where ASPT values were
396 predicted in the present study. Based on this assessment, the need for countermeasures

397 such as the establishment or refinement of effluent standards is further examined for
398 regulating a given chemical (Naito et al. 2010). In this process, the biological status at
399 the environmental reference points has not been considered even if the objective of
400 environmental water quality standards is the protection of aquatic organisms. Yet, river
401 ecosystems are subject to multiple influences (Birk et al. 2020), and thereby the
402 regulation of individual chemicals may result in limited conservation benefits,
403 particularly in areas where biological communities are already severely impacted
404 (Iwasaki et al. 2018). Information about biological status, such as that can be inferred
405 from ASPT in the present study, should be valuable as foundational knowledge for
406 implementing the effective managements in freshwater ecosystems.
407



409

410 Fig. 5. Relationships between average score per taxon (ASPT) and macroinvertebrate
 411 metrics for taxon richness (number of taxa per 625 cm²) and abundance (number of
 412 individuals per 625 cm²).

413 Asterisks indicate $p < 0.05$. Macroinvertebrate metrics are averages of three 25 × 25 cm
 414 quadrat samples collected per site. Note that for the illustration on a log₁₀-scale, we
 415 added 1 to each observed value (i.e., $X + 1$) to avoid any zero values.

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418

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425

426 **Author contribution**

427 **Yuichi Iwasaki:** Conceptualization, Methodology, Formal analysis, Investigation, Data
428 curation, Writing – original draft, Writing – review & editing, Visualization, Funding
429 acquisition. **Tomomi Suemori:** Investigation, Data curation, Writing – review &
430 editing. **Yuta Kobayashi:** Methodology, Formal analysis, Investigation, Resources,
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437

438 **Data availability**

439 All data and R code used are available from a GitHub repository at
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441

442 **Declarations**

443 **Ethical approval:** Not applicable.

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449

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