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Diversity and distribution of caddisflies (Insecta, Trichoptera) in Singapore's freshwater streams

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3
4 **Diversity and distribution of caddisflies (Insecta, Trichoptera) in Singapore’s freshwater**
5 **streams**

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22
23 **Abstract**

24 In land-scarce Singapore where fresh water is a critical resource, Trichoptera communities can
25 serve as a biomonitoring tool yet are poorly known. This study seeks to address this gap by
26 establishing a preliminary understanding of trichopteran diversity and distribution in Singapore’s
27 freshwater streams. From October 2023–January 2024, 11 stream sites across forested nature
28 reserves (n = 5), buffer parks (n = 4), and urban areas (n = 2) were surveyed for trichopteran larvae.
29 Only eight sites (four in forest streams, four in buffer streams) yielded Trichoptera, totalling 107
30 larval specimens comprising six families (Calamoceratidae, Ecnomidae, Hydropsychidae,
31 Leptoceridae, Odontoceridae, and Polycentropodidae). Leptoceridae were most abundant, while
32 Ecnomidae was the rarest, and Hydropsychidae were the most widely distributed. In this study,
33 Trichoptera were absent from the urban streams, which had greater depth and total dissolved solids,
34 but similar Trichoptera assemblages and environmental parameters were recorded in both forest and
35 buffer streams. Although differences between the latter two stream types were not statistically
36 significant, buffer streams had the highest abundance and taxonomic richness. Comparison with
37 past literature also reveals differences in recorded Trichoptera diversity, thus this study presents an
38 updated record for Trichoptera in Singapore’s streams. These findings further add to a baseline for
39 future biomonitoring, research, and informing long-term freshwater conservation efforts in
40 Singapore.

41
42 **Key words.** bioindicator, ecology, insects, macroinvertebrate, urbanisation

43 Introduction

44
45 Trichoptera, also known as caddisflies, comprise one of the world's most diverse orders of insects
46 with aquatic larvae, with over 17,000 species currently described globally from all biogeographical
47 regions save Antarctica (Morse et al. 2019). Trichoptera are amphibiotic, with larval stages
48 inhabiting aquatic environments and adult stages occupying terrestrial habitats near water bodies,
49 linking ecosystems and enhancing functional diversity (Morse et al. 2019). Trichoptera also display
50 great functional diversity in their larval stages, having a wide variety of feeding modes and retreat
51 forms in freshwater systems (Holzenthall et al. 2015; Morse et al. 2019). Similar to other
52 macroinvertebrates, Trichoptera functional diversity is also greatly shaped by environmental factors
53 including water temperature (as affected by altitude, latitude, riparian shade, etc.), water flow rate,
54 dissolved substances, and substrate type (Hynes 1970; Huryn and Wallace 1988; Lamouroux et al.
55 2004; Hughes 2006). As a result, trichopteran assemblages fulfil diverse niches within freshwater
56 ecosystems, generating many essential ecosystem services (Morse et al. 2019). Consequently,
57 Trichoptera are important bioindicators of water quality and environmental stress, especially given
58 their wide range of pollution tolerances (Blakely et al. 2014; Kalaninová et al. 2014; Morse et al.
59 2019). Freshwater life stages of many caddisfly species are highly susceptible to physico-chemical
60 changes in streams, which are increasingly attributed to urbanisation and anthropogenic impacts
61 (Pickett et al. 2011). Therefore, the long-term monitoring of Trichoptera populations is crucial in
62 the management and conservation of freshwater habitats and for evaluation of urbanisation impacts
63 (Wiederkehr et al. 2020).

64
65 While male adults are required for identification to species level, larval characteristics are sufficient
66 for identification of most of the 30 Southeast Asian trichopteran families (Morse 2025). The
67 Oriental Region, where Singapore is situated, has the highest known diversity of Trichoptera among
68 all biogeographical regions, with 5,854 species recorded as of 2019 (Morse et al. 2019). Yet despite
69 the increasing focus on the diversity and importance of Trichoptera within the academic and
70 biomonitoring community, no published systematic work on Singapore species are present to date.
71 Little is also known about trichopteran distribution in Singapore, apart from families and genera
72 discovered in a dedicated survey of macroinvertebrates in Singapore's last remnant freshwater
73 swamp forest (Ho et al. 2018) and a study of macroinvertebrate assemblages across forest, buffer
74 and urban streams (Wiederkehr et al. 2020).

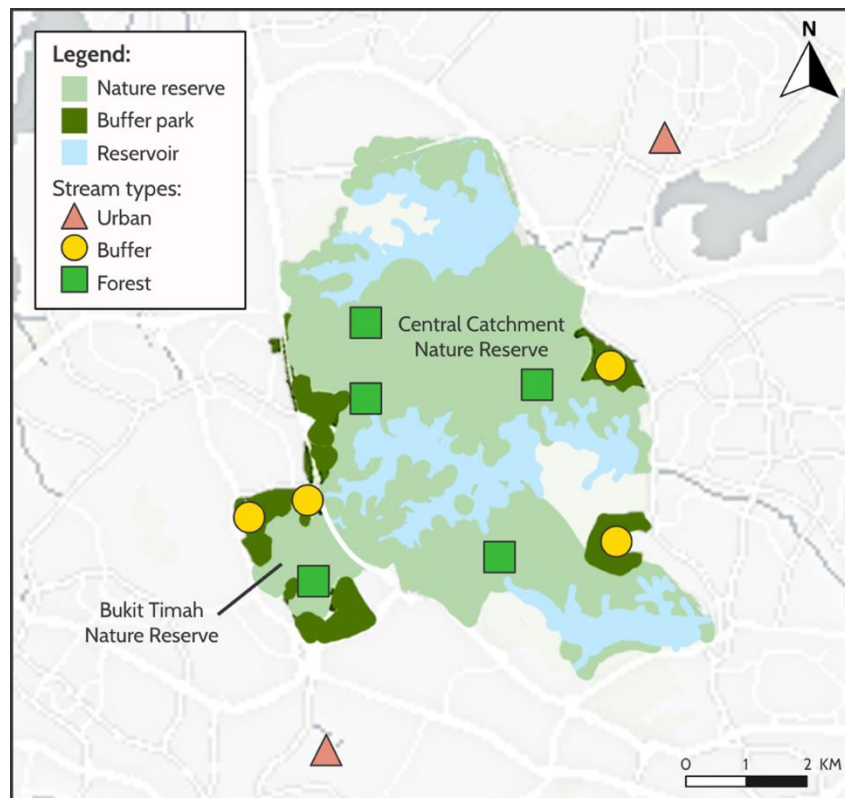
75
76 To deepen current understanding of Trichoptera in Singapore, this study aimed to discover and
77 describe Singapore's Trichoptera diversity and distribution in freshwater streams along an
78 urbanisation gradient. Specifically, this study determined and compared the taxonomic diversity of
79 trichopteran larvae (1) across forest, buffer and urban streams, and (2) in relation to differences in
80 environmental conditions along the continuum of stream types. Study findings contribute to global
81 records of Trichoptera, furthering the understanding and identification of this diverse taxon while
82 also informing local conservation and management of Singapore's freshwater streams and
83 Trichoptera assemblages.

84 85 Methods

86 87 *Sampling sites*

88 Eleven sites (Table A1) in freshwater streams of Singapore were sampled for Trichoptera larvae
89 during the Northeast Monsoon months of October 2023–January 2024 in the mornings (0930h–
90 1330h). These sites were categorised into three stream types (forest, buffer, and urban) depending
91 on where they were located, following the geographical boundaries of nature reserves and buffer
92 parks determined by the Singapore National Parks Board (NParks). Given that nature reserves
93 contain Singapore's remaining primary forest patches as well as native flora and fauna, they are
94 thus of highest conservation priority and most protected from urbanisation impacts (NParks, 2025).

95 Buffer parks fringe the nature reserves, thus buffering the latter from urbanisation impacts while
 96 providing spaces for recreation (NParks, 2025). Urban or open-country areas are not managed for
 97 the purpose of biodiversity conservation, hence these three stream types generally reflect an
 98 urbanisation gradient. Accordingly, forest streams are situated in nature reserves, buffer streams in
 99 buffer parks and urban streams in urban or open-country areas not immediately adjacent to the
 100 nature reserves. This study sampled five forest streams in Central Catchment Nature Reserve
 101 (excluding Nee Soon Swamp Forest; NSSF) and Bukit Timah Nature Reserve, four buffer streams
 102 in buffer parks like Thomson Nature Park and Windsor Nature Park, and two urban streams in the
 103 residential towns of Yishun and Clementi (Fig. 1).
 104



105
 106 Figure 1. Map showing 11 sampling sites included in the present study. These include sites on five
 107 forest streams (green), four buffer streams (yellow), and two urban streams (red). Sites are shown in
 108 relation to the Central Catchment Nature Reserve and the Bukit Timah Nature Reserve in
 109 Singapore.
 110

111 *Collection of specimens and environmental parameters*

112 At each stream site, a 10-m longitudinal transect was measured and environmental parameters were
 113 collected at 0 m, 5 m, and 10 m. These included temperature (°C), dissolved oxygen (DO; mg/L),
 114 conductivity (µS/cm), total dissolved solids (TDS; mg/L), salinity (ppt) and pH, all of which were
 115 measured using a multi-meter probe (YSI® Professional Plus; Xylem Analytics). Wetted width
 116 (cm) and water depth (cm) were measured using a ruler. General hardness of the water was
 117 determined using a hardness test (Salifert®), and surface flow rate (km/h) was determined either
 118 using a flow meter (Flowatch®; JDC Electronics SA) or a stopwatch (timings were recorded for a
 119 floating object to travel 1 m). Finally, canopy cover (%) was recorded using a spherical densiometer
 120 (Spherical Crown Densiometer® Concave Model C; Forestry Suppliers).
 121

122 Subsequently, the kick and tray-netting method (Ho et al. 2018) was employed in the 10-m transect
 123 using kick nets (36 × 30 cm, 500 µm pore size) for sampling stream macroinvertebrates, and was
 124 standardised across sites. Substrate at both the sides and centre of the stream were disturbed
 125 vigorously by two field surveyors concurrently for five minutes, from downstream to upstream.
 126 After removal of large debris, the remaining collected material from the kick nets was passed

127 through two sieves, first through a mesh size of 2 mm and then, 250 µm. Any vertebrates or
 128 decapods collected were returned to the stream, and any visible Trichoptera larvae and
 129 macroinvertebrates were stored separately in labelled tubes containing 75% ethanol. Remaining
 130 material in the 250 µm sieve was then bagged, labelled, and kept separately on ice for transportation
 131 and storage.

132
 133 *Sample processing and Trichoptera larvae identification*

134 Upon returning to the laboratory, the bagged material was washed and stored in 96% ethanol (Fig.
 135 A1). This material was subsequently sorted, without magnification, for Trichoptera larvae and other
 136 macroinvertebrates (Fig. A2), which were verified under a stereo microscope (Leica EZ4, 35x
 137 magnification) and then stored with those collected at the same site in 75% ethanol. For sites where
 138 no Trichoptera were detected during sampling, a subsample comprising 30% of collected material
 139 was sorted without magnification to confirm the absence of Trichoptera, and the remaining material
 140 was not sorted. Finally, all collected Trichoptera larvae were removed from cases, if any, (but
 141 retained with the specimen, Fig. A3) and thereafter identified to family level (Fig. A4) under a
 142 microscope (Leica M205 C, 160x magnification) using a Trichoptera identification key developed
 143 for the Malaysian region (Morse 2004).

144
 145 *Data analysis*

146 All statistical analyses were performed using R version 4.2.2 (R Core team, 2022) at 5%
 147 significance level.

148
 149 *Diversity, richness and abundance of Trichoptera assemblages*

150 Family abundance and richness of the Trichoptera assemblage at each site was first determined, and
 151 the relative abundance (pi) of each family (i) was calculated. The Shannon Index (H') was then
 152 computed as a measure for diversity using the following equation:

153
 154 Shannon Index (H') = - $\sum[(pi) \times \ln(pi)]$

155
 156 Comparison of abundance, richness, and Shannon Index was made across stream types. Differences
 157 were tested for statistical significance using Mann-Whitney U-test and Student's t-test for non-
 158 Normally and Normally distributed data, respectively.

159
 160 *Comparison of Trichoptera assemblages and environmental parameters across stream types*

161 Trichoptera assemblages were compared across stream types by conducting non-metric dimensional
 162 scaling (NMDS) using the Bray-Curtis distance measure to identify any clustering of families or
 163 sites. Any variation was then tested for statistical significance via permutational multivariate
 164 analysis of variance (PERMANOVA) using the *adonis2* function with the Bray-Curtis distance
 165 measure. Environmental parameters were then compared across stream types via a Principal
 166 Component Analysis (PCA), in which environmental data used were site averages of three readings
 167 per transect. Differences in environmental parameters were then tested for statistical significance
 168 using either Mann-Whitney U-test or Student's t-test depending on Normality of data.

169
 170 *Relating Trichoptera distribution to environmental parameters across sites*

171 To relate Trichoptera distribution with environmental parameters across sites, a Constrained
 172 Analysis on Principal Coordinates (CAP) was done using the *vegan* R package with Bray-Curtis
 173 distance to identify any environmental correlates determining Trichoptera family distribution.
 174 Overlapping environmental vectors revealed through a preliminary CAP biplot were removed to
 175 obtain better separation of vectors in the final biplot. PERMANOVA was then conducted using the
 176 *adonis2* function with the Bray-Curtis distance measure to determine the statistical significance of
 177 the associations between environmental parameters and family distribution across sites.

178

179 **Results**

180

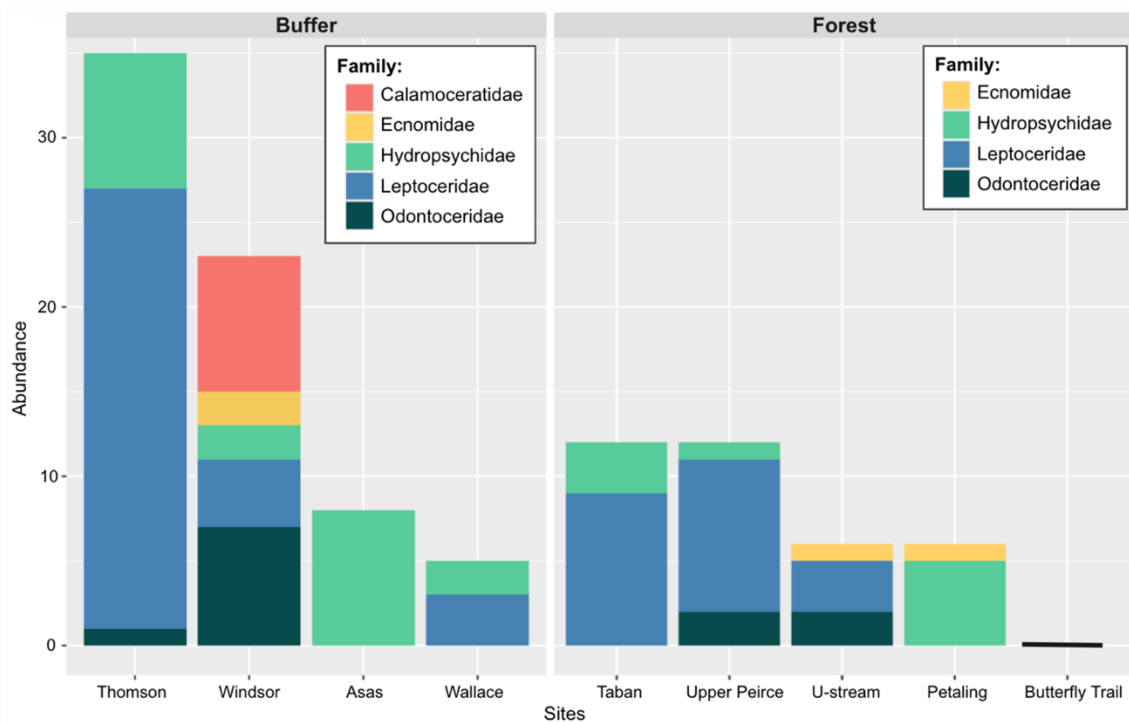
181 *Trichoptera assemblages across sites and stream types*

182 The six families of Trichoptera collected comprise Leptoceridae, Hydropsychidae, Odontoceridae,
 183 Calamoceratidae, Polycentropodidae, and Ecnomidae. None were found in urban streams, five
 184 families were found in forest streams, and Calamoceratidae occurred only in buffer streams along
 185 with the five found in forest streams (Table 1). Across sites, Leptoceridae was the most abundant at
 186 54 individuals, with highest relative abundance in both forest and buffer streams at 58.3% and
 187 46.5%, respectively. In contrast, Ecnomidae was the least abundant, with two individuals across all
 188 sites, contributing 1.9% of relative abundance in total.

189

190 When comparing Trichoptera assemblages across individual forest and buffer stream sites (Fig. 2),
 191 all four buffer streams had Trichoptera, while only four out of five forest streams had Trichoptera.
 192 The highest Trichoptera abundance and richness were recorded in buffer streams, which also had
 193 the highest variability in abundance (5–35 individuals per site) and richness (1–5 families per site).
 194 In comparison, forest streams with Trichoptera had less variation in both abundance (6–12
 195 individuals per site) and richness (2–3 families per site). Comparing all six Trichoptera families,
 196 Hydropsychidae was the most widespread, with specimens collected at seven sites, followed by
 197 Leptoceridae at six sites (Fig. 2). Calamoceratidae was the least widespread, occurring at only one
 198 buffer stream site (Fig. 2).

199



200

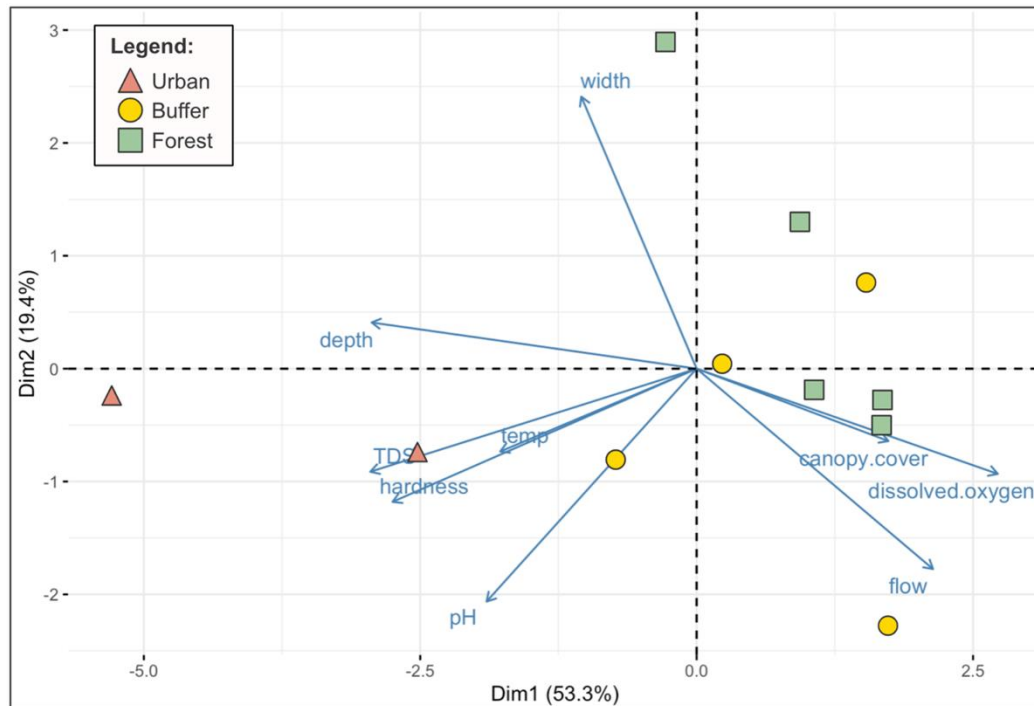
201 Figure 2. Stacked barplots of Trichoptera family composition, richness, and abundance at buffer and
 202 forest stream sites.
 203

204 Using NMDS to compare Trichoptera assemblages across stream types (Fig. A5), the 95%
 205 confidence ellipses of buffer and forest streams overlap and there is no statistically significant
 206 difference between Trichoptera assemblages across buffer and forest streams (p -value = 0.769).
 207 Further analysis revealed higher Trichoptera diversity, family richness, and abundance in buffer
 208 streams than in forest streams (Fig. A6), but again the differences were not statistically significant.
 209

210 *Environmental parameters across stream types*

211 The PCA of environmental parameters collected across all three stream types reveals separation of
 212 urban sites from buffer and forest sites along PCA axis 1 (Dim1) and axis 2 (Dim2), which accounts

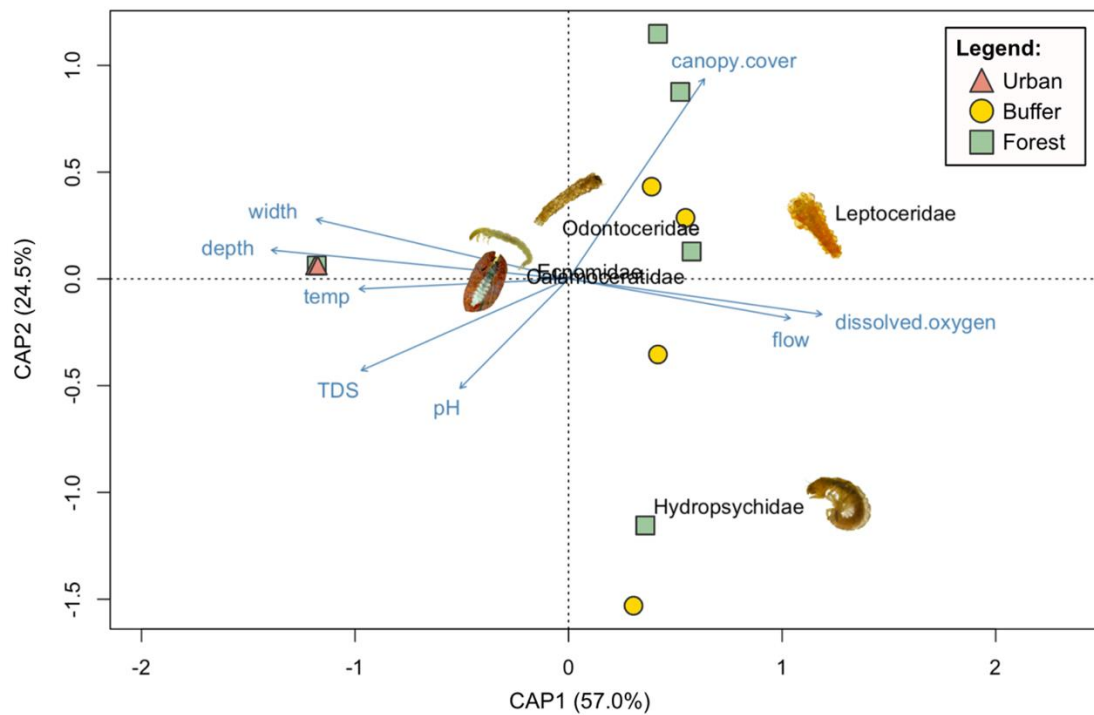
213 for 72.7% of total variation (Fig. 3). Dim1 appears to be associated with wetted depth, TDS,
 214 hardness, pH, dissolved oxygen, and flow, accounting for 53.3% of total variation. On the other
 215 hand, Dim2 is strongly associated with width and accounts for a further 19.4% of total variation.
 216 The urban sites are situated on the left, driven mostly by greater depth, TDS, hardness and pH. In
 217 comparison, the buffer and forest sites are situated more toward the right, and this distinction from
 218 urban sites is driven by higher canopy cover, dissolved oxygen, and greater flow rate. Additionally,
 219 buffer and forest sites overlap, varying in terms of wetted width. Comparison of collected
 220 environmental parameters between buffer and forest streams reveal higher hardness, DO,
 221 conductivity, TDS, salinity, count, and population density in buffer streams than in forest streams,
 222 which are more acidic (Table A2). These differences, however, were not statistically significant (p -
 223 value > 0.05).
 224



225
 226 Figure 3. Principal component analysis (PCA) plot of environmental parameters. Vectors (blue) for
 227 urban (red), buffer (yellow) and forest (green) stream sites are shown, accounting for 72.7% of total
 228 variance. PCA axis 1 (Dim1) and axis 2 (Dim2) account for 53.3% and 19.4% total variance,
 229 respectively.
 230

231 *Relating Trichoptera diversity to stream environmental parameters*

232 The CAP biplot (Fig. 4), accounting for 74.8% of total variance, shows a separation of sites without
 233 and with Trichoptera. Three sites without Trichoptera (two urban, one forest) are situated in the left
 234 half of the plot, while the four buffer and four forest streams with Trichoptera overlap in the right
 235 half of the plot, showing no distinct clustering (Fig. 4). CAP1 explains 50.8% of total accounted
 236 variance, and appears to be associated with flow rate, dissolved oxygen, temperature, depth, width,
 237 and TDS. Canopy cover and pH appear to be associated with CAP2, which explains 24% of total
 238 accounted variance.
 239



240
 241 Figure 4. A Constrained Analysis on Principal Coordinates (CAP) biplot. Urban (red), buffer
 242 (yellow), and forest (green) stream sites are shown with environmental parameters represented by
 243 vectors (blue) as well as the six Trichoptera families collected. CAP1 and CAP2 account for 50.8%
 244 and 24.0% of variance, totalling 74.8% of accounted variance.
 245

246 Hydropsychidae appears to be associated with higher flow rate, dissolved oxygen, TDS, and pH;
 247 Leptoceridae with higher TDS and pH (Fig. 4). Ecnomidae, Calamoceratidae, and Odontoceridae
 248 are clustered near the centre of the biplot and appear to be associated with greater depth and width,
 249 higher temperature, as well as lower flow and dissolved oxygen (Fig. 4). Odontoceridae and
 250 Leptoceridae appear to be associated with greater canopy cover (Fig. 4). Among all eight
 251 environmental parameters included in the CAP, further statistical analysis confirmed that depth (p -
 252 value = 0.036) and flow (p -value = 0.029) were significant in explaining community differences.
 253

254 Discussion

256 *Urbanisation gradient*

257 The taxonomic diversity of trichopteran larvae varied across forest, buffer and urban streams, and in
 258 relation to stream environmental condition, buffer streams had the highest Trichoptera diversity
 259 among all sampled stream types in this study. Along this urbanisation gradient comprising a
 260 continuum of stream types, urban, buffer, and forest streams experience different types and extents
 261 of urbanisation impacts (Pickett et al. 2011), which often result in distinct physico-chemical
 262 properties, productivity, and macroinvertebrate community across stream types (Urban et al. 2006;
 263 Wiederkehr et al. 2020).
 264

265 In the present study, stream types were determined based on the geographical designation of
 266 forested nature reserves and buffer parks (forest edges adjacent to nature reserves) by NParks
 267 (2024). Consequently, these streams receive different extents of protection against disturbance and
 268 anthropogenic impacts, depending on how the surrounding area is managed (Wiederkehr et al.
 269 2020). Urban streams in this study are located in urban, open-country areas not immediately
 270 adjacent to the nature reserves that are not specifically managed or monitored by NParks, while
 271 forest streams likely receive the most protection due to strict regulation pertaining access and the
 272 use of nature reserves for biodiversity conservation (NParks 2015). Buffer parks fall in the middle
 273 of this spectrum, being situated at forest edges, serving the fundamental purpose of buffering

274 urbanisation and anthropogenic impacts on the core biodiversity areas, which are within the larger
 275 nature reserves (NParks 2015). Hence, streams within these buffer parks likely experience more
 276 disturbance than those in nature reserves, but less disturbance compared to urban streams, which
 277 often experience flash floods and high pollutant and allochthonous input due to run-off (Delong and
 278 Brusven 1994).

279
 280 The disturbance that buffer parks experience can be further discussed in both a historical and
 281 current context. Buffer parks are often situated near past settlements or in areas previously
 282 transformed (e.g. redirected, straightened, canalised, etc.) by human activities (Ong et al. 2023), and
 283 trash like broken pots and cement blocks were found at some buffer stream sites, along with finer
 284 metal pieces in the sediment (Fig. 5). As a result of this historical disturbance, active restoration
 285 efforts were undertaken prior to the opening of these buffer parks (Ong et al. 2023), with the first
 286 buffer park established in 2001 and subsequent ones established mostly in the 2010s (Otterman
 287 2015). This history of disturbance coupled with the recency of establishment for these buffer parks
 288 thus suggest that buffer streams may have experienced high levels of historical disturbance.
 289



290
 291 Figure 5. Trash observed in the present study. (A) A bottle, a pipe and a cement block in Asas
 292 stream. (B) Metal pieces in the sediment collected from the stream in Thomson Nature Park.
 293

294 In the current context, buffer parks are slated for forest restoration efforts (Fig. A7) under the Forest
 295 Restoration Action Plan (NParks 2019). Open all-year round, buffer parks also receive constant
 296 visitorship (NParks 2024), with some of these streams situated near hiking trails, unlike in nature
 297 reserves where streams tend to be situated further away from hiking trails, or even in areas closed to
 298 public access (personal observation). Heavy usage of hiking trails results in increased soil erosion
 299 and, over time, soil compaction, both of which would cause greater runoff into streams (Salesa and
 300 Cerdà 2020). The resulting physico-chemical changes that might occur include increased turbidity,
 301 TDS and nutrients, which would affect Trichoptera larval assemblages (Kalaninová et al. 2014). In
 302 the present study, higher TDS, conductivity and salinity was observed in buffer streams when
 303 compared against forest streams, along with differences in observed Trichoptera diversity between
 304 stream types.
 305

306 Continuous disturbance from these various sources would likely prevent the establishment of
 307 climax communities, causing stream communities to alternate between establishment and recovery
 308 (Death 1996). Human disturbance may also allow for greater co-existence of species between rapid
 309 coloniser species as well as highly competitive species, preventing the dominance of either group

310 (Wilson 1994) which, following the Intermediate Disturbance Hypothesis (Mayor et al. 2012), may
311 thus account for the highest diversity found in sampled buffer streams.

312

313 *Absence of Trichoptera in urban streams*

314 In this study, no Trichoptera were found in the two urban streams surveyed. Urban streams are often
315 associated with poor water quality, and thus lower abundance of intolerant taxa (Hughes 2006;
316 Urban et al. 2006; Violin et al. 2011). In the present study, the PCA revealed that urban sites were
317 associated with higher TDS, which is often a result of anthropogenic input from runoff (Köse et al.
318 2014) and suggests that the sampled urban streams experience a greater extent of pollution and
319 anthropogenic disturbance (da Silva et al. 2020; Correa-Araneda et al. 2022) than buffer and forest
320 streams. Using SingScore, a macroinvertebrate biotic index developed for Singapore streams, the
321 assigned scores for the six families collected in the present study range from 6–10, suggesting
322 medium to low pollution tolerance (cf., the full range of SingScore-Tolerance scores from 1 (most
323 pollution-tolerant) to 10 (least pollution-tolerant)) (Blakely et al. 2014). Therefore, the absence of
324 Trichoptera from urban streams, coupled with higher TDS, may indicate poorer water quality as
325 opposed to buffer and forest streams, and aligns with the findings by Wiederkehr et al. (2020).

326

327 *No significant difference between buffer and forest streams*

328 Similarities in environmental conditions and Trichoptera assemblages were observed between
329 buffer and forest streams. Taxonomic and functional structure of Trichoptera assemblages are
330 strongly associated with stream order and other environmental conditions such as temperature, flow,
331 and substrate type (Huryń and Wallace, 1988; Lamouroux et al. 2004; Hughes 2006; Kalaninová et
332 al. 2014). Hence, given the similarity of environmental conditions between buffer and forest
333 streams, their trichopteran assemblages could be expected to be similar as well.

334

335 The similarities in Trichoptera assemblages could also be due to Singapore's geographical features,
336 with a relatively small area, a generally flat topography, and a large proportion of land area being
337 urbanised (Chia et al. 1991). This results in a compression of stream orders (Wiederkehr et al. 2020)
338 such that most of Singapore's naturally occurring streams are lower order and therefore ecologically
339 similar in that characteristic. Furthermore, buffer parks in Singapore are close to nature reserves,
340 being situated along their peripheries (NParks 2024). This proximity increases the likelihood of
341 movement between buffer parks and nature reserves, aiding in adult Trichoptera dispersal across
342 stream types (Curry and Baird 2015) and widening the range of the organisms (NParks 2015).
343 Additionally, buffer and forest streams are often situated within the same drainage basins,
344 potentially supporting a metacommunity of Trichoptera families in a single basin across buffer
345 parks and nature reserves (Brown and Swan 2010), further explaining why similar Trichoptera
346 assemblages are observed across these two stream types.

347

348 *Comparison with past studies in Singapore*

349 The six Trichoptera families collected in the present study are a subset of the 18 families found in
350 Peninsular Malaysia and Singapore (Morse 2004). Within Singapore, only two recent studies have
351 documented Trichoptera larvae (Ho et al. 2018; Wiederkehr et al. 2020).

352

353 A study on aquatic macroinvertebrate diversity in Nee Soon Swamp Forest (NSSF), Singapore's
354 only remaining freshwater swamp forest, recorded 11 families, with Ecnomidae as the most
355 abundant family (Ho et al. 2018). Trichoptera were collected during 2013–2015 using methodology
356 similar to that of the present study, except for overall higher sampling effort owing to many more
357 sampling sites within NSSF covering diverse microhabitats (n = 40). Additionally, Ho et al. (2018)
358 discussed the characteristic waterlogged 'freshwater swamp forest' stream environmental
359 conditions within NSSF—lower flow, higher tannins and higher acidity—as opposed to faster-
360 flowing forest streams with visibly less tannins that were sampled in this present study, which
361 excluded NSSF streams. These environmental conditions may potentially account for the high

362 diversity of Trichoptera (Lamouroux et al. 2004; Hughes 2006; Kalaninová et al. 2014); however,
363 this can only be confirmed by further analysis of the environmental conditions of sampling sites and
364 their corresponding trichopteran assemblages within NSSF. There also exists high heterogeneity
365 within NSSF, comprising both lentic and lotic freshwater habitats, in addition to periodic reservoir
366 freshwater input in the northeast edge (Ho et al. 2018)—in contrast to the present study, which
367 focused only on lotic environments without reservoir input. Given the specificity of niches filled by
368 various Trichoptera families (Morse 2004; Holzenthal et al. 2015), the wider range of freshwater
369 habitats covered by Ho et al. (2018) within NSSF might account for the higher Trichoptera diversity
370 reported in comparison to the present study.

371
372 Another study investigating macroinvertebrates across 11 urban, buffer, and forest streams in
373 Singapore (Wiederkehr et al. 2020) recorded Trichoptera in all three stream-types; however,
374 diversity was limited to only two families, Ecnomidae and Polycentropodidae. Ecnomidae was the
375 more abundant family (similar to NSSF by Ho et al. 2018), contrasting with the present study which
376 found no Trichoptera in sampled urban streams and Ecnomidae to be the least abundant family.
377 This could be attributed to the difference in collection method used by Wiederkehr et al. (2020),
378 which made use of leaf-litter bags that selectively attract leaf-litter dwelling macroinvertebrates.
379 Given the passive and targeted nature of that collection method, the two Trichoptera families
380 collected in the study by Wiederkehr et al. (2020) are likely not as representative of the entire
381 stream Trichoptera assemblage. In the present study, the six families collected comprise a variety of
382 functional feeding groups, including filtering-collectors and facultative predators (Ecnomidae,
383 Hydropsychidae, Polycentropodidae), obligate predators (some Leptoceridae), shredding
384 detritivores (most Calamoceratidae, most Leptoceridae, Odontoceridae), and scrapers (some
385 Calamoceratidae). Furthermore, collected families in the present study differed between case-
386 making larvae that build portable cases of plant matter (Calamoceratidae, some Leptoceridae) or
387 minerals (some Leptoceridae, Odontoceridae) and fixed-retreat-making larvae (Ecnomidae,
388 Hydropsychidae, Polycentropodidae), the fixed retreats suggesting preference for harder sediment
389 types (Morse 2004). These two reasons could further explain why a greater diversity of Trichoptera
390 was found in the present study than in the study by Wiederkehr et al. (2020).

391 392 **Conclusion**

393
394 Overall, the present study has confirmed the continued presence of Trichoptera in Singapore,
395 providing a snapshot of Singapore's Trichoptera diversity and distribution within the Northeast
396 Monsoon months of October 2023 to January 2024. Differences in Trichoptera assemblages and
397 environmental conditions were observed across an urbanisation gradient, comprising forest, buffer
398 and urban streams, in which Trichoptera were recorded to be absent from urban streams. This thus
399 affirms the effectiveness of current freshwater conservation efforts by NParks, while also
400 emphasising the need to continue managing urbanisation impacts to allow such pollution-sensitive
401 and diverse taxa to continue existing. Future work could thus be done to investigate temporal trends
402 in Trichoptera diversity, which would shed light on Trichoptera dispersal patterns that will be
403 important to inform conservation efforts of this taxa. Additionally, taxonomic work identifying
404 Trichoptera specimens to genus or species would also be useful to deepen current knowledge of
405 Trichoptera diversity in Singapore.

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544

545 Figure captions

546 Figure 1. Map showing 11 sampling sites included in the present study. These include sites on five
547 forest streams (green), four buffer streams (yellow), and two urban streams (red). Sites are shown in
548 relation to the Central Catchment Nature Reserve and the Bukit Timah Nature Reserve in
549 Singapore.

550
551 Figure 2. Stacked barplots of Trichoptera family composition, richness, and abundance at buffer and
552 forest stream sites.

553
554 Figure 3. Principal component analysis (PCA) plot of environmental parameters. Vectors (blue) for
555 urban (red), buffer (yellow) and forest (green) stream sites are shown, accounting for 72.7% of total
556 variance. PCA axis 1 (Dim1) and axis 2 (Dim2) account for 53.3% and 19.4% total variance,
557 respectively.

558
559 Figure 4. A Constrained Analysis on Principal Coordinates (CAP) biplot. Urban (red), buffer
560 (yellow), and forest (green) stream sites are shown with environmental parameters represented by
561 vectors (blue) as well as the six Trichoptera families collected. CAP1 and CAP2 account for 50.8%
562 and 24.0% of variance, totalling 74.8% of accounted variance.

563
564 Figure 5. Trash observed in the present study. (A) A bottle, a pipe and a cement block in Asas
565 stream. (B) Metal pieces in the sediment collected from the stream in Thomson Nature Park.

566
567

568 Table 1. Checklist of Trichoptera families with absolute and relative abundances across sampled
 569 buffer and forest streams.
 570

Family	Buffer		Forest		Total abundance	Total relative abundance (%)
	Abundance	Relative Abundance (%)	Abundance	Relative abundance (%)		
Leptoceridae	33	46.5	21	58.3	54	50.5
Hydropsychidae	20	28.2	9	25	29	27.1
Calamoceratidae	8	11.3	0	0	8	7.5
Odontoceridae	7	9.9	4	11.1	11	10.3
Polycentropodidae	2	2.8	1	2.8	3	2.8
Ecnomidae	1	1.4	1	2.8	2	1.9
Total	71	100	36	100	107	100

571