Using invertebrate functional traits to improve flow variability assessment within European rivers

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1 **Using invertebrate functional traits to improve flow variability assessment within European**

2 **rivers**

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25   **Abstract**

26   Rivers are among the most threatened ecosystems worldwide and are experiencing rapid

27   biodiversity loss. Flow alteration due to climate change, water abstractions and augmentation is a

28   severe stressor on many aquatic communities. Macroinvertebrates are widely used for

29   biomonitoring river ecosystems although current taxonomic approaches used to characterize

30   ecological responses to flow have limitations in terms of generalisation across biogeographical

31   regions. A new macroinvertebrate trait-based index, Flow-T, derived from ecological functional

32   information (flow velocity preferences) currently available for almost 500 invertebrate taxa at the

33   European scale is presented. The index was tested using data from rivers spanning different

34   biogeographic and hydro-climatic regions from the UK, Cyprus and Italy. The performance of

35   Flow-T at different spatial scales and its relationship with an established UK flow assessment tool,

36   the Lotic-invertebrate Index for Flow Evaluation (LIFE), was assessed to determine the

37   transferability of the approach internationally. Flow-T was strongly correlated with the LIFE index

38   using both presence-absence and abundance weighted data from all study areas (r varying from 0.46

39   to 0.96). When applied at the river reach scale, Flow-T was effective in identifying communities

40   associated with distinct mesohabitats characterised by their hydraulic characteristics (e.g., pools,

41   riffles, glides). Flow-T can be derived using both presence/absence and abundance data and can be

42   easily adapted to varying taxonomic resolutions. The trait-based approach facilitates research using

43   the entire European invertebrate fauna and can potentially be applied in regions where information

44   on taxa-specific flow velocity preferences is not currently available. The inter-regional and

45   continental scale transferability of Flow-T may help water resource managers gauge the effects of

46   changes in flow regime on instream communities at varying spatial scales.

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48   **KEYWORDS:** bioassessment, flow velocity preference, traits theory, river ecosystems,

49   hydrological alteration, functional ecology

1. Introduction

Rivers are among the most threatened ecosystems worldwide (Tickner et al., 2020; Albert et al., 2021) with their flow regimes (discharge) widely altered, and their longitudinal and lateral connectivity fragmented due to weirs, impoundments and floodplain development (Grill et al., 2019; Belletti et al., 2020). The natural flow regime acts as major driver in fluvial ecosystems (Rolls et al., 2018) with hydrological variability and alteration having wide ranging effects instream, as well as on aquatic–terrestrial linkages (Tockner et al., 2010). The predicted increase in extreme flow events frequency and intensity for numerous areas worldwide (Prudhomme et al., 2014; Chang et al., 2018), coupled with the increasing demands of water abstraction for anthropogenic activities, are likely to exacerbate the effects of flow regime alteration (Tonkin et al., 2018; Dudgeon, 2019).

Streamflow affects many abiotic characteristics, from water temperature and streambed stability to channel and floodplain morphology (see Richter et al., 1997 and reference inside). Riverine aquatic communities display a range of life history traits and morphological adaptations that enable individuals to persist and maintain populations within lotic ecosystems (Statzner and Holm, 1989; Mims and Olden, 2012). However anthropogenic alteration of flow regimes (e.g., longitudinal impoundments, abstraction and augmentation) may constrain biological communities (Krajenbrink et al., 2019; Mellado-Díaz et al., 2019; White et al., 2021) and prevent unpolluted or otherwise relatively pristine rivers from achieving their maximum ecological capacity (Theodoropoulos et al., 2020). Although the importance of hydromorphological alterations on rivers and their flow regime are widely recognised, tools to address this stressor have rarely been integrated in biomonitoring policies or environmental legislations (Poikane et al., 2020; Theodoropoulos et al., 2021).

Macroinvertebrate communities are crucial components of aquatic and riparian ecosystems (Suter and Cormier, 2015) that are frequently used for biomonitoring of rivers worldwide (Buss et al., 2015), and have been shown to be sensitive to flow regime variability and alteration (Wood et al.,

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75   2000; Belmar et al., 2019; Doretto et al., 2019). Since the late Twentieth Century, an increasing

76   number of biological indices have been developed to characterise the flow preferences of lotic

77   macroinvertebrates. For example, the Rheo-index (Banning, 1990, 1998) was one of the first to

78   characterise faunal associations with river flow velocity and is one of the core metrics for

79   evaluating the quality status of rivers in Germany (Buchner et al., 2019). Similarly, Timm et al.

80   (2011) proposed an index (MESH), able to assess hydromorphological quality of Estonian surface

81   waters (lakes and rivers) focussing on macroinvertebrate taxonomic composition. Theodoropoulos

82   et al. (2020) proposed a dual-index system, called ELF (Hellenic Flow Index), to assess the effects

83   of hydrological alterations upon Greek streams based on optimal discharge conditions for

84   macroinvertebrates, utilising prior knowledge of the pollution status of the site being considered.

85   One of the most widely tested macroinvertebrate-based indices for characterising faunal river flow

86   preferences is the Lotic Invertebrate Index for Flow Evaluation (LIFE - Extence et al., 1999). The

87   index was developed using data from UK rivers and was specifically designed to reflect faunal

88   associations with flow velocity and its variability via a scoring system. This index and its

89   derivatives are currently used in countries spanning 3 continents and multiple countries including

90   the UK (e.g., White et al., 2021), Canada (e.g., Armanini et al., 2011), New Zealand (e.g.,

91   Greenwood et al., 2016) and Costa Rica (e.g., Quesada-Alvarado et al., 2020). However, all these

92   indices are based on locally derived scores for each macroinvertebrate taxon, typically recorded at

93   family or species level, according to their flow velocity preferences. In general, the determinations

94   of velocity-preference were derived from an information-driven expert panel process, rather than

95   empirical and contemporary measures of State-Pressure-Response. The benefit of this approach is a

96   mechanistic independence of output. There is, however, an inbuilt element of subjectivity. Given

97   natural biogeographical constraints, most historic approaches are often limited in terms of the

98   geographical application to the areas where they were originally developed (e.g., lack of specific

99   taxonomic information, scores not available for local endemics or families unique to a specific

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region/ country, or presence of new / alien taxa). Therefore, there is the need to increase the

potential transferability and applicability of tools to monitor and characterise hydrological

variability and stress where it occurs (Friberg et al., 2011; Alonso et al., 2013; Poff, 2018).

Ecological functional information used to characterise floral and faunal communities has received

increasing attention in recent years, as it potentially overcomes the constraints imposed by

traditional taxonomic approaches (Mouillot et al., 2013; Kunz et al., 2021). Within freshwater

ecosystems, a wide range of faunal traits (e.g., body size, voltinisms, feeding habits, life-history

strategies) have been assigned to macroinvertebrates. These traits are commonly used in ecological

and ecohydrological research to assess the effects of flow alteration on macroinvertebrate

communities, providing complementary information to traditional taxonomic approaches

(Guareschi et al., 2014; Ruhi et al., 2018; but also see limitations in Hamilton et al., 2020). As the

combination of species traits determines their likelihood and ability to withstand disturbance events,

such as flow alteration, a non-random taxa selection is expected along environmental gradients and

associated with specific disturbances (Mouillot et al., 2013).

This study aimed to develop a new aquatic macroinvertebrate trait-based index, called Flow-T,

derived from the "current velocity" trait proposed by Tachet et al. (2010) in a comprehensive

international trait dataset encompassing nearly 500 macroinvertebrate taxa. We compared the

performance of the newly developed index with those of an already established index, LIFE, in

different biogeographic and climatic regions and using both family and mixed-level resolution (e.g.

species, genera and families within the same dataset). Flow-T was calculated using: i) the original

UK macroinvertebrate dataset used to develop and test the LIFE index; ii) Mediterranean rivers

from Cyprus focussed on riffle and pool habitats and iii) Italian rivers encompassing 5 different

habitats characterised by different flow velocity characteristics. Flow-T was expected to perform

better than the existing index (LIFE) when applied in geographical areas where local / region

specific flow-sensitivity indexes are currently lacking because of increased taxa coverage. In

addition, it overcomes the inherent issue of applying flow-sensitivity taxa lists outside regions they were developed in. Specifically, we predicted similar responses of both indices in the UK and better performances of Flow-T over LIFE in Italy and Cyprus because the first includes more taxa than the second. We also predicted better performance of mixed-level taxonomic resolution compared to family data because not all flow velocity preferences are preserved or represented at the family level.

2. Materials and Methods

2.1 Biological and trait datasets

LIFE is a macroinvertebrate-based index for the assessment of flow conditions within streams and rivers (Extence et al., 1999). It was developed in the UK by the Environment Agency of England (EA) using historic macroinvertebrate data from 1986 to 1997. The core dataset comprised of multi-habitat 3-minute kick samples from three chalk streams: River Lark and Waithe Beck (Anglian region), the R. Kennet (Thames region) and two Midlands rivers: R. Derwent and R. Wreake (detailed information regarding all sampling methods and study areas is provided in S1). In this study macroinvertebrate community data from the original sites used by Extence et al. (1999) were employed to derive the values of LIFE and Flow-T using the original dataset from 1986 to 1997 (for a total of 133 samples) and an extended dataset from the same rivers spanning 1986 to 2019 (285 samples). The taxonomic resolution was mostly at species level except for some groups (e.g., Diptera). Briefly, the LIFE index is calculated as follows. first, each taxon (both family and species level) is assigned to one of the 6-flow groups (from I: Rapid flow velocity to VI: Drought resistant). Flow sensitivity scores are then assigned based on a two-entry table where the entries reflect the flow group and the abundance category of individual taxa on a log-scale. For each sample, the final score is derived by dividing the sum of individual taxon flow scores by the number of scored taxa. Higher flow velocities typically result in communities characterised by higher LIFE scores.

The second dataset used to compare the LIFE methodology and Flow-T was from the eastern Mediterranean island of Cyprus. Macroinvertebrates were sampled quantitatively from distinct habitats (using a Surber sampler) and identified to family level according to the national biomonitoring method (Water Development Department, 2006). Samples were collected from both riffle and pool habitats, where the latter included both instream pools and glides (see below for mesohabitat nomenclature). The Cypriot dataset comprised a total of 872 samples from 77 rivers and 143 sites, spanning the period from 2005 to 2018.

LIFE and Flow-T were also calculated using a dataset derived from three Italian braided rivers within the Po River basin (R. Taro, Trebbia and Enza within the Emilia Romagna Region, Northern Italy). The three rivers were sampled once during spring and summer 2020, providing a total of 180 samples (60 macroinvertebrate samples per-river). River reaches of 300 m in length for the Enza River and of ~1000 m for the wider channels of the Trebbia and Taro Rivers were used and mesohabitats (riffle, pool, isolated pond, backwater and glide) identified before the collection of macroinvertebrate samples according to Belletti et al. (2017). Samples were collected from each mesohabitat type identified trying to encompass the entire range of flow velocity/depth available throughout the reach. At each sampling point, mean flow velocity and water depth and the mesohabitat type was recorded.

The Tachet et al. (2010) database (hereafter TAC) represents the most widely used trait resource for European freshwaters. It has been successfully applied in previous research at both national and continental scales (Mellado-Díaz et al., 2008; Bonada and Dolédec, 2017; Laini et al., 2019) and has been integrated into the online European dataset *freshwaterecology.info* (Schmidt-Kloiber and Hering, 2015). This later database includes 22 ecological and biological traits available for the majority of European freshwater taxa and was developed using information gathered from around 6,000 sources (Bonada and Dolédec, 2017). Information regarding flow velocity preferences for macroinvertebrate taxa is available within the ecological trait "current velocity" which is divided in 4 modalities (lentic, slow, moderate and fast) where each taxon affinity is coded using a fuzzy

175 coding from 0 (no affinity) to 3 (strong affinity). Information is available mostly at genus level, but

176 species-level (when genera are monospecific, e.g., *Ancylus fluviatilis*), subfamily-level (e.g., family

177 Chironomidae, for which information at subfamily level is reported) and family-level (e.g.,

178 Tipulidae and Tubificidae) are also present.

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180 *2.2. Flow-T calculation*

181 Flow-T is calculated using the current velocity preference categories for each sample using:

182
$$Flow - T = \frac{\sum_i^n \log(A_i + 1) \times (m_i + f_i)}{\sum_i^n \log(A_i + 1)} \times 100$$

183 Where n represents the number of taxa in a sample, A_i the abundance of the ith taxon, m_i and f_i the

184 "moderate" and "fast" velocity preference classes of the ith taxon according to Tachet et al. (2010).

185 The logarithm of the abundance can be replaced by 1 to obtain the presence-absence version of the

186 Flow-T index (both tested in the present study). Hereafter, Flow-T_{abu} and Flow-T_{pa} will refer to Flow-

187 T based on abundance and presence-absence data, respectively. Furthermore, two other versions of

188 the Flow-T index were calculated for both presence-absence and abundance data (a reduced and a

189 weighted version respectively). The first version excludes Chironomidae and Oligochaeta from the

190 calculation because of the absence of a clear association for them at the family level with flow velocity

191 (due to the large number of species). The second version assigns more weight to the taxa that are low

192 flow specialists (velocity classes "lentic" and "slow") or high flow specialists (velocity classes

193 "moderate" and "fast"). Weights were calculated using the trait specialisation index (TSI) developed

194 by Mondy and Usseglio-Polatera (2014). TSI calculations were undertaken using two aggregated

195 velocity classes, the first being the sum of the lentic and slow velocity classes and the second the

196 moderate and fast velocity classes. Complete outputs for these two latter approaches are presented in

197 Appendix S2. A summary of the different versions of the Flow-T index used in this research is

198 presented in Table 1. Flow-T can be calculated with a mixed taxonomic resolution (e.g., family, genus

199 and species resolution in the same dataset) and family level, where family level traits are obtained by

200 averaging traits at a finer taxonomic level. Trait categories were converted to percentages of affinity

201 prior to the Flow-T and TSI calculation.

202

203 *2.3 Data analysis*

204 For the UK dataset, the association between LIFE and Flow-T was tested using Pearson

205 correlation coefficients for family and species level data. Species level LIFE was compared to Flow-

206 T at the mixed taxonomic level. To investigate how the choice of family level scores may influence

207 the correlation between Flow-T and LIFE indices, taxa within each family were randomly sampled

208 and taken as representative of the family scores (e.g., *Ecdyonurus* for Heptageniidae in the first run,

209 *Heptagenia* in the second). Two different values of both Flow-T_{pa} and Flow-T_{abu} were thus obtained:

210 i) considering the mean genus values as a proxy for the family value and ii) considering the random

211 selection of finer taxonomic levels within a family (e.g., genus, subfamily for some dipterans). This

212 later process was repeated 1,000 times and the median and the percentile confidence interval (2.5% -

213 97.5%) calculated.

214 Flow-T and LIFE indices for Cyprus rivers were calculated at the family level. Linear mixed

215 effect models were used to test the association between both indices and mesohabitat as a fixed effect

216 and sampling site as a random effect. Indices values were centred and standardized prior to the

217 analysis to allow comparison of the results. Models with and without the mesohabitat were compared

218 and the best model selected according to deviance information criterion - DIC (Spiegelhalter et al.,

219 2002). The greater the difference between the DIC of two competing models the greater the support

220 for the best model (which is the one with the lowest DIC). Like the Akaike Information Criterion,

221 DIC differences < 7, between 7 and 14 and > 14 provide plausible, equivocal and implausible support

222 for the model with larger DIC (Burnham et al., 2011).

223   For Italian data, the LIFE index was calculated at the family level and Flow-T derived at both

224   the family, the taxonomic resolution used for biomonitoring purposes at the national level (Buffagni

225   and Erba, 2007), and at a mixed taxonomic level (family and genus). Linear mixed effect models

226   were used to regress both indices in association with mesohabitat, flow velocity and their interaction

227   as fixed effects. For each index, two different error structures were used to account for spatial

228   autocorrelation and the best model selected according to Ventrucci et al. (2020) (method description

229   and full results are presented in S3). Models including an interaction and site as the random effect

230   were compared to additive models to select the best performing model based on the DIC. In addition,

231   models considering "site" as a fixed effect were derived to validate the results obtained with the linear

232   mixed model approach because the estimation of the random effect could be affected by the low

233   number of sites used (n = 3). According to Zuur et al. (2009) we used the random effect approach in

234   the final analysis because it focuses on the variables of interest (flow velocity values and

235   mesohabitat). Similarly, we tested the response of LIFE and Flow-T to discriminate between the five

236   mesohabitats, without including flow velocity in the model and to discriminate riffles from pools, by

237   joining pools and glides.

238   Marginal and conditional R^2_m and R^2_c values were calculated according to Nakagawa and Schielzeth

239   (2013) for each model for the Cypriot and Italian datasets. R^2_m represents the variance explained by

240   the fixed effects, while R^2_c represents the variance explained by both the fixed and random effects. A

241   summary of the analysis performed for each dataset is given in Table 2.

242   All models were fitted using a Bayesian approach using the package INLA (Rue et al., 2009) for the

243   R statistical computing software (R Core Team, 2020). We selected a Bayesian approach because of

244   its ability to model complex error structure and to provide estimates of uncertainty for all the

245   parameters involved (e.g., R^2). Models were also computed with a 'frequentist' approach and the

246   results   presented   in   appendix   S4.   The   package   biomonitoR

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247 (https://github.com/alexology/biomonitoR) was used to calculate both LIFE and Flow-T indices,

248 while the package ggplot2 (Wickham, 2009) was used for plotting the results.

249

3. Results

251 *3.1 Dataset 1, UK: Flow-T and LIFE relationship*

252 A total of 406,576 organisms belonging to 99 families and 411 taxa were identified. All families with

253 a LIFE score also were allocated a score for Flow-T. Flow-T_{pa} and Flow-T_{abu} displayed strong

254 correlations with family-level LIFE of 0.86 and 0.96 (Fig. 1, $p < 0.001$) for the original dataset and

255 0.80 and 0.95 for the extended dataset ($p < 0.001$). The random selection of taxa for the family level

256 Flow-T resulted in a median correlation of 0.93 (0.87 – 0.96) for Flow-T_{abu} and of 0.80 (0.62 – 0.89)

257 for Flow-T_{pa}. The reduced version of Flow-T behaved similarly to the full version while the weighted

258 version displayed lower correlation coefficients (Table 3, appendix S5). The correlation between

259 species level LIFE and Flow-T derived using the mixed taxonomic level of presence and log-

260 abundance data were 0.82 and 0.92 respectively (appendix S6 for full results).

261

262 *3.2 Dataset 2, Cyprus: Comparison and performance at different spatial scale*

263 A total of 624,958 organisms, belonging to 103 families and 105 taxa were recorded. The number of

264 families allocated a score was 70 (68%) for LIFE and 97 (94%) for Flow-T. Most of the mismatches

265 for the LIFE index were due to absence of specific dipteran families, some of which are not present

266 in the UK, or excluded from LIFE owing to a lack of necessary knowledge (e.g., Athericidae,

267 Blephariceridae and Chironomidae) and oligochaete families, and included rarely occurring families

268 (e.g., Euphaeidae, Bibionidae and Melanopsidae). LIFE displayed a strong correlation with Flow-T_{abu}

269 ($r = 0.72$) but not with Flow-T_{pa} ($r = 0.46$). The correlation between both versions of Flow-T

270 (abundance-based and presence-absence) was 0.80. The random selection of taxa for family level

271 Flow-T resulted in a median correlation of 0.55 (0.17 – 0.82) for Flow-T$_{abu}$ and of 0.30 (-0.01 – 0.64)

272 for Flow-T$_{pa}$ with the family-level LIFE score.

273 LIFE, Flow-T$_{pa}$ and Flow-T$_{abu}$ scores based on abundance data were higher for riffles than for pools

274 (Fig. 2). The difference between riffles and pools was most marked when using both Flow-T

275 approaches. The R^2 explained by mesohabitat was low for the LIFE index (median 0.06, Bayesian

276 credible intervals = 0.04 – 0.08) but was higher for Flow-T$_{pa}$ (0.17, 0.14 – 0.19) and Flow-T$_{abu}$ (0.15,

277 0.12 – 0.17), respectively. Similarly, the R^2 explained for both mesohabitat and random effects was

278 0.70 (0.65 – 0.74), 0.66 (0.62 – 0.70) and 0.71 (0.67 – 0.75) for LIFE, Flow-T$_{pa}$ and Flow-T$_{abu}$,

279 respectively.

280

281 *3.3 Dataset 3, Italy: Comparison and performance at different spatial scale*

282 A total of 32,426 organisms from 64 families and 106 taxa were identified within the Italian dataset.

283 The number of families allocated a score was 45 (70%) for LIFE, with 64 (100%) and 106 (100%)

284 allocated for the family and mixed taxonomy-level Flow-T, respectively. The reduced number of taxa

285 used in the LIFE index was due to the absence of specific dipterans and oligochaetes and because of

286 the absence of the family Oligoneuriidae in the UK. The most frequently occurring mesohabitat was

287 riffle (n = 59), followed by glide (48), pool (35), isolated pool (22) and backwater (16). Median flow

288 velocity was 0.10 ms^{-1} and ranged from 0 ms^{-1} to 1.68 ms^{-1}.

289 Flow-T$_{pa}$ and Flow-T$_{abu}$ displayed a correlation with family-level LIFE of 0.73 and 0.90 for family

290 level data and a correlation of 0.79 and 0.82 for mixed- level taxonomy. The random selection of taxa

291 for the family level Flow-T resulted in a median correlation of 0.81 (0.60 – 0.91) for Flow-T$_{abu}$ and

292 0.48 (-0.28 – 0.83) for Flow-T$_{pa}$.

293 LIFE and both versions of Flow-T were able to differentiate the two main mesohabitats in rivers

294 displaying significantly higher values in riffles than in pools (details in appendix S7) with the same

response for both Flow-T approaches. Similarly, LIFE, Flow-T_{abu} and flow-T_{pa} displayed consistent patterns when compared to flow velocity and reflected the lentic-lotic gradient between the 5 mesohabitats (from isolated ponds to riffles, Fig. 3). There was a clearer distinction between glides and the slow flowing and lentic habitats (pools, backwaters, isolated ponds) for both Flow-T approaches compared to LIFE scores (appendix S8).

Models considering the interaction between flow velocity and mesohabitat type performed better than the additive model for all the combinations of indices and taxonomic levels tested based on the Δ DIC (Table 4). Indices based on abundance data (LIFE, Flow-T_{abu}) obtained greater Δ DIC than Flow-T_{pa} at the family level suggesting greater differences in the relationship between flow velocity and the indices among different mesohabitats. In contrast, Flow-T based on presence-absence data displayed a higher Δ DIC compared to the abundance-based Flow-T when considering the mixed taxonomic level. Indices calculated at the mixed taxonomic level yielded greater ΔDIC values compared to those calculated at family level. Moreover, mixed-level indices generally performed better than those calculated at the family level, as indicated by the non- or marginally overlapping credible R^2_m intervals.

4. Discussion

To predict the effect of flow variability and alteration on biodiversity and ecosystem functioning is a major goal for the management of lotic ecosystems (Ruhi et al., 2018; Tonkin et al., 2018). The development of indices that can be applied across multiple geographic regions or globally would be ideal (Poff, 2018), but challenging because of the high number of macroinvertebrate taxa involved and the limited information regarding their flow velocity preferences in some geographical regions. To address this issue we developed an index, Flow-T, based on an extensive dataset of trait flow velocity-preferences proposed by Tachet et al. (2010) for European freshwater fauna. Our analyses demonstrate that the new index can successfully track the effect of flow variation at the both micro

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320 and meso-scale and that it displayed consistent responses in different geographical regions (England

321 - oceanic, Cyprus - hot-summer Mediterranean and Italy – humid sub-tropical) using both presence-

322 absence and abundance data.

323

LIFE and Flow-T comparison

325 We compared and assessed the new trait-based index for riverine macroinvertebrate communities

326 Flow-T with the LIFE index, an established flow assessment tool using 3 different datasets. Both

327 versions of the newly developed Flow-T index (abundance based and presence-absence) displayed

328 consistently high and statistically significant correlations with the LIFE index calculated on the

329 original dataset used to establish the LIFE methodology (UK dataset 1986-1997) and on datasets

330 from different European climatic regions. Stronger correlations were recorded for the abundance-

331 based version of Flow-T (Flow-T$_{abu}$) compared with the method based on presence only data (Flow-

332 T$_{pa}$). The reduced versions of Flow-T (excluding Chironomidae and Oligochaeta) displayed similar

333 responses to versions of Flow-T using all data, while the weighted version (that assigns more

334 weight to specialist taxa) generally performed less well.

335 When focussing on datasets from Italy and Cyprus from which samples had been collected from

336 specific mesohabitats, all three indices were able to discriminate between riffle and pool

337 mesohabitats consistently. However, Flow-T$_{pa}$ and Flow-T$_{abu}$ demonstrated a clearer differentiation

338 compared to LIFE, based on the R^2_m values for the more arid Mediterranean rivers in Cyprus. The

339 indices performed similarly well when analysing a greater gradient of habitats within the Italian

340 dataset (from lentic to lotic). However, both Flow-T approaches demonstrated a greater sensitivity

341 in differentiating between mesohabitats, especially glides and pools, indicating its ability to inform

342 local scale assessments and its potential application within widely applied meso-scale habitat

343 models (e.g., Parasiewicz et al., 2013; Vezza et al., 2017). Within the Italian dataset, the significant

344 interaction between flow velocity and mesohabitat indicated that the association between current

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345 speed and faunal communities can vary within individual mesohabitat types. For instance, areas

346 with low flow velocity within riffles are more likely to be colonized via passive drift from nearby

347 areas upstream with higher flow velocity and may display higher index values than low flow

348 velocity areas of lentic mesohabitats. The results therefore support the documented interactive

349 effects between river flow conditions and local environmental factors (e.g., substratum composition,

350 temperature, dissolved oxygen) on riverine faunal communities (White et al., 2017; Karaouzas et

351 al., 2019).

352 At family level, the better performance of Flow-T compared with LIFE in detecting the effect of

353 meso- and micro-scale flow variable probably reflects the occurrence of taxa that were not

354 originally considered in the LIFE methodology (e.g., Athericidae, Chironomidae and Oligochaeta

355 among others, for the Mediterranean dataset, but also Oligoneuriidae - a mayfly family widely

356 recorded throughout Central and South Europe) and other taxonomic issues (e.g., differences in

357 taxonomic resolution and classification). For example, the number of families receiving a score, and

358 so considered in the final index, was different for LIFE and Flow-T, especially within the Cyprus

359 dataset (70 for LIFE and 97 for Flow-T). This may also partially explain the weaker correlations

360 recorded between LIFE and Flow-T (based on presence/absence data) for the Mediterranean

361 dataset, although the use of abundance data appeared to largely mitigate this issue.

362 The taxonomic resolution considered affected the strength of association of Flow-T for both LIFE

363 and mesohabitat/flow velocity. The taxonomic resolution affected the correlation between LIFE and

364 Flow-T and was sensitive to the random selection of taxa within families, especially for the Cyprus

365 and Italian datasets and for Flow-T$_{pa}$. Sensitivity to random selection of taxa could reflect the

366 relatively poor representation of some eastern European taxa in the TAC database (Usseglio-

367 Polatera et al., 2000b). For the Italian dataset, the mixed taxonomic resolution version of Flow-T

368 (family and genus level) performed better than the family level data, being particularly sensitive

369 when using the presence data for both flow velocity and distinct mesohabitats types. The improved

performance of the mixed taxonomy dataset probably reflects among-genus variability of flow

velocity preferences within some macroinvertebrate multi-genus families such as the mayfly larvae

Baetidae and Heptageniidae, the black fly larvae Simuliidae and the caddisfly larvae Limnephilidae

(Dolédec et al., 2007). The increased sensitivity captured when using the mixed taxonomic

resolution may be important when deriving indices where there may be low overall taxa richness or

when using presence only data. Our results suggest that Flow-T performs best when using that

finest taxonomic resolution available on either presence-absence or abundance data. The optimal

taxonomic resolution should be the one that matches the TAC database, where currently velocity

preferences are reported primarily at genus level.

On the wider application of macroinvertebrates flow preferences

A wider geographical application of Flow-T would require flow velocity preferences being fixed

(non-labile) rather than labile traits. Labile traits respond directly to the environment through local

adaptation or phenotypic plasticity, leading some to question their geographical generality. For

example, Bonada and Dolédec (2017) reported that some genera within the TAC dataset have a

greater prevalence of multivoltine life cycles in Mediterranean basins and of univoltine/semivoltine

life cycles in Scandinavia because of major differences in thermal regimes. In contrast, non-labile

traits are considered fixed in space and time i.e., they are not malleable within the local

environmental setting or over the lifetime of an individual, and are usually constrained by

phylogeny (Poff et al., 2006). Examples of non-labile traits include respiration and locomotion type.

In the European TAC dataset, traits have been assigned using fuzzy coding, which allows

interpolation of trait variability and trait changes alongside the life history (ontogeny) for some

taxonomic groups (e.g., feeding habits of Coleoptera larvae and adults) (Richoux 1994).

Ecological flow preferences (Usseglio-Polatera et al., 2000a) do not appear to be particularly labile

in the current study. The flow velocity preference information summarized in Flow-T does not

behave as a labile functional trait, at least over space (displaying stable patterns across multiple European climatic regions). In addition, a high degree of consistency in taxon hydraulic preferences have been identified for benthic macroinvertebrates (e.g., Belmar et al., 2013), but some exceptions at specific taxonomic levels have also been recognised (Dolédec et al., 2007). An individual taxon can then have a flow velocity optimum/preference (i.e., high association with a specific flow) but that in itself is not a labile/non-labile trait, instead that optima can be influenced by other biological features including body shape, size and mode of respiration or locomotion; representing a mixture of both labile and non-labile features (see Chessman, 2018). Similarly, according to Verberk et al. (2013) the preference of a taxon is not a trait or attribute in itself, but rather the result of how a trait has interacted with environmental conditions. The term "flow velocity preference" has been also discussed in ecohydrology suggesting that it may only be intended as "association" (Lancaster and Downes, 2010). Indeed, the latter was also the approach and terminology used within the original LIFE index (Extence et al., 1999).

Novelty and wider applications

Our results indicate that using the original LIFE index within different climatic regions results in a loss of predictive power in relation to flow variation and mesohabitat types because of the reduced number of taxa that have been assigned a score. The assignment of scores to these taxa would potentially address this issue and would be crucial for the wider applicability and improved performance of the LIFE index beyond the UK. This research also clearly demonstrates how improving the taxonomic resolution from family level to a mixed taxonomic resolution (family and genus) improved the response of Flow-T to both flow velocity and mesohabitat type. Flow-T represents a potentially attractive ecological functional biomonitoring tool because it: i) facilitates trans-national comparisons in flow ecology associations as it is based on a pre-existing western-European taxa trait dataset ; ii) has been shown to perform effectively across a gradient of riverine

17

mesohabitats based on flow characteristics (particularly riffles and pools); iii) it is relatively easy to

derive using abundance and presence/absence data: iv) it can be used when LIFE scores for specific

families are not considered in the original metric, but are present at sites being studied; v) its range

from 0 - 1 (or 0 - 100 if needed) means that it is very easy to interpret. Flow-T is based on a freely

available and regularly updated dataset that has become the gold standard for functional analysis of

macroinvertebrate communities in Europe. The same approach used in this research could be

applied to other biological/ecological traits, leading the way towards the development of a suite of

pressure specific indices. Moreover, unlike many taxonomic-based indices, Flow-T can readily

integrate new fauna within its calculation by using faunal functional information available for

almost 500 invertebrate taxa in the TAC database. This is particularly pertinent considering that

colonisation and spread of new alien species seems likely in the future and is increasingly linked

with the modification of the riverine flow regime (e.g., Comte et al., 2021).

Including species traits in current ecological metrics to achieve broader spatial transferability is still

a challenge in environmental flow management (Poff, 2018). Flow-T overcomes this challenge by

relying on an extensive database of flow velocity traits and identifying the direction of a

mechanistic understanding of flow-ecology relationships, linking hydraulic and hydrological

alterations to variation in macroinvertebrate communities. Potential applications of the newly

developed index include both routine monitoring and predictive modelling across multiple spatial

scales. Flow-T will thus help guide managers in evaluating the effects of flow alterations

consistently and for the first time at a truly international scale.

Author Contributions

AL conceived the idea and designed the methodology; AL, IT, RC provided the raw data; AL and MV analysed the data; AL and SG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

Data are available in the supplementary material.

Declaration of competing interest

The authors have no conflicts of interest to report.

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Table 1 Different versions of the Flow-T index used in this research. The equation of Flow-T$_{sub}$ is the same of Flow-T$_{pa}$ and Flow-T$_{abu}$ for presence-absence and abundance data but excludes Chironomidae and Oligochaeta from the computation. Flow-T$_{tsi}$ flow velocity preferences are weighted by tsi, calculated according to the reported equation. Weighted flow velocity preferences are standardized to sum 1 for each species prior to the calculation of Flow-T$_{tsi}$. The presence-absence equation is reported for Flow-T$_{tsi}$ for illustrative purposes, but the abundance-based equation can also be used.

ACRONYM	MEANING	EQUATION
Flow-T$_{pa}$	Presence-absence data using all the taxa receiving a score.	$Flow - T_{pa} = \dfrac{\sum_i^n (m_i + f_i)}{n} \times 100$
Flow-T$_{abu}$	Abundance data using all the taxa receiving a score.	$Flow - T_{abu} = \dfrac{\sum_i^n \log A_i \times (m_i + f_i)}{\sum_i^n \log A_i} \times 100$
Flow-T$_{sub}$	Excluding Chironomidae and Oligochaeta.	-
Flow-T$_{tsi}$	More weight assigned to flow-specialist taxa.	$Flow - T_{tsi} = \dfrac{\sum_i^n (m_i + f_i) * tsi_i}{n} \times 100; \; tsi = \dfrac{\sum_i^k F_k \times \frac{1}{k}}{1 - \frac{1}{k}}$

n = number of taxa in a sample; Ai = abundance of the ith taxon; mi and fi = "moderate" and "fast" velocity preference classes of the ith taxon according to Tachet et al. (2010); k is the number of flow velocity classes; F the kth scores transformed to relative frequencies for the ith taxon.

Table 2. Dataset used in this work with an indication of the sample size, taxonomic resolution, available metadata and the type of analysis performed on each dataset (N = number of samples).

COUNTRY	TIME PERIOD	N	TAXONOMIC LEVEL	METADATA	ANALYSIS
UK	1986-1997	133	Family, mixed-level	-	Correlation with the LIFE index, Sensitivity of family level index to random selection of finer taxonomic levels
	1986-2019	285			
CYPRUS	2005-2018	872	Family	mesohabitat	Correlation with the LIFE index, Sensitivity of family level index to random selection of finer taxonomic levels, Mixed model of index vs mesohabitat_1
ITALY	2020	180	Family, mixed-level		Correlation with the LIFE index,

	mesohabitat, current velocity	Sensitivity of family level index to random selection of finer taxonomic levels, Mixed model of index vs mesohabitat_1 and mesohabitat_2, Mixed model of index vs mesohabitat*current velocity

[*]mesohabitat_1 refers to the subdivision of mesohabitat in riffles and pools used for biomonitoring in Italy and Cyprus. Mesohabitat_2 refers to the subdivision in mesohabitat according to Belletti et al. (2017), frequently used in habitat modelling.

Table 3 Correlation between LIFE and flow-T index for each dataset and typology (pa = presence-absence, abu = abundance; sub = reduced Flow-Tl, tsi = weighted flow-T). Data are shown for family level information.

Index	UK	Cyprus	Italy
Flow-T$_{pa}$	0.86	0.46	0.73
Flow-T$_{abu}$	0.96	0.72	0.90
Flow-T$_{sub\_pa}$	0.87	0.52	0.82
Flow-T$_{sub\_abu}$	0.96	0.73	0.89
Flow-T$_{tsi\_pa}$	0.54	0.23	0.51
Flow-T$_{tsi\_abu}$	0.86	0.40	0.68

Table 4: R^2 of the mixed model analysis performed using LIFE and the two versions of Flow-T as dependent variables and mesohabitat, velocity and their interaction as independent variables for the Italian dataset. LIFE was calculated at family level while Flow-T at both family and mixed taxonomic level for presence absence (pa) and abundance (abu) data. Both median values and credible intervals are reported, together with the Deviance Information Criteria (DIC) for the models with and without interaction and their differences (Δ DIC). See main text and S2 for further results about Cyprus and other versions of Flow-T.

Index	Taxonomic level	$R2_{m\_median}$	$R2_{m\_ci}$	$R2_{c\_median}$	$R2_{c\_ci}$	$DIC_{interaction}$	$DIC_{additive}$	Δ DIC
LIFE	Family	0.54	0.21-0.63	0.66	0.58-0.86	207.8	222.6	14.8
Flow-T$_{pa}$	Family	0.49	0.42-0.56	0.50	0.42-0.56	1197.5	1205.2	7.7
Flow-T$_{abu}$	Family	0.67	0.61-0.71	0.70	0.65-0.74	1200.9	1218.8	17.9
Flow-T$_{pa}$	Mixed	0.74	0.70-0.77	0.77	0.72-0.79	1294.0	1333.8	39.8
Flow-T$_{abu}$	Mixed	0.74	0.70-0.77	0.76	0.72-0.79	1237.9	1266.1	28.2

FIGURE CAPTION

Fig. 1. Relationship between LIFE values and both Flow-T based on the UK using the original dataset from 1986 to 1997.

Fig. 2. Median effect of mesohabitat in LIFE and both Flow-T indices for the Cyprus dataset. Both standardized (left y-axis) and raw (right y axis) of the indices are displayed to ease the comparison among indices. Bayesian credible intervals are also displayed.

Fig. 3. a) Relationships of the LIFE and Flow-T indices with flow velocity for the Italian dataset (pa = presence-absence, abu = abundance). The trend line was computed with a loess function and the grey band represents the 95% confidence intervals; b) boxplot of the association between LIFE and Flow-T indices and mesohabitats for the same dataset (i_p= isolated pond, b=backwater, p=pool, g=glide, r=riffle). Boxplots report median and interquartile ranges and the whiskers represent the maximum/minimum value of the data that lie within 1.5 times the interquartile range. Box plots with the same letter indicate that no significant difference between groups were recorded according to a model with mesohabitat as a fixed effect and collection site as a random effect. Statistical significance was determined via credible intervals: full details of the analysis are reported in supplementary material 8.