# Sub-basin and temporal variability of macroinvertebrate assemblages in Alpine streams: when and where to sample?

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## ABSTRACT

The seasonal dynamics and spatial variability of macroinvertebrate assemblages in Alpine streams are becoming better understood. However, the implications of this knowledge for indices used in monitoring programs have yet to be fully considered. Establishing the ecological status of such streams using macroinvertebrates may then be difficult if what is expected to be found, and where, varies across small distances between streams at a given altitude, or between years in response to different climatic characteristics. In this paper, we evaluated the degree of spatial variability in

25   macroinvertebrate abundance and diversity for 14 tributaries at similar altitude that feed a 6-km

26   reach of an Alpine stream, and how this spatial variability differs within and between years.

27   Environmental variables and macroinvertebrates were sampled on 10 dates for assessment. The data

28   showed that differences in environmental characteristics over relatively short distances lead to

29   between tributary differences in the "windows of opportunity" and hence when a tributary should be

30   sampled for monitoring purposes. Superimposed on this spatial variability was inter-annual

31   variability linked to climate differences that shifted this "window of opportunity" in time, and thus

32   altered when monitoring was optimal for any one tributary in any one year. If biological indices are

33   not rendered sensitivity to these variations, the results obtained may reflect more natural variability

34   than possible human impacts.

35

36   Aquatic ecology; climate change; spatial variability; bioassessment; ecological index, intermittent

37   streams

38    **INTRODUCTION**

39    It is well established that human activities have modified the instream habitats of many rivers and

40    streams through changing hydrology (Poff et al., 1997), water quality (e.g. temperature, organic

41    matter availability) and river morphology. Such modifications may lead to the degradation of

42    ecological quality (Poff et al., 1997; Hart & Finelli, 1999; Bunn & Arthington, 2002; Poff &

43    Zimmerman, 2010). For this reason, substantial attention has been given to quantifying the extent of

44    stream degradation: (1) to prioritise mitigation measures and improve ecological status; and (2),

45    although less common (Wohl et al., 2015), to determine the success of those measures. For decades,

46    ecological degradation has been expressed in terms of changes in the biological status of streams,

47    and measured using freshwater flora, macrozoobenthos and fish. Benthic macroinvertebrates have

48    been a particular focus (Sandin & Hering 2004; Dohet et al., 2008; Bo et al., 2016). As a result of

49    natural, between-stream variability in flora and fauna, measured biological status also must be

50    compared to a reference state, the latter a measure of what might be expected in a non-, or partially-

51    degraded state. For this reason, stream typologies have been developed that describe what would be

52    expected in a given stream based on reference conditions (e.g. Wright, 1995; Reynoldson et al.,

53    1997; Brown et al., 2003; Clarke et al., 2003; Adriaenssens et al., 2007; Brown et al., 2009; Feio &

54    Poquet, 2011).

55    Reference conditions may be complicated by natural variability in space or time (e.g. Ghetti &

56    Bonazzi, 1977; Rico et al., 1992; Charvet et al., 1998; Sandin & Johnson, 2000; Pinto et al., 2004;

57    Sandin & Hering, 2004; Aroviita et al., 2010). How streams are sampled spatially is important because

58    habitat and associated macroinvertebrate communities can vary within a stream over quite small

59    spatial scales (meters or 10s of meters) (Keizer-Vlek et al., 2012; Leitão et al., 2014; Burgazzi et al.,

60    2017). If characterisation of ecological status is required over larger scales, then sampling protocols

61    should be adapted to the range of stream habitats present in the landscape; e.g., inter-tributary

62    differences in environmental characteristics within a basin (Li et al., 2001). Similarly, the time when

63    samples are obtained can be important. For instance, in some cases (e.g. Lorenz & Clarke, 2006, but

64    see Sánchez-Montoya et al., 2009) season can have a major influence on macroinvertebrate

65    community composition (Armitage et al., 1995; Clarke et al., 2002).

66    These points emphasise that careful attention must be given to the definition of the reference

67    condition, something that may have a major impact on whether or not ecological status is defined as

68    degraded (Raven et al., 2002). In Alpine streams, these issues may be more complicated because of

69    strong environmental gradients that influence macroinvertebrate assemblages. For instance, the

70    balance between kryal, nival and krenal contributions to flow and their variation through time can

71    differ greatly between nearby streams within a basin and macroinvertebrate assemblages may

72    reflect this variability (Milner & Petts, 1994; Ward, 1994; Füreder, 1999; Malard et al., 1999; Ward et

73    al., 1999; Füreder et al., 2001; Brown et al., 2003). This balance has been conceptualized as

74    producing "windows of opportunity", periods when conditions are most optimal for flora and fauna

75    (see Uehlinger et al., 2010) and which may reflect inter-species differences in adaptation to cold

76    environments (Robinson et al., 2001).

77    Environmental conditions can vary inter-annually, altering the timing of these "windows of

78    opportunity" among streams as a result of climatic variability, and thus when sampling should occur.

79    There are few systematic studies of the temporal variation (seasonality) of Alpine stream fauna (e.g.

80    Brown et al., 2006) and little guidance as to when to sample. Annex 1, for example, shows when

81    researchers have typically sampled Alpine streams. There is surprising inconsistency. There are very

82    few studies that have included any winter (December through February) or spring (March through

83    May) sampling (exceptions include Robinson et al., 2001 and Zah et al., 2001). The most popular

84    sampling month is September (e.g. Lods-Crozet et al., 2001; Robinson et al., 2001; Malard et al.,

85    2003; Ilg & Castella, 2006; Brown et al., 2007; Lencioni et al., 2007; Bizzotto et al., 2009; Maiolini et

86    al., 2011). Further, very few studies have measured the same stream in the same month for more

87    than one year (e.g. Lodz-Crozet et al., 2001; Snook and Milner, 2001; Brown et al., 2007).

88    The above discussion indicates the need to think carefully about how to sample (where and when)

89    Alpine stream macroinvertebrates. Such sampling should be the basis of identifying more precisely

90    the reference conditions for such streams; an aspect necessary to evaluate human impacts on

91    mountain stream ecosystems (e.g. flow regulation for hydropower). This study aims to fill this gap by

92    testing two hypotheses. (1) There is substantial variability in macroinvertebrate composition

93    between similar altitude sites in tributaries of the main stem of an Alpine stream, and that this

94    variability is related to tributary characteristics. (2) _There is inter-annual variability in

95    macroinvertebrate composition at the same site as a result of inter-annual climatic variability. The

96    hypotheses were addressed by (1) quantifying the extent of macroinvertebrate community

97    differences between tributaries with different physical characteristics; and (2) by sampling tributaries

98    across all seasons of the year, repeated at least twice in different years at each sample site, to

99    evaluate how community composition changes as a function of time.

100

**MATERIALS AND METHODS**

102    **STUDY SITE**

103    The study was conducted in tributaries of a glacier-fed stream, the Borgne d'Arolla in southwest

104    Switzerland. The Borgne flows in a glacial valley and is fed by a series of both glacial and nival

105    tributaries (Figure 1a). The climate in the area is temperate, with cold, fairly wet winters (Arnold,

106    2005) and warm, relatively dry summers, mainly due to the effects of the southern Alpine divide as a

107    topographic barrier against precipitation from the south and west (Micheletti et al., 2015). Annual

108    precipitation is generally between 900 and 1300 mm and mean annual air temperature at 2000 m

109    was 3.4°C for the period 1991 to 2014 (Micheletti et al., 2015, based on Meteosuisse, 2014). The

110    average monthly temperature and the total monthly precipitation are shown for Evolène

111    (Meteosuisse, 2018), at about the same altitude (1800 m) as the study area and very close (about 8

112    km) (Figure 1b). Glacier-melt, snowmelt water, groundwater and direct precipitation contribute to

113    runoff according to the tributary under consideration (Petts & Bickerton, 1994). The main tributaries

114    (notably those of the Haut Glacier d'Arolla, and Glaciers of Tsijiore Nouve, Pièce, Aiguilles Rouge,

115    Bertol and Douves Blanches) are heavily modified by flow abstraction for hydroelectric power

116    production. Thus, in this study, we focused on those tributaries unaffected by flow abstraction, with

117    only one abstraction-influenced tributary retained.

118    Geologically, the study area is part of the Dent Blanche nappe, gneiss and granite series (Stampfli,

119    2015). The Borgne flows in a glacially-incised valley, fed by steep tributaries that cross morainic and

120    related glacial deposits and rockfalls. Some of the tributaries cross alluvial fans before they enter the

121    Borgne. Vegetation is generally pioneer or secondary successional species (shrubs, larch (*Larex* sp.),

122    Alpine grasses, etc.) (Stampfli, 2015). In this region of the European Alps, the current tree line is

123    between c. 2000 and 2100 m. Above this altitude, to about 2400 m, land use is almost entirely

124    extensive but low intensity summer pasture. Above 2400 m, land cover is mainly weathered debris,

125    with occasional permanent snow packs, rock glaciers or small glaciers. The main differences between

126    the tributaries relate to (1) their altitudinal distribution, (2) geomorphic differences (e.g. in slope), (3)

127    geological differences, and (4) extent of glacial influence. The altitudinal range within the basin

128    contributing to each tributary controls the amount of precipitation, the tributary-integrated depth of

129    accumulated snow and the timing of snow melt, stream temperature and the percentage of each

130    tributary below and at tree line, and hence organic matter production.

131

132    **DATA COLLECTION**

133    A total of 14 tributaries were considered (Figure 1), with a maximum longitudinal distance of 1.5 km

134    between them. Each tributary was sampled at the same altitude, between 1800 and 2000 m. After a

135    winter field visit, we identified 7 tributaries that were permanent (T05, T08, T09, T10, T11, T12, T13

136    in dark blue) and 7 that were temporary (T01, T02, T03, T06, T07, T14, T15 in purple) (Figure 1) and

137    either completely frozen or dry during winter. T01, T02, T06, T07 and T15 are glacial streams (fed by

138    rock glaciers or small glaciers). None of the tributaries has a gauging station along its stream except

139    T04, which has most of its flow extracted for hydropower flow generation and hence no water or

140    instream life; it was not considered in the current study.

141    Sampling stations were located as close as possible to the tributary junction with the Borgne without

142    being influenced by flow and/or sediment transport in the Borgne. A sampling station was defined as

143    corresponding to a representative section of the river whose length is 10 times the mean width of

144    the wetted bed of the stream (OFEV, 2010). Except for periphyton, where samples were taken only

145    on 9-10 August 2016, and parameters that do not vary as a function of time (e.g. altitude and slope),

146    all other parameters were measured on the dates shown in Table 1, thereby providing a

147    comparatively high frequency data set. To help ease of reading, only the months are used to describe

148    sample dates , for example "May" means "data collected on 12-13 May".

149

150    **PHYSICAL MEASURES AND PERIPHYTON SAMPLING**

151    Velocity (m s$^{-1}$), temperature (°C), electrical conductivity (µS cm$^{-1}$), wetted width (m) and water depth

152    (m) were measured inside a predetermined representative reach of each stream. Temperature was

153    measured in the middle of a profile and conductivity was measured as an indicator of dissolved load

154    and hence captures geological differences between tributaries. These parameters were measured

155    using a ProfiLine Cond 3110 multi-parameter sampler. Velocity was measured with an

156    electromagnetic Valeport BFM001 & 002 Open Channel Flow Meter in the middle of each stream at

157    40% of the flow depth, corresponding to the approximate mean velocity within a water column

158    (Richards, 1982). Depth and velocity were measured at 10 equally defined intervals within a study

159    reach. The percentages of coarse (> 64 mm) and fine (< 2 mm) sediment were classified using a

160    standard protocol (OFEV, 2010).

161    Mean slope (%) of each stream was estimated from a SwissTopo© altimetric Digital Elevation Model

162    (DEM) (0.5 m resolution). The altitude of the measuring station and mean altitude of each stream

163    were determined from the DEM. The length of each stream was estimated from the origin of flow

164    (considered as the extremity of the glacier for glacial streams and as the first detectable water source

165    according to the SwissTopo© Swiss Map (1:10'000)) until its junction with the Borgne. With the

166    support of the SwissTopo© Swiss Map (1:10'000), the percentage of forest cover directly connected

167    to each tributary also was calculated. All information was obtained using the GIS software ArcGIS.

168    Periphyton was sampled on 9-10 August 2016 because summer conditions are thought to be less

169    influenced by disturbance in Alpine tributaries (Rott et al., 2006). Collection was based upon

170    quantitative sampling of a predefined area (3.5 cm diameter cylinder) on the upper surface of a stone

171    (Robinson et al., 2001). Five stones in each tributary were selected, chosen to be representative of

172    the heterogeneity of the system. Samples were stored in anti-UV bags on ice. Half of each filter was

173    used for determination of chlorophyll-a by HPLC (Uehlinger, 1991), and the other half was used for

174    determination of periphyton AFDM (Uehlinger, 1991). Lastly, the autotrophic index (AI) was

175    expressed as the ratio of heterotrophic to autotrophic production, based on dividing the AFDW (mg

176    $cm^{-2}$) values by the CHL (mg $cm^{-2}$) values (Wu, 2017).

177

178    **MACROINVERTEBRATE SAMPLING**

179    Macroinvertebrate sampling followed Swiss IBCH guidelines (see OFEV, 2010). The IBCH recognises

180    that altitude will determine when a stream is likely to be suitable for life. It sets temporal windows

181    according to broad altitudinal bands based on the lag of temperature with elevation (0.6°C/100m)

182    and corresponding theoretically to macroinvertebrate life stages. Insect emergence, egg laying and

183    early development typically occur during the summer melt period in non-glacial streams, while glacial

184    stream populations must adapt life cycles to peak flows, high suspended sediment delivery and large

185    temperature variations (Füreder et al., 2001; Saltveit et al., 2001; Brown et al., 2015). A single sample

186    per year is compulsory with an optional window for sampling also proposed (Figure 2), depending on

187    study aims. Hydrologically stable periods must be used and buffer periods are imposed to allow for

188    early or late onset of suitable living conditions due to climatic variability.

189    After sampling a section of river, the organisms, classified by families, are divided into 9 indicator

190    groups (GIs) according to their sensitivity to water quality, where class 9 represents the more

191    sensitive taxa (OFEV, 2010). Based on the indicator group and taxonomic diversity, the index is

192    subsequently projected on a scale from 1 to 20, representing 5 levels (bad, poor, average, good, very

193    good) where 20 indicates the best quality of a stream (OFEV, 2010). Macroinvertebrates were

194    collected using a kick-sampling method with a 25x25 cm kicknet and a net mesh size of 1 mm. The

195    acquisition method depended on the type of substrate. The Swiss IBCH recognises 11 substrates

196    listed in descending order of habitat suitability, where 8 must be selected to allow the collection of

197    almost 95% of the taxa present at a station (Verneaux, 1982; Agence de l'eau, 2000).

198    Because of particular conditions related to the altitude of sample sites, only some habitats were

199    present in the field, with only three substrate types commonly present at > 5% (minimal cover to be

200    considered as representative) and thus selected: large stones (> 250 mm), mixed pebbles (250 mm >

201    Ø > 25 mm) and finer sands (Ø < 2.5 mm), with or without organic matter (according to season). For

202    each substrate category, sampling was conducted for the velocity class where the substrate was

203    most represented, remembering that the higher the width and depth of water, the higher the

204    diversity and surface area of habitats, and the likely the richer the system (Newson & Newson, 2000).

205    A current speed between 0.25 to 0.75 ms$^{-1}$ is an important habitat characteristic as it allows good

206    oxygenation of the environment without transporting material downstream (OFEV, 2010). This

207    methodology was validated by a cantonal specialist in macroinvertebrate surveys. The collected

208    macroinvertebrates of the three samples were combined and roughly sorted into: (1) stoneflies,

209    Plecoptera; (2) caddis flies – Trichoptera; (3) mayflies – Ephemeroptera; (4) Diptera; and (5) others,

210    and preserved in 97% ETOH. This produced 140 replicate samples (14 tributaries (with 3

211    undifferentiated/mixed substrates) and 10 months of sampling). The collected animals were

212    identified using a binocular microscope and determined using Tachet et al. (2010) to at least family

213    level.

214

215    **DATA ANALYSIS**

216    Statistical analyses were completed within the *R* system (R Development Core Team, 2017) *ade4* and

217    with the library *ade4TkGUI* (see http://pbil.univ-lyon1.fr/ade4/ and Thioulouse et al., 1997).

218    First, a Principal Component Analysis (PCA) (Eklöv et al., 1999) was performed for the data from

219    August 2016, as this month included periphyton measures as well, to sort tributaries along a gradient

220    of (dis)similarity and to bring out the dominant drivers explaining this gradient (Dray et al., 2014).

221    The method identifies the components that maximise variance (Principal Components or PC).

222    Eigenvalues (variance among the scores) over 1 were kept (Eklöv et al., 1999). Each original variable

223    was correlated with the retained principal components. A *critical r* threshold was calculated to

224    determine significant correlations based on a two-tailed Pearson correlation to obtain 95%

225    significance or confidence in the variables used to explain retained components. This means that

226    each PC (or axis) was defined by the combination of the physical drivers that were significantly

227    brought out by the PCA and which explained the spatial repartition of the tributaries along the

228    gradient in question.

229    Second, the biotic measures were standardised. Macroinvertebrates were listed at the family/taxa

230    level to insure comparison at the lowest common level. Following standard protocols in Switzerland

231    (OFEV, 2010), the faunistic indicator group must be represented by at least 3 individuals, and 10 for

232    the taxa of Limnephilidae, Ephemerellidae, Baetidae, Caenidae, Elmidae, Gammaridae,

233    Chironomidae, Asellidae, and Oligochaeta, to take into account the influence of organism drift (OFEV,

234    2010). If this number is not reached, the cell is entered as null, i.e. as containing zero individuals.

235    Respective abundances of each taxon by tributary were then transformed by log(x+1) to avoid

236    extreme spreading and null values. A NMDS (Non-metric MultiDimensional Scaling) was performed

237    to sort the tributaries along a gradient of (dis)similarity according to taxa composition and

238    abundance, i.e. diversity and richness.

239    Third, an ordination was conducted. Here, the values of each tributary along the respective dominant

240    PCA axis are positioned in one, reduced dimension along each principal component; i.e. a coordinate

241    is given to each site along each axis and arranged by similar sites in terms of physical drivers and

242    biotic communities (Goodall, 1954; Pielou, 1984; Ter Braak, 1987; Dray et al., 2014). A coefficient of

243    determination, $R^2$, was determined between the two ordinated axes (ordination of PCA vs ordination

244    of NMDS) to show the relationship between taxa abundance and physical measures among sites

245    (Digby & Kempton, 1987; Dray et al., 2014; Bailly & Carrère, 2015). These analyses provided a general

246    overview of the spatial variability of tributaries within the catchment.

247    Fourth, to consider macroinvertebrate community change over time, the Bray-Curtis Dissimilarity

248    Index was calculated from log(x+1) abundances, and results represented by a dendrogram (Bray &

249    Curtis, 1957; Thioulouse et al., 1997; Legendre & Legendre, 1998). A value of 0 indicates a perfect

250    similarity between two stations (same taxa and abundances), while a value of 1 indicates a null

251    similarity. This index was used to define population patches; i.e. taxa sharing the same habitats

252    present in different tributaries and to compare biological dynamics over time according to:

253    $$BCjk = 1 - \frac{2\sum_{i=1}^{p} \min(N_{ij}, N_{ik})}{\sum_{i=1}^{p}(N_{ij}+N_{ik})} \tag{1}$$

254    With $N_{ij}$ = abundance of a taxa *i* in the sample *j* and $N_{ik}$ = abundance of a taxa *i* in the sample *k* (Bray

255    & Curtis, 1957). Taxa of each tributary in each month were compared from May to October 2016. In

256    addition, a comparison of the data for August and October was performed for 2015/2016. Samples

257    without individuals were deleted to avoid the group of similarity equal to zero. The node level of 6 in

258    the dendrogram was chosen as a relevant level to obtain broadly representative groups/patches of

259    trends displaying group similarities. While all tributary values were compared, a graphical distinction

260    was established to highlight differences between intermittent and permanent streams.

261    Lastly, the Shannon index (H) was calculated:

262                                      $$H = -\sum_{i=1}^{s} p_i \ln p_i$$                                      [2]

263    where $p_i$ is the proportion of individuals of one particular taxon (n) divided by the total number of

264    individuals (N) and $s$ the number of taxa of the sample. By taking into account the number of

265    individuals per taxa, it allowed comparison of sites that included wide variability in taxa and numbers

266    of individuals (Shannon & Weaver, 1949; Peet, 1974). Maximum diversity occurs when the

267    proportion of individuals is the same for all taxa. Taxa with fewer than 3 to 10 individuals were

268    excluded, which represents between 2% and 8% of the initial sample and the elimination of

269    Empididae, Thaumaleidae and Tipulidae taxa. Boxplots (quartiles, median, mean) were used to

270    display biodiversity results.

271    To test the significance of comparing independently the tributaries as two groups (temporary and

272    permanent), a two-way ANOVA (analysis of variance) test was performed. This test allowed

273    comparison of the mean differences between groups along two independent factors (McDonald,

274    2014). The two-way ANOVA test was applied with replication to assess whether there is an

275    interaction between the two independent variables (the groups of tributaries) (McDonald, 2014). If

276    the F statistics (test of the variance of means) is higher that its critical value the null hypothesis can

277    be rejected and the independence of the two groups can be confirmed (see McDonald, 2014 for

278    methodological details).

279

280    **RESULTS**

281    **RELATIONSHIPS BETWEEN PHYSICAL PARAMETERS AND SPECIES ABUNDANCE IN AUGUST 2016**

282    Annex 2 details the measured physical parameters in August 2016 that were used as basis for the

283    PCA. All tributaries had a mean velocity <0.8 ms$^{-1}$, ranging between 0.3 and 0.6 ms$^{-1}$, and linked to

284    local slopes between 2 and 18%, except T07 and T09 where cascades were present. T01, T02 and T03

285    had a mean temperature >9°C, whilst all others were between 5.0 and 7.3°C. Mean basin altitude

286    was between 2020 (T13) and 2410 m (T03). Although sites were all within a 200 m altitude range;

287    there was some association between altitude and parameters such as conductivity. This pattern was

288    because of a weak negative trend in site altitude from upstream to downstream along the 6 km main

289    stem of the stream, corresponding to a small temperature gradient (about 1.3°C), but which was

290    correlated with other variables that have systematic spatial variability such as geology, soil

291    composition, periphyton (autotrophic index) and water origin. Forest cover varied from 0 to 64%,

292    with higher cover found in the downstream part of the catchment, regardless of stream length.

293    Permanent streams were predominantly at lower altitude than temporary ones, except for T14 and

294    T15 (glacier-fed tributaries).

295    The PCA is illustrated in Figure 3. The spatial repartition of the original values (here as vectors)

296    defined axes by the dominant physical parameters that explained them. The three first axes or

297    Principal Components (PC) with eigenvalues >1 were kept, explaining in total almost 70% of the

298    variation in the original data. Table 2 shows the correlations between the original variable and each

299    PC, marked in bold where the scores were above the threshold r value required for $p<0.05$ with two

300    tails. Axis 1 was positively associated with higher velocity and width, whereas coarser bed sediment

301    and lower temperature had high values on this axis. The combination of these characteristics was

302    used as an index of *torrentiality.* Axis 2 was associated with altitude and periphyton characteristics,

303    being negatively correlated with altitude (measuring station and mean) and positively with

304    autotrophic index and forest coverage; it was used as an index of *organic matter availability*. Axis 3

305    represented scale effects, showing the prominence of catchment size and distance from water origin;

306    the term *catchment* was used for this axis. These three axes represent three gradients of

307    (dis)similarity along which the tributaries were positioned in PCA space (Figure 3) and they bring out

308    the dominant physical parameters that distinguish the tributaries.

309    To better visualise these gradients in relation to whether tributaries were temporary or permanent, a

310    coordinate was given to each site along each axis based on the ordinations and displayed along a

311    one-dimensional gradient in Figure 4. Tributaries were quite widely distributed along PC1. However,

312    torrentiality clearly showed association with flow temporality, with more torrential streams likely to

313    be permanent. Distribution of tributaries is more concentrated along PC2. The categorisation

314    temporary/permanent separated organic matter availability less clearly and did not separate the

315    catchment component values. The values in catchment showed that temporary tributaries are of

316    medium length while permanent ones are more variable.

317    Beyond physical drivers, tributaries were compared in terms of their community composition. Annex

318    3 shows the abundance of macroinvertebrate families (log(x+1) transformed values, family level) in

319    each tributary in August 2016. The following taxa were present in at least one stream: Planariidae

320    (Platyhelminthes, Turbellaria), Oligochaeta (Annelida, order level), Hydracarina (Arthropoda,

321    Arachnida), Baetidae - Heptageniidae (Insecta Ephemeroptera), Leuctridae - Nemouridae - Perlodidae

322    (Insecta, Plecoptera), Limnephilidae - Rhyacophilidae (Insecta, Trichoptera), Chironomidae -

323    Limoniidae - Simuliidae (Insecta, Diptera). With fewer than 3 to 10 individuals by family, T03

324    appeared as a stream without macroinvertebrates, while T11 and T12 had, respectively, 11 and 10

325    taxa of the 13 detected taxa in all samples. Insects largely dominated the other tributaries. In

326    particular, Chironomidae, well-adapted to cold conditions and disturbance, were prevalent in most

327    streams. Baetidae, Nemouridae, Perlodidae and Simuliidae were numerous, and Planariidae were

328    mainly present in permanent streams.

329    Tributaries were widely distributed along the axis describing the complexity of communities (Figure

330    5) resulting from the NMDS. This resulting biotic ordination showed a clear separation between

331    permanent and temporary tributaries. Taxa richness and diversity was higher in permanent

332    tributaries than in temporary ones.

333    Tributaries hence can be distinguished according to biotic (Figure 5) and abiotic (Figure 4)

334    characteristics, or both. Figure 6 shows the three PCAs describing physical variables plotted against

335    community complexity. Torrentiality and community complexity were positively associated (Figure

336    6a), but the results showed that this positive association reflected stream permanence. Permanent

337    streams were more likely to be torrential and had more complex communities, whereas temporary

338    streams were less likely to be torrential and had less complex communities.

339    The relation between organic matter availability and biotic communities was less clear (Figure 6b).

340    The higher biotic complexity of permanent streams was evident, but the correlation was less strong.

341    This result indicated a relation between habitat conditions and taxa presence. Some tributaries with

342    low organic matter had a well-developed community complexity, while some tributaries with greater

343    organic matter composition were biologically poor. For example, T11 and T07 were similar in terms

344    of organic matter availability (Figure 4), but had very different community complexity. This result

345    revealed that taxa habitat preferences played a major role among tributaries. Even if

346    geomorphologically relatively similar, T07 possessed only rheophilic taxa (Simuliidae, Planaridae,

347    Nemouridae, Perlodidae and Baetidae), while T11 also had limnophilous taxa (Limnephilidae and

348    Limoniidae).

349    A higher diversity reflected higher community complexity, and organic matter availability was lower

350    at higher altitudes. In addition, organic matter sources may have a greater effect on assemblages in

351    streams at high mountain altitudes near tree-line than at lower altitudes, reflecting a general

352    resource limitation (Zah et al., 2001). At higher elevations, there were fewer deciduous trees, and fir

353    and larch needles were the predominant source of organic matter. At lower altitudes, the percentage

354    of the basin supplying organic matter increases (Annex 2) and the variety of trees are greater.

355    Autotrophic differences were distinct between lower (heterotrophic) streams with variable food

356    sources and upper streams (except T11, which was completely open canopy) that were generally

357    autotrophic (Annex 2). Thus, organic matter differences were less pronounced for low altitude

358    tributaries than for tributaries higher than 1800 m. Lastly, the diversity and richness of

359    macroinvertebrates were not dependent on catchment size (Figure 6c), showing that the distance

360    from the water source was not a dominant factor in community assembly.

361    By considering physical characteristics and biotic complexity in combination, we can say that (1)

362    physical factors can be represented along three primary components of torrentiality, organic matter

363    availability and catchment characteristics; (2) how the tributaries map onto these components,

364    notably torrentiality, relates to conditions of stream intermittence with permanent streams more

365    likely to be torrential (high velocity and width, coarser bed sediment and lower temperature); and (3)

366    permanent streams tend to have more complex communities, provided that habitat conditions are

367    favourable throughout the year.

368

369    **SIMILARITY AND BIOTIC CHANGE THROUGH SEASONS AND YEARS**

370    Temporal variability was assessed using the Bray-Curtis Dissimilarity Index (Equation [1]). Abundance

371    and diversity of macroinvertebrates for the 14 tributaries from May to October 2016 were compared

372    and groups with similar complexity were color-coded. Except for the white group of null data, three

373    main groups were distinguished over the seasons (Figure 7a) and some streams were permanent

374    throughout the period (T01, T07, T08, T09, T10, T11, T12, T13, in blue). The yellow group identified

375    the permanent tributaries T08, T09, T11, T12 and T13 as similar in terms of macroinvertebrate

376    complexity, except for T08 in September and T11 in June. T05, also permanent, contained

377    comparable communities in August and September. Some temporary streams also were classified in

378    this group in spring and autumn: T02 in October, T07 in June and T15 in May. The blue group

379    identified the permanent streams T10, throughout, and T05 in October, and the temporary streams

380    T07 in May and from July to October, T06 in May and September, T14 in August and October, T15 in

381    June and July, and T02 in September and T03 in May. Finally, the red group included the permanent

382    stream T05 from May to July with the temporary streams T01 from June to September, T02 from

383    May to August, T03 in July and September, T06 in June, July and August, T14 in May, June, July and

384    September and T15 in August, September and October. The color groups remained more consistent

385    throughout the months for temporary tributaries than permanent ones.

386    The variation between tributaries in space was high, even higher than the variation over time. Spatial

387    variability can be high even between nearby tributaries: the columns of Figure 7a are ordered from

388    upstream to downstream in terms of tributary connection with the main river channel (see Figure 1).

389    Figure 8 shows how group membership changed between years (2015 versus 2016) for the months of

390    August and October. Tributaries in August 2015 were more similar, shown by the dominance of the

391    green group, but this was not the case in 2016. In August, 6 of the 14 tributaries remained in the

392    green group for both 2015 and 2016, showing a certain stability and similarity of communities in

393    summer. Without considering null samples, October displayed a higher similarity between 2015 and

394    2016 for permanent streams. Temporary streams, by definition, were mainly frozen in 2015 during

395    the transition season due to cold conditions (Figure 1b) that influenced the onset of flow between

396    years. Hence, again, intermittence played a major role in characterising tributary similarity,

397    suggesting temporal variability may be high.

398

399    **BIODIVERSITY PATTERNS OVER THE SEASONS**

400    The richness, abundance and Shannon Index (Equation [2]) of the macroinvertebrate communities

401    were determined for each tributary from May 2016 to October 2016, plus January and March 2017;

402    the respective mean of each calculation by month was added, plus the means for the temporary and

403    permanent streams (Annex 3). Table 3 gives the mean richness, abundance and Shannon Index by

404    month for all tributaries, but also for tributaries classified as permanent or temporary. To illustrate

405    the monthly patterns, Figure 9 shows boxplots of taxa richness, abundance and the Shannon Index,

406    separately for permanent and temporary streams.

407    Based on annex 3, Table 4 indicates the two-way ANOVA results. As the F value is greater than its

408    critical value, there is a significant difference between the permanent tributaries and the temporary

409    tributaries and supports that this is meaningful to compare these two groups of tributaries.

410    Results showed the highest number of families, number of individuals and Shannon Index values

411    were found for permanent streams throughout the year. The highest richness (number of taxa) was

412    found in July and the highest abundance (number of individuals) was found in August, while June and

413    October had the highest mean Shannon Index. Shannon indices for winter were almost as high as

414    summer for permanent tributaries, particularly in March. By focusing only on permanent tributaries,

415    the maximum diversity also occurred in July. Permanent tributaries contained a richer number of

416    families, a higher abundance and a higher Shannon Index throughout the year. Some permanent

417    tributaries (T09, T10, T11, T12 and T13) had a higher diversity than temporary tributaries across all

418    seasons (i.e., a large number of taxa and individuals) (Annex 3). Tributaries with high Shannon indices

419    corresponded to permanent streams at lower altitude (T09, T10, T11, T12 and T13). The variation in

420    Shannon index was greater between tributaries in space than between tributaries over time (Annex

421    3). Winter values were not that different to other seasons, flagging the importance of this season for

422    community turn-over.

423

424

425    **DISCUSSION**

426    Physical variables measured at 14 tributaries of the Arolla catchment could be classified along three

427    principal components. The torrentiality of a stream, linked to velocity, width, coarse bed sediment

428    and low temperature, was the first and most important component (Table 2, Figure 4a). The second

429    main driver was organic matter availability, which correlated with altitude, autotrophic index and

430    forest coverage (Table 2, Figure 4b). This observation underlines the need to consider physical

431    characteristic (dis)similarities when comparing geographically-proximal streams at the catchment

432    scale, especially as the components, notably torrentiality were found to impact community

433    complexity. That said, at least some of this association was due to the fact that component scores

434    were also differentiated by stream intermittence. Indeed, there was a general conclusion from the

435    data that permanent streams behave differently to temporary ones. Permanent streams were

436    predominantly torrential in character (Figure 4a), a hydro-morphological characteristic that reduces

437    the possibility of freezing. These streams thus provide more suitable habitat conditions for

438    macroinvertebrates, especially during relatively more stable periods, in particular during early

439    summer between snow melt and ice melt, and in autumn before the decrease in temperatures (see

440    Schütz et al., 2001; Zah & Uehlinger, 2001). Permanent tributaries had more diverse and abundant

441    taxa (Table 3, Figure 9, Annex 4) and a higher community complexity (Figure 5) over the year. Clearly,

442    categorising streams as permanent or temporary is crucial for comparing the ecological status of

443    Alpine waters (see also Cottenie, 2005).

444    Macroinvertebrate assemblages were present throughout the year in these relatively harsh high

445    altitude Alpine tributaries, especially in permanent streams. Pioneer families such as Chironomidae

446    were predominant, but pollution- and disturbance-sensitive taxa such as Perlodidae also were

447    common (Annex 3). This result was probably due to the adaptive traits of many high altitudinal taxa

448    that allow them to inhabit the relatively harsher streams found at high elevation (Zbinden et al.,

449    2008), thus underlining the greater diversity observed in summer (Table 3, Annex 3). Rheophilic taxa,

450    able to live in rapidly-flowing waters, were prevalent in the study tributaries (Annex 2). Limnophilous

451    taxa (Oligochaeta, Limneplilidae, Limoniidae), more common in stagnant waters, were present

452    mostly in August in a few tributaries: T05, T10, T11, T12, T13, T14 (Annex 3). This observation

453    emphasizes the need to define the desired target species in order to determine the most significant

454    tributaries to survey.

455    Macroinvertebrate communities were found to vary in space and time. Some similarities in

456    community complexity were found in mid-summer (June-July, July-August and August-September)

457    (Figure 7). Permanent tributaries were more stable, indicating that they are less influenced by

458    external variability in physical factors than in-stream conditions. Our study also indicated higher taxa

459    richness, abundance and heterogeneity during this period (Annex 4). This contrasts with previous

460    research (e.g. Burgherr & Ward, 2001; Füreder et al., 2001; Robinson et al., 2001; Schütz et al., 2001)

461    that found higher richness and abundance in spring and autumn in high altitude streams. Bray-Curtis

462    dissimilarity indices (Figure 7) suggested that spring and autumn (respectively, May-June and

463    September-October) (Figure 7) had less stable conditions for macroinvertebrates than summer in

464    these tributaries. Hence, inter-annual variability in meteorological conditions (e.g. late onset of

465    spring; early onset of winter) means that either spring or autumn sampling may produce less

466    consistent results among years than summer sampling. This is supported by the greater inter-annual

467    consistency in August than in October (Figure 8).

468    In addition to temporal diversity, spatial diversity is significant. Spread across a maximum

469    longitudinal distance of 1.5 km, the 14 tributaries were heterogeneous in terms of their community

470    complexity (Figure 7). Elevation also played a role. For instance, one group (poor complexity)

471    comprised tributaries T01 to T06 plus T14 and T15, whereas a second group (high complexity)

472    comprised T08 to T13 (except T10) (Figure 7). This latter group of permanent streams had a well-

473    developed abundance and richness of macroinvertebrates, earlier than higher altitude permanent

474    streams (Annex 4). Slightly lower altitude permanent streams also displayed good stability in

475    macroinvertebrate community composition over the summer (Figure 8), whilst composition varied

476    substantially in slightly higher altitude permanent streams (Figure 7, Annex 3). These findings further

477    emphasise the high sensitivity of Alpine streams to small altitudinal gradients.

478    Given the high sensitivity of high altitude streams to spatio-temporal variation, it is perhaps

479    surprising given the number of investigations of Alpine stream macroinvertebrates (Annex 1) that the

480    guidance for sampling remains relatively basic. Even within Europe, countries tend to use their own

481    indices and protocols, despite attempts to adopt a European-standardized method. The AQEM

482    (Integrated assessment system for the ecological quality of streams and rivers throughout Europe

483    using benthic macroinvertebrates) method proposes a framework to assess all types of water bodies

484    in Europe (AQEM Consortium, 2002). However, whilst altitudinally-variable sampling windows are

485    defined in the AQEM scheme, all streams above 800 m are combined and classified into a single

486    window as "Alpine". In Germany, following Schmedtje et al. (2001) and Pottgiesser & Sommerhäuser

487    (2004), ecoregions have been distinguished, but these also have adopted a single window (above 800

488    m) for Alpine streams. In Italy, the older IBE (Extended Biotic Index) was replaced by the

489    Standardisation of River Classifications – Intercalibration Common Metrics (STAR_ICM) after the

490    Water Framework Directive, but no distinction of high-altitude streams was made, except that they

491    should be considered as particularly sensitive (OCDE, 2002). In France, based on the Biotic Index

492    established by Verneaux and Tuffery (1967), the IBGN (Standardized Global Biological Index)

493    conceived in 2000 does not distinguish specific altitudinal bands. Only a note indicates that scores in

494    high altitude streams must be carefully interpreted as they are areas with basically poor diversity,

495    thus outcomes must be contextualized before being analysed (Agence de l'eau, 2000). Finally, in

496    Switzerland, the IBCH (Swiss Biological Index) (OFEV, 2010) has been adopted as a standardized

497    procedure for the collection and the analysis of macrozoobenthos samples at the regional level. Sites

498    above 1800 m are grouped together into a single category, with sampling targeted for June, possibly

499    late May or early July if conditions require it, and an optional sampling period from mid-September

500    to mid-October (figure 2). Using data in the Swiss Hydrological Atlas (Breinlinger et al., 1992), which

501    classifies Switzerland into small river basins, ca. 30% of these basins (by surface area) have a mean

502    basin altitude greater than 1800 m.

503    Although it was not possible to confirm that any one month displayed the best representation for

504    macroinvertebrates in the study catchment, summer seemed to be the best period for

505    macroinvertebrate monitoring (Figure 7), even though summer is commonly assumed as the period

506    for flying adults (i.e., less invertebrates at the larvae stage) (Füreder et al., 2005). Importantly, by

507    sampling during the previously established optimal window for life (June), even with the addition of

508    an optional campaign period of late September, it is possible to miss the optimal sampling period in

509    long-term monitoring. This result is probably the effect of altitude, emphasising the need to sample

510    high altitude monitoring sites (> 1800 m in this region) later in summer. When developing large-scale

511    recommendations, using a threshold like 1800 m may not be wise because altitude is not the only

512    effect on local climate. For instance, there is a marked continental gradient from the western Alps to

513    the eastern Alps in Europe, as well as climatic differences between the northern and southern Alps,

514    both partly independent of altitude. Consequently, the required altitudinal threshold for the optimal

515    sampling period is likely to vary with climatic region as well as annual conditions. Many strategies

516    advocate sampling macroinvertebrates in high altitude streams during late Spring (e.g. the Swiss

517    IBCH) on the basis that it is this period that has the highest and most abundance and richness.

518    However, some research has reported significant macroinvertebrate richness and abundance in

519    winter at high altitudes (Burgherr & Ward, 2001; Füreder et al., 2001; Brown et al., 2015). This might

520    appear to be surprising because it doesn't seem to fit with the observation that colder temperatures

521    negatively impact macroinvertebrate populations (as reflected in population recovery with distance

522    downstream from a glacier margin; Milner and Petts, 1994). However, it can arise in basins which,

523    whether for natural reasons or due to human impacts, summer macroinvertebrate populations are

524    strongly impacted by glacial melt cycles and the associated stream bed disturbance and high

525    suspended sediment loads (Gabbud et al., 2018). High altitude basins may show quite significant

526    differences in the relative importance of kryal, nival and krenal contributions, such as due to the

527     changing relative proportion of glacier melt, over very small distances and sampling needs to be

528     much more sensitive to the physical geography of individual basins and less focused on "simplistic"

529     parameters such as theoretical altitudinal ranges.

530     The defining characteristic to take into account to answer the question of when and where to sample

531     macroinvertebrates in high elevation streams remains a challenge. Indices such as the Swiss IBCH

532     consider only permanent flows as significant (OFEV, 2010) for sufficient representativeness of

533     macroinvertebrates, and for the most part, this finding is confirmed here (Figure 5, Figure 6a, Figure

534     9, Annex 4). In the study catchment, all glacial streams (T01, T02, T06, T07 and T15) were temporary

535     and thus should not be considered as reference systems for permanent streams. This result revealed

536     the likely underestimated problem of how to characterise Alpine glacial streams, as central providers

537     of water, sediment and organisms to low elevation waters. Hence, to insure that a stream is

538     permanent, and so to be a candidate as reference to an "undisturbed" state, suitable sensors should

539     be installed for measuring flow presence/absence throughout the year. Regardless, the extent of

540     intermittent streams in Alpine catchments can be high and should not be neglected in understanding

541     the ecology of high elevation drainage networks (Robinson et al. 2015). Shannon Indices also can be

542     quite high in winter in these tributaries (Table 3, Annex 4), showing that despite cold conditions, the

543     winter period allows larval development of macroinvertebrates (Schütz et al., 2001; Sertic Peric et al.,

544     2015). Clearly, a single sampling period is too restrictive to represent the temporal variability of

545     macroinvertebrate assemblages in streams. While the season of sampling certainly matters (Füreder

546     et al., 2005; Callanan et al., 2008), our results showed more differences between streams in space

547     than within a stream through time.

548

549     **CONCLUSIONS**

550     In this paper, we established the degree of spatial and temporal variability in macroinvertebrate

551     abundance and diversity for 14 tributaries at similar altitude that feed a 6-km reach of an Alpine

552 glacial stream. We showed that biotic assemblages of temporary and permanent streams were

553 clearly different. Hydrologically less prone to freeze during winter (torrential), permanent streams

554 with continuous water running throughout the year provide more suitable conditions for

555 macroinvertebrates, and macroinvertebrate communities that are more diverse and taxa rich. This

556 result supports the conclusion that only permanent streams should be defined as a reference for

557 "undisturbed" streams (as requested by many indices), but establishing them as candidate streams

558 needs confirmation by winter campaigns or deployment of sensors. Further, intermittent streams

559 should not be ignored but perhaps be grouped as a separate stream class for Alpine streams.

560 Spring and autumn were more likely to vary between years because of climatic conditions, and thus

561 when conditions become suitable for macroinvertebrates also vary inter-annually. This result implies

562 that when to sample likely varies between years to correspond with differences in macroinvertebrate

563 life cycles. Lastly, the variation between adjacent streams at the same altitude (space) was higher

564 than the variation between tributaries over time. Even if sampled at the same time within a year, the

565 reference condition of streams may be very different, thus sampling conditions should focus on

566 sampling more streams in space.

567 It seems clear that "optimal windows of opportunity" for macroinvertebrates, and hence when a

568 tributary should be sampled, cannot be fixed to a given period of time in the year. We showed that

569 this variability differs both within and between years, leading to a need to shift the "window of

570 opportunity" of any one tributary to either earlier or later. Data analysis suggested that even over

571 very short distances, environmental differences in tributary characteristics lead to different

572 macroinvertebrate assemblages. Altitudinal classification of sites did not fully account for

573 environmental differences among streams and probably underestimates the spatio-temporal

574 variability of high elevation streams, as demonstrated through this field-based research. If this

575 spatio-temporal complexity is not taken into account in Alpine streams, there is a risk that the

576 qualification of ecological status reflects natural geographical and temporal variability and not the

577    true ecological status of the streams being sampled. Alpine streams are notably complex and this

578    complexity must be incorporated into long-term monitoring programs to ensure better

579    understanding and better river protection.

580

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809    **TABLES**

810    *Table 1 : Date of sampling in 2015, 2016 and 2017.*

| Year | 2015 | 2016 | 2017 |
|------|------|------|------|
| | | | 30-31 January |
| | | | 28-29 March |
| | | 12-13 May | |
| **Dates and Month of sampling** | | 13-14 June | |
| | | 12-13 July | |
| | 04-05 August | 9-10 August | |
| | | 14-15 September | |
| | 12-13 October | 11-12 October | |

811

812    **Table 2** *Correlation between the principal components of the PCA and original variables; in bold the scores above critical r =*

813    *0.6.*

814

|  |  | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|---|
| Velocity | (m/s) | **0.91** | -0.18 | -0.18 | 0.12 |
| Temperature | (°C) | **-0.73** | -0.38 | -0.39 | -0.06 |
| Conductivity | (µS/cm) | 0.55 | 0.36 | 0.14 | **0.67** |
| Width | (m) | **0.75** | -0.06 | -0.18 | -0.23 |
| Depth | (m) | 0.51 | -0.31 | 0.52 | -0.06 |
| Coarse sediment | (% > 64mm) | **0.87** | -0.16 | 0.06 | -0.25 |
| Fine sediment | (% < 2mm) | **-0.66** | 0.40 | 0.28 | 0.13 |
| Autotrophic index | (-) | -0.15 | **0.66** | 0.52 | -0.36 |
| Slope | (%) | 0.51 | -0.41 | -0.38 | -0.18 |
| Measuring station altitude | (m) | -0.50 | **-0.64** | -0.29 | 0.29 |
| Mean altitude | (m) | -0.30 | **-0.74** | 0.36 | 0.17 |
| Forest coverage | (%) | 0.30 | **0.65** | -0.40 | 0.42 |
| Length | (m) | 0.26 | -0.54 | **0.63** | 0.30 |

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818    ***Table 3*** *Taxa richness (mean number of taxa), abundance (mean number of individuals) and Shannon Index (H mean –*

819    *Heterogeneity; considered only when H ≠ 0) for each month; (a) for all tributaries together; (b) distinguished between*

820    *permanent and temporary streams*

| TOTAL | Mean nb of families | Mean nb of individuals | Shannon Index (H) |
|---|---|---|---|
| May 2016 | 5 | 116 | 1.51 |
| June 2016 | 6 | 135 | 1.53 |
| July 2016 | 7 | 180 | 1.46 |
| August 2016 | 7 | 233 | 1.38 |
| September 2016 | 6 | 189 | 1.28 |
| Octobrer 2016 | 5 | 128 | 1.56 |
| January 207 | 2 | 70 | 1.44 |
| March 2017 | 3 | 69 | 1.55 |
| **PERMANENT** | | | |
| May 2016 | 7 | 176 | 1.48 |
| June 2016 | 7 | 176 | 1.40 |
| July 2016 | 8 | 238 | 1.75 |
| August 2016 | 8 | 350 | 1.66 |
| September 2016 | 7 | 270 | 1.30 |
| Octobrer 2016 | 7 | 207 | 1.65 |
| January 207 | 5 | 140 | 1.03 |
| March 2017 | 5 | 138 | 1.33 |
| **TEMPORARY** | | | |
| May 2016 | 4 | 56 | 0.90 |
| June 2016 | 6 | 95 | 1.23 |
| July 2016 | 5 | 122 | 0.96 |
| August 2016 | 5 | 116 | 0.91 |
| September 2016 | 5 | 108 | 1.07 |
| Octobrer 2016 | 3 | 49 | 0.80 |
| January 207 | 0 | 0 | 0 |
| March 2017 | 0 | 0 | 0 |

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823    *Table 4 Two-way ANOVA test with F value, P value and F critical value (McDonald, 2014)*

| Source of variations | F value | P value | F critical value |
|---|---|---|---|
| Sample | 22.77 | 2.22067E-05 | 4.073 |
| Column | 78.30 | 6.77729E-15 | 3.22 |
| Interaction | 20.983 | 4.80687E-07 | 3.21 |

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827  **FIGURE CAPTIONS**

828  *Figure 1a* Arolla catchment with study sites indicated

829  *Figure 1b* Mean monthly temperature and sum of monthly precipitations for Evolène meteorological station (Meteosuisse,

830  2018) for the period of study

831  *Figure 2* Temporal windows according to broad altitudinal bands; above, compulsory campaign periods (F) and buffer

832  periods (T); below, optional campaign periods (modified from OFEV, 2010

833  *Figure 3* Resulting PCA from measured physical variables; Axis 1 expresses Torrentiality; Axis 2 expresses Organic matter

834  availability; Axis 3 expresses Catchment; Red triangles for permanent tributaries, blue squares for temporary tributaries.

835  *Figure 4* Ordination of the PCA axes: PC1-Torrentiality (top), PC2-Organic matter availability (middle), PC3-Catchment

836  (bottom); Red triangles for permanent and blue squares for temporary tributaries.

837  *Figure 5* Ordination of the taxa PCA according to PC1-log abundance; Red triangles for permanent and blue squares for

838  temporary tributaries.

839  *Figure 6* Correlation between biotic complexity (taxa richness and diversity) and physical measures of (a) PC1-Torrentiality,

840  (b) PC2-Organic matter availability, and (c) Catchment; Red triangles for permanent and blue squares for temporary

841  tributaries.

842  *Figure 7* Similar groups of tributary complexity (same color) from May to October 2016 shown by the Bray-Curtis

843  dissimilarity index; (a) from T01 to T15 from upstream to downstream, (b) according to group similarities; in white are null

844  data (no sampling in May and June in T08; devoid of macroinvertebrates for T01 in May, T03 in June and August, and T06 in

845  October; T01 and T03 were frozen in October). Permanent streams labelled in red and temporary in blue.

846  *Figure 8* Groups of tributary complexity (same color) shown by the Bray-Curtis dissimilarity index; (a) August 2015/2016; (b)

847  October 2015/2016; in white are null data (T01 and T03 frozen in October 2015 and 2016; T06 devoid of macroinvertebrates

848  in October 2016; T08 no sampling in October 2015; T15 frozen in October 2015). Permanent streams in red and temporary in

849  blue.

850  *Figure 9* Boxplot of (a) Taxa richness (mean number of taxa); (b) Abundance (mean number of individuals); (c) Shannon

851  Index (H mean – Heterogeneity; considered only when $H \neq 0$) for each month; Permanent streams in red and temporary in

852  blue; dotted boxplot for winter results

853 **APPENDIX**

854 ***Annex 1*** *Months and altitudinal range of macroinvertebrate streams in Alpine basins with x =sampled, 2x = sampled during*

855 *two consecutive years, xx = sampled two times during the same month; the total represents the number of studies effecting*

856 *samplings at least once during the mentioned month, without the current study (personal communication: Y. Riffault).*

| Study | J | F | M | A | M | J | J | A | S | O | N | D | Altitudinal range of measurement sites |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bizzotto et al. 2009 | | | | | | x | x | | x | x | | | 2000m |
| Brown et al. 2007 | | | | | | 2x | 2xx | 2xx | 2x | | | | 2000m |
| Burgherr and Ward 2001 | | | | | | x | | x | | x | x | | 1760-2150m |
| Ilg and Castella 2006 | | | | | | | | | x | | | | 1755-2610m |
| Lencioni et al. 2007 | | | | | | x | | x | x | | | | 1800-3000m |
| Lods-Crozet et al. 2001 | | | | | | 2x | | 2x | 2x | | | | 1880-2600m |
| Maiolini et al. 2011 | | | | | | x | | x | x | x | x | | (170-)1790-2792m |
| Malard et al. 2003 | | | | | | x | x | x | x | x | | | 1773-2122m |
| Petts and Bickerton 1994 | | | | | | | x | | | | | | 1800-2500m |
| Robinson et al. 2001 | x | x | x | x | x | x | x | x | 2x | x | x | x | 1320-2070m |
| Snook and Milner 2001 | | | | | | x | 2x | 2x | x | | | | 1800-2500m |
| Zah et al. 2001 | | x | | | | | | | x | | | | 1768-2159m |
| This study | x | | x | | x | x | x | xx | x | xx | | | 1800-2000m |
| | | | | | | | | | | | | | |
| *Total* | 2 | 2 | 1 | 1 | 1 | 9 | 6 | 8 | 10 | 5 | 3 | 1 | |

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859    **Annex 2** *Physical characteristics of tributaries used as basis of the PCA; August 2016.*

|  | Velocity (m/s) Veloc | Temperature (°C) Temp | Conductivity (µS/cm) Cond | Width (m) Width | Depth (m) Depth | Coarse sedim. (% >64mm) Bould64 | Fine sedim. (% <2mm) Fine2 | Autotrophic Index AI | Slope (%) Slope | Station altitude (m) AltSta | Mean altitude (m) Altmean | Forest coverage (%) Forest | Length (m) Length |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T01** | 0.343 | 11.8 | 51.3 | 0.7 | 0.02 | 15 | 25 | 5.298 | 12 | 2005 | 2240 | 9.4 | 1117 |
| **T02** | 0.362 | 10.4 | 54.2 | 0.84 | 0.07 | 25 | 40 | 5.89 | 5.6 | 2012 | 2085 | 15.6 | 762 |
| **T03** | 0.332 | 9.3 | 79.5 | 0.9 | 0.04 | 40 | 40 | 2.942 | 3.4 | 2000 | 2410 | 0 | 1420 |
| **T05** | 0.571 | 6 | 278 | 1.44 | 0.12 | 60 | 15 | 8.911 | 7.8 | 1938 | 2268 | 13.7 | 3340 |
| **T06** | 0.382 | 4.6 | 168.2 | 1.3 | 0.11 | 50 | 50 | 17.858 | 10 | 1940 | 2090 | 7.6 | 803 |
| **T07** | 0.493 | 6.6 | 85.5 | 1 | 0.08 | 55 | 25 | 8.339 | 27.9 | 1922 | 2330 | 26 | 2010 |
| **T08** | 0.639 | 5.6 | 282 | 0.85 | 0.05 | 75 | 10 | 9.106 | 12 | 1903 | 2075 | 59 | 1030 |
| **T09** | 0.627 | 6.3 | 169.3 | 2 | 0.04 | 85 | 10 | 3.717 | 35.7 | 1876 | 2050 | 44 | 990 |
| **T10** | 0.464 | 5.5 | 220 | 0.97 | 0.04 | 15 | 60 | 10.48 | 3.2 | 1863 | 2050 | 64 | 721 |
| **T11** | 0.711 | 5.2 | 93 | 1.57 | 0.15 | 80 | 20 | 6.487 | 17.9 | 1854 | 2235 | 0 | 1910 |
| **T12** | 0.292 | 7.3 | 135 | 0.7 | 0.06 | 30 | 60 | 30.551 | 3.1 | 1841 | 2220 | 44 | 1630 |
| **T13** | 0.597 | 6.5 | 132 | 2.3 | 0.09 | 60 | 15 | 15.438 | 3.3 | 1834 | 2020 | 49 | 863 |
| **T14** | 0.361 | 6.8 | 128 | 0.88 | 0.05 | 50 | 30 | 41.23 | 2.9 | 1832 | 2050 | 14 | 1040 |
| **T15** | 0.347 | 5.8 | 121 | 0.75 | 0.03 | 65 | 30 | 21.912 | 10 | 1826 | 2160 | 13 | 1470 |

860

861      **Annex 3** *Taxa (families) present in each tributary expressed with log(x+1) transformed values; August 2016.*

| | Planariidae | Oligochaeta | Hydracarina | Baetidae | Heptageniidae | Leuctridae | Nemouridae | Perlodidae | Limnephilidae | Rhyacophilidae | Chironomidae | Limoniidae | Simuliidae |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T01 | 0.00 | 0.00 | 0.00 | 1.36 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.49 | 0.00 | 0.00 |
| T02 | 0.00 | 0.00 | 0.00 | 2.39 | 0.78 | 0.00 | 1.58 | 0.70 | 0.00 | 0.00 | 1.43 | 0.60 | 0.60 |
| T03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| T05 | 1.54 | 0.00 | 0.00 | 2.13 | 1.30 | 0.00 | 1.58 | 1.30 | 1.34 | 0.00 | 2.03 | 0.78 | 0.95 |
| T06 | 0.00 | 0.00 | 0.00 | 1.91 | 0.60 | 0.00 | 0.60 | 0.70 | 0.00 | 0.00 | 1.36 | 0.00 | 0.90 |
| T07 | 0.70 | 0.00 | 0.00 | 1.23 | 0.00 | 0.00 | 0.70 | 0.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.60 |
| T08 | 1.15 | 0.00 | 0.00 | 1.83 | 0.00 | 0.00 | 1.45 | 1.59 | 0.00 | 0.00 | 1.97 | 0.90 | 0.00 |
| T09 | 1.68 | 0.00 | 0.00 | 1.26 | 0.00 | 0.00 | 1.23 | 0.70 | 0.00 | 0.85 | 2.17 | 0.90 | 1.15 |
| T10 | 1.20 | 0.00 | 0.00 | 0.00 | 0.90 | 0.00 | 1.46 | 0.95 | 1.28 | 0.00 | 0.00 | 0.00 | 1.20 |
| T11 | 1.23 | 0.00 | 0.00 | 1.49 | 1.85 | 1.30 | 2.03 | 1.15 | 1.20 | 0.70 | 2.22 | 1.04 | 1.85 |
| T12 | 1.54 | 1.53 | 0.60 | 1.79 | 0.00 | 0.78 | 0.95 | 1.04 | 1.52 | 0.00 | 2.11 | 1.11 | 0.00 |
| T13 | 1.28 | 0.00 | 0.00 | 2.24 | 0.00 | 0.00 | 2.08 | 1.00 | 0.00 | 0.00 | 2.33 | 0.78 | 2.02 |
| T14 | 0.78 | 1.38 | 0.00 | 1.96 | 0.78 | 0.78 | 1.30 | 0.70 | 0.00 | 0.00 | 0.00 | 0.00 | 1.34 |
| T15 | 0.00 | 0.00 | 0.00 | 1.93 | 0.00 | 0.00 | 1.08 | 0.95 | 0.00 | 0.60 | 1.04 | 0.00 | 0.00 |

862

863  ***Annex 4*** *Taxa richness (mean number of taxa), abundance (mean number of individuals) and Shannon Index (H mean –*

864  *Heterogeneity; considered only when H ≠ 0) for each tributary by month; Permanent streams in red and temporary in blue.*

| | Nb of families | Nb of individuals | Shannon Index (H) | | Nb of families | Nb of individuals | Shannon Index (H) |
|---|---|---|---|---|---|---|---|
| T01_0516 | 0 | 0 | 0.00 | T01_0816 | 3 | 52 | 0.68 |
| T02_0516 | 4 | 69 | 0.90 | T02_0816 | 7 | 321 | 0.87 |
| T03_0516 | 1 | 9 | 0 | T03_0816 | 0 | 0 | 0 |
| T05_0516* | 6 | 172 | 1.32 | T05_0816* | 9 | 381 | 1.76 |
| T06_0516 | 2 | 13 | 0.62 | T06_0816 | 6 | 119 | 1.05 |
| T07_0516 | 4 | 40 | 1.27 | T07_0816 | 5 | 30 | 1.33 |
| T08_0516* | 0 | 0 | 0 | T08_0816* | 6 | 243 | 1.51 |
| T09_0516* | 9 | 181 | 1.83 | T09_0816* | 8 | 257 | 1.38 |
| T10_0516* | 5 | 66 | 1.47 | T10_0816* | 6 | 91 | 1.69 |
| T11_0516* | 9 | 207 | 1.87 | T11_0816* | 11 | 516 | 1.93 |
| T12_0516* | 10 | 215 | 1.99 | T12_0816* | 10 | 325 | 1.80 |
| T13_0516* | 9 | 391 | 1.89 | T13_0816* | 7 | 638 | 1.53 |
| T14_0516 | 6 | 135 | 1.56 | T14_0816 | 8 | 172 | 1.50 |
| T15_0516 | 8 | 125 | 1.93 | T15_0816 | 5 | 116 | 0.95 |
| **Mean_Perm0516** | **7** | **176** | **1.48** | **Mean_Perm0816** | **8** | **350** | **1.66** |
| **Mean_Temp0516** | **4** | **56** | **0.90** | **Mean_Temp0816** | **5** | **116** | **0.91** |
| **Mean_Total0516** | **5** | **116** | **1.51** | **Mean_Total0816** | **7** | **233** | **1.38** |
| T01_0616 | 4 | 64 | 1.23 | T01_0916 | 5 | 48 | 1.38 |
| T02_0616 | 7 | 148 | 1.34 | T02_0916 | 7 | 141 | 1.49 |
| T03_0616 | 0 | 0 | 0 | T03_0916 | 5 | 343 | 0.89 |
| T05_0616* | 8 | 260 | 0.98 | T05_0916* | 9 | 492 | 1.50 |
| T06_0616 | 5 | 51 | 0.97 | T06_0916 | 1 | 6 | 0 |
| T07_0616 | 11 | 173 | 1.88 | T07_0916 | 2 | 25 | 0.67 |
| T08_0616* | 0 | 0 | 0 | T08_0916* | 5 | 53 | 1.39 |
| T09_0616* | 8 | 249 | 1.68 | T09_0916* | 7 | 135 | 1.27 |
| T10_0616* | 6 | 37 | 1.53 | T10_0916* | 4 | 48 | 1.01 |
| T11_0616* | 6 | 77 | 1.56 | T11_0916* | 12 | 349 | 2.10 |
| T12_0616* | 11 | 381 | 2.00 | T12_0916* | 6 | 497 | 0.86 |
| T13_0616* | 9 | 227 | 2.06 | T13_0916* | 5 | 315 | 1.00 |
| T14_0616 | 8 | 115 | 1.82 | T14_0916 | 8 | 92 | 1.53 |
| T15_0616 | 7 | 113 | 1.33 | T15_0916 | 6 | 98 | 1.50 |
| **Mean_Perm0616** | **7** | **176** | **1.40** | **Mean_Perm0916** | **7** | **270** | **1.30** |
| **Mean_Temp0616** | **6** | **95** | **1.23** | **Mean_Temp0916** | **5** | **108** | **1.07** |
| **Mean_Total0616** | **6** | **135** | **1.53** | **Mean_Total0916** | **6** | **189** | **1.28** |
| T01_0716 | 4 | 39 | 0.93 | T01_1016 | 0 | 0 | 0 |
| T02_0716 | 8 | 289 | 1.12 | T02_1016 | 6 | 171 | 1.64 |
| T03_0716 | 1 | 23 | 0 | T03_1016 | 0 | 0 | 0 |
| T05_0716* | 6 | 160 | 1.37 | T05_1016* | 5 | 154 | 1.31 |
| T06_0716 | 6 | 141 | 1.12 | T06_1016 | 0 | 0 | 0 |
| T07_0716 | 5 | 65 | 0.81 | T07_1016 | 4 | 39 | 1.16 |
| T08_0716* | 6 | 263 | 1.55 | T08_1016* | 5 | 310 | 1.46 |
| T09_0716* | 6 | 90 | 1.42 | T09_1016* | 10 | 109 | 2.03 |
| T10_0716* | 9 | 93 | 2.06 | T10_1016* | 6 | 128 | 1.63 |
| T11_0716* | 9 | 154 | 1.94 | T11_1016* | 10 | 364 | 1.86 |
| T12_0716* | 10 | 259 | 2.14 | T12_1016* | 8 | 196 | 1.57 |
| T13_0716* | 10 | 650 | 1.75 | T13_1016* | 7 | 186 | 1.70 |
| T14_0716 | 7 | 99 | 1.68 | T14_1016 | 6 | 75 | 1.46 |
| T15_0716 | 7 | 200 | 1.05 | T15_1016 | 4 | 56 | 1.34 |
| **Mean_Perm0716** | **8** | **238** | **1.75** | **Mean_Perm1016** | **7** | **207** | **1.65** |
| **Mean_Temp0716** | **5** | **122** | **0.96** | **Mean_Temp1016** | **3** | **49** | **0.80** |
| **Mean_Total0716** | **7** | **180** | **1.46** | **Mean_Total1016** | **5** | **128** | **1.56** |

| | Nb of families | Nb of individuals | Shannon Index (H) | | Nb of families | Nb of individuals | Shannon Index (H) |
|---|---|---|---|---|---|---|---|
| T01_0117 | 0 | 0 | 0 | T01_0317 | 0 | 0 | 0 |
| T02_0117 | 0 | 0 | 0 | T02_0317 | 0 | 0 | 0 |
| T03_0117 | 0 | 0 | 0 | T03_0317 | 0 | 0 | 0 |
| T05_0117* | 11 | 563 | 1.75 | T05_0317* | 6 | 259 | 1.71 |
| T06_0117 | 0 | 0 | 0 | T06_0317 | 0 | 0 | 0 |
| T07_0117 | 0 | 0 | 0 | T07_0317 | 0 | 0 | 0 |
| T08_0117* | 3 | 64 | 0.97 | T08_0317* | 4 | 62 | 1.33 |
| T09_0117* | 4 | 34 | 1.30 | T09_0317* | 4 | 39 | 1.29 |
| T10_0117* | 4 | 55 | 1.15 | T10_0317* | 5 | 65 | 1.41 |
| T11_0117* | 0 | 0 | 0 | T11_0317* | 0 | 0 | 0 |
| T12_0117* | 10 | 267 | 2.05 | T12_0317* | 9 | 268 | 1.86 |
| T13_0117* | 0 | 0 | 0 | T13_0317* | 7 | 273 | 1.70 |
| T14_0117 | 0 | 0 | 0 | T14_0317 | 0 | 0 | 0 |
| T15_0117 | 0 | 0 | 0 | T15_0317 | 0 | 0 | 0 |
| **Mean_Perm0117** | **5** | **140** | **1.03** | **Mean_Perm0317** | **5** | **138** | **1.33** |
| **Mean_Temp0117** | **0** | **0** | **0.00** | **Mean_Temp0317** | **0** | **0** | **0.00** |
| **Mean_Total0117** | **2** | **70** | **1.44** | **Mean_Total0317** | **3** | **69** | **1.55** |

865
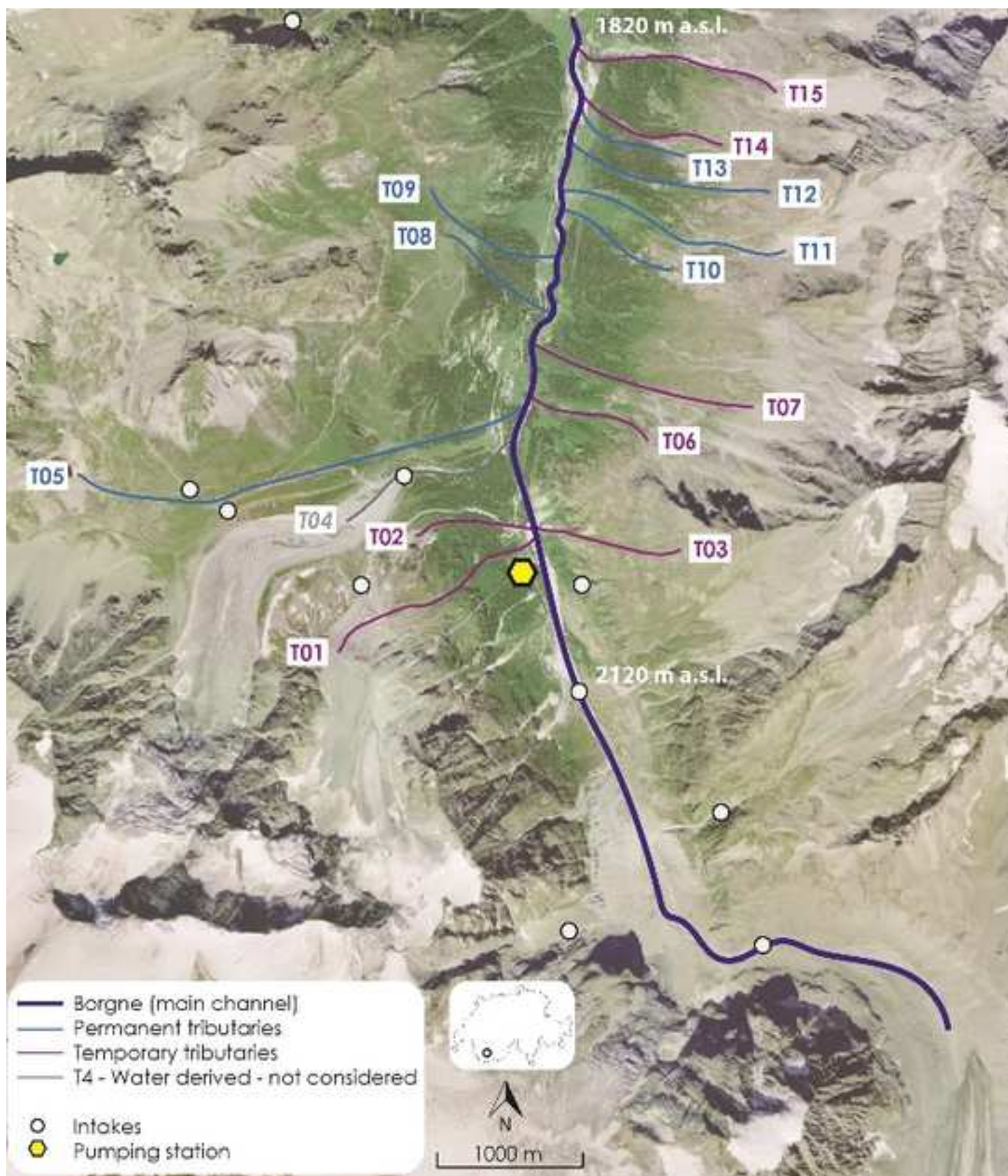
| Month | January | | February | | March | | April | | May | | June | | July | | August | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15days/elevation | 01.–15. | 16.–31. | 01.–15. | 16.–28. | 01.–15. | 16.–31. | 01.–15. | 16.–30. | 01.–15. | 16.–31. | 01.–15. | 16.–30. | 01.–15. | 16.–31. | 01.–15. | 16.–31. |
| 200–600 m | | | | T | F | F | T | | | | | | | | | |
| 601–1000 m | | | | | | T | F | F | T | | | | | | | |
| 1001–1400 m | | | | | | | T | F | F | T | | | | | | |
| 1401–1800 m | | | | | | | | T | F | F | T | | | | | |
| > 1800 m | | | | | | | | | | T | F | F | T | | | |

F = Sampling window     T = Buffer for particular hydrological situations

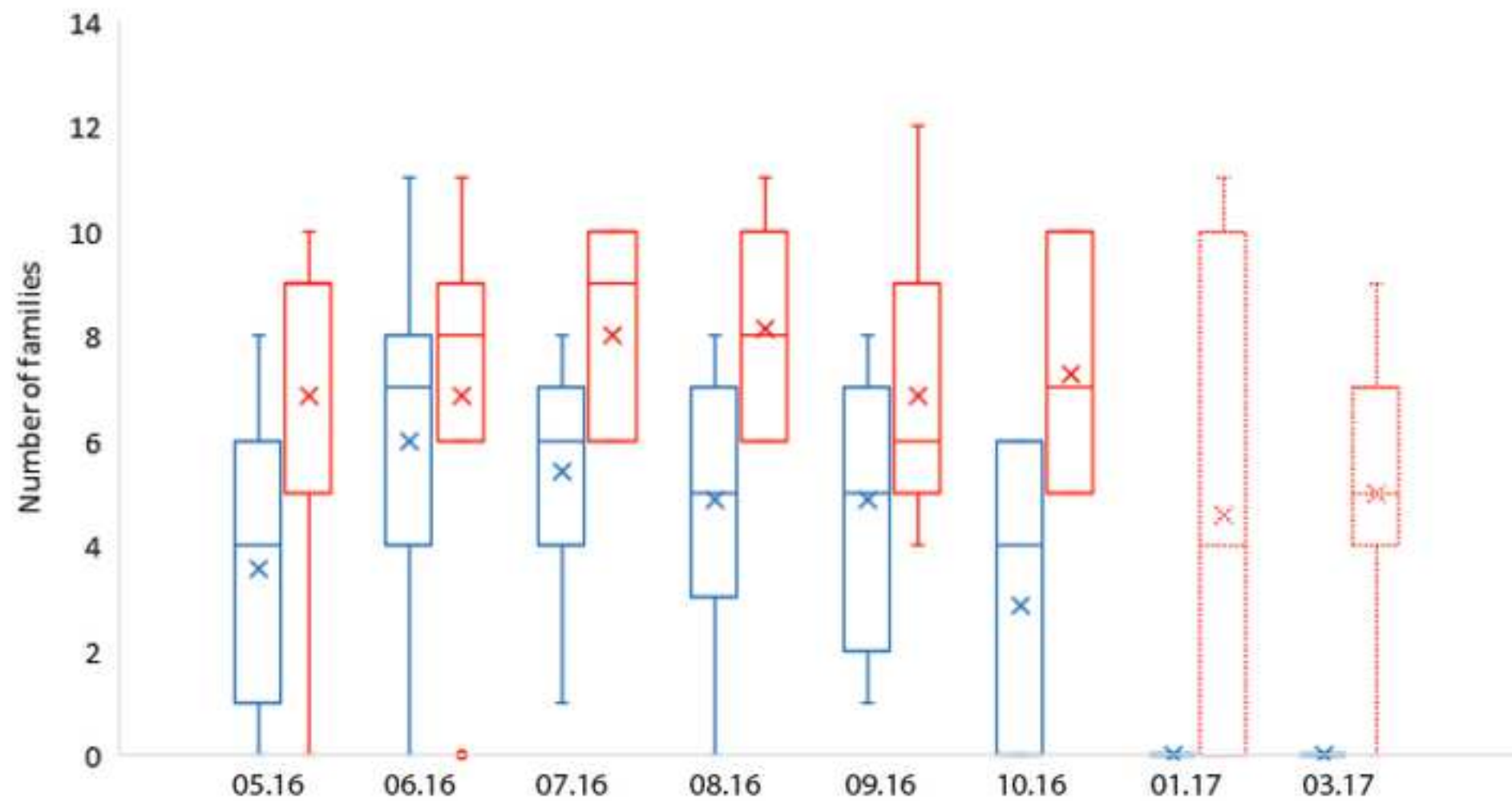| | 200–600 m | 601–1000 m | 1001–1400 m | 1401–1800 m | > 1800 m |
|---|---|---|---|---|---|
| Optional campaign | 16.05.–15.06. | 16.06.–15.07. | 01.07.–30.07. | 16.09.–15.10. | 16.09.– 15.10. |

Complexity of communities
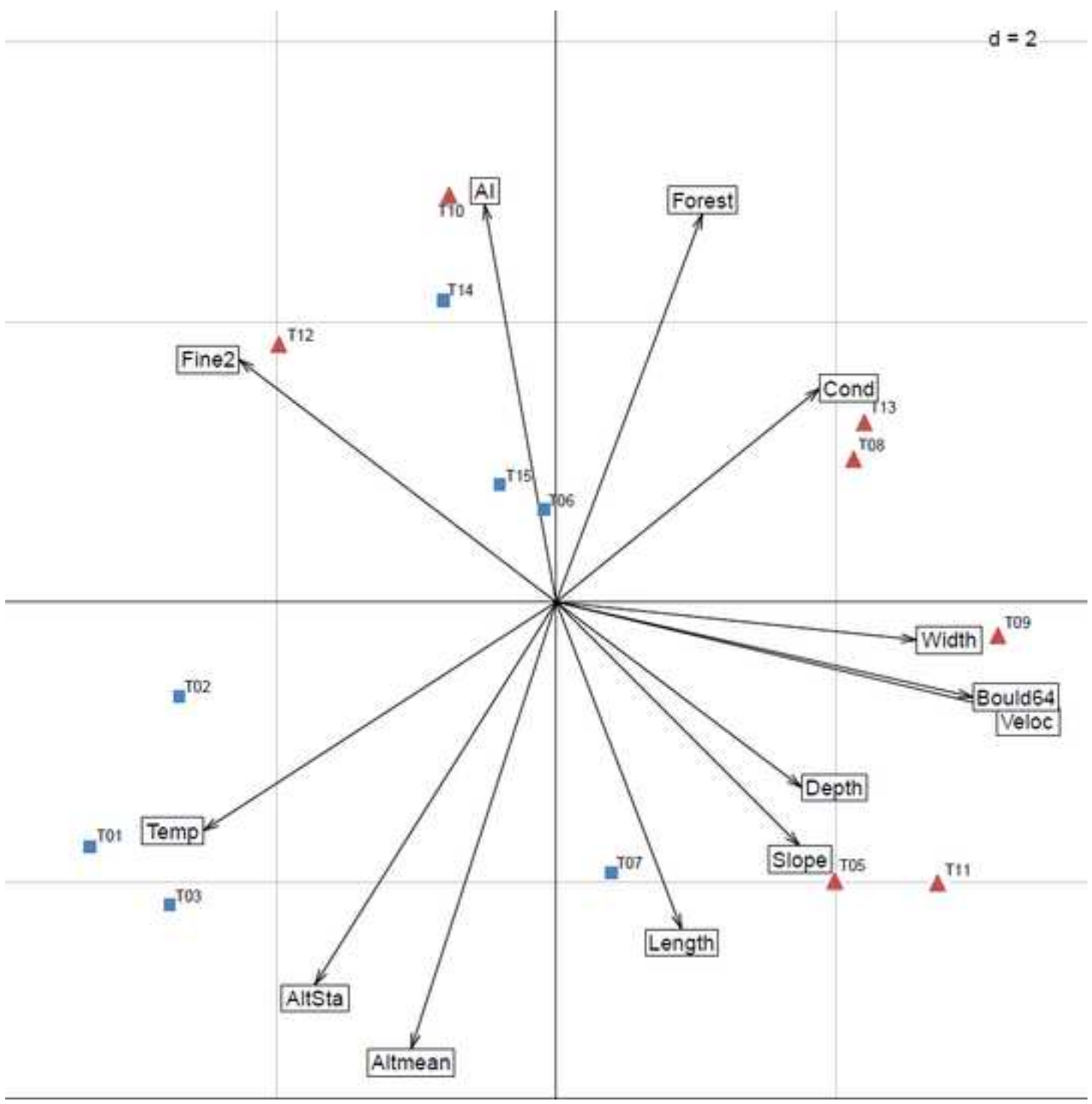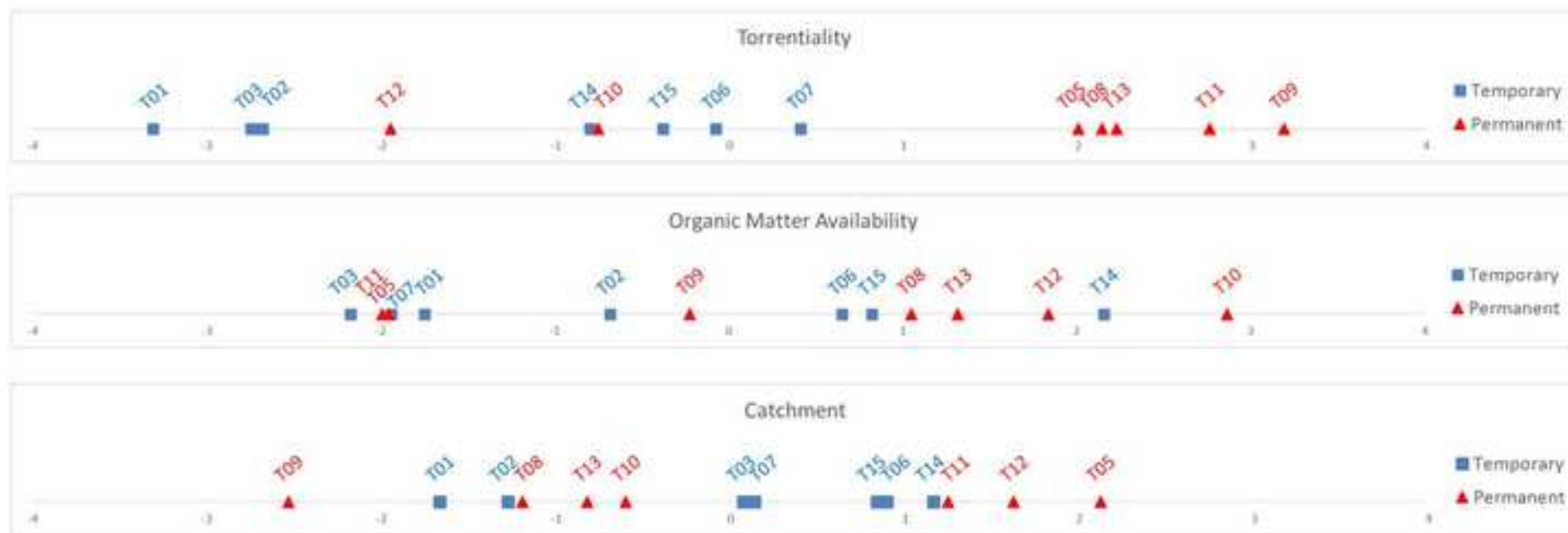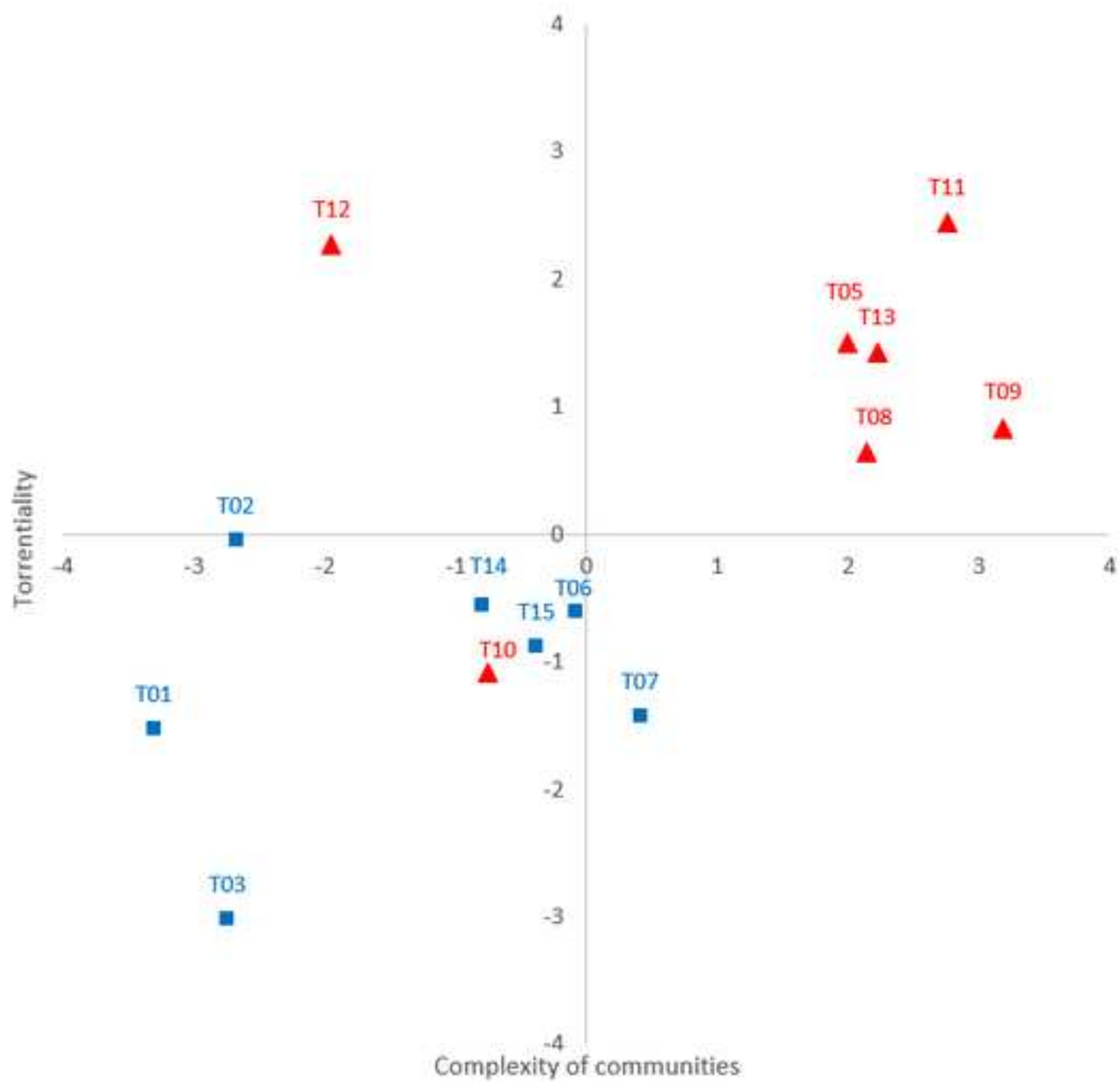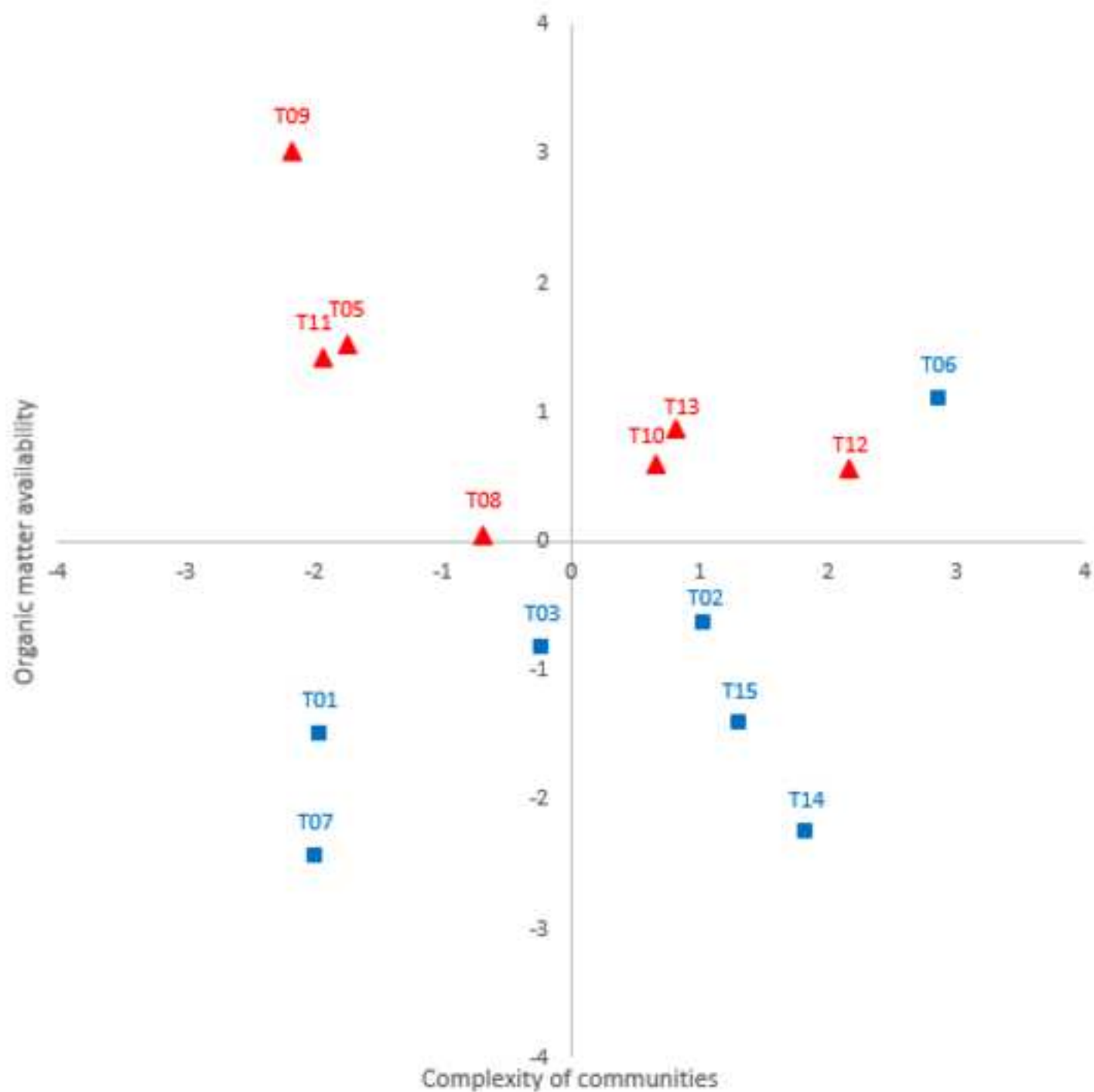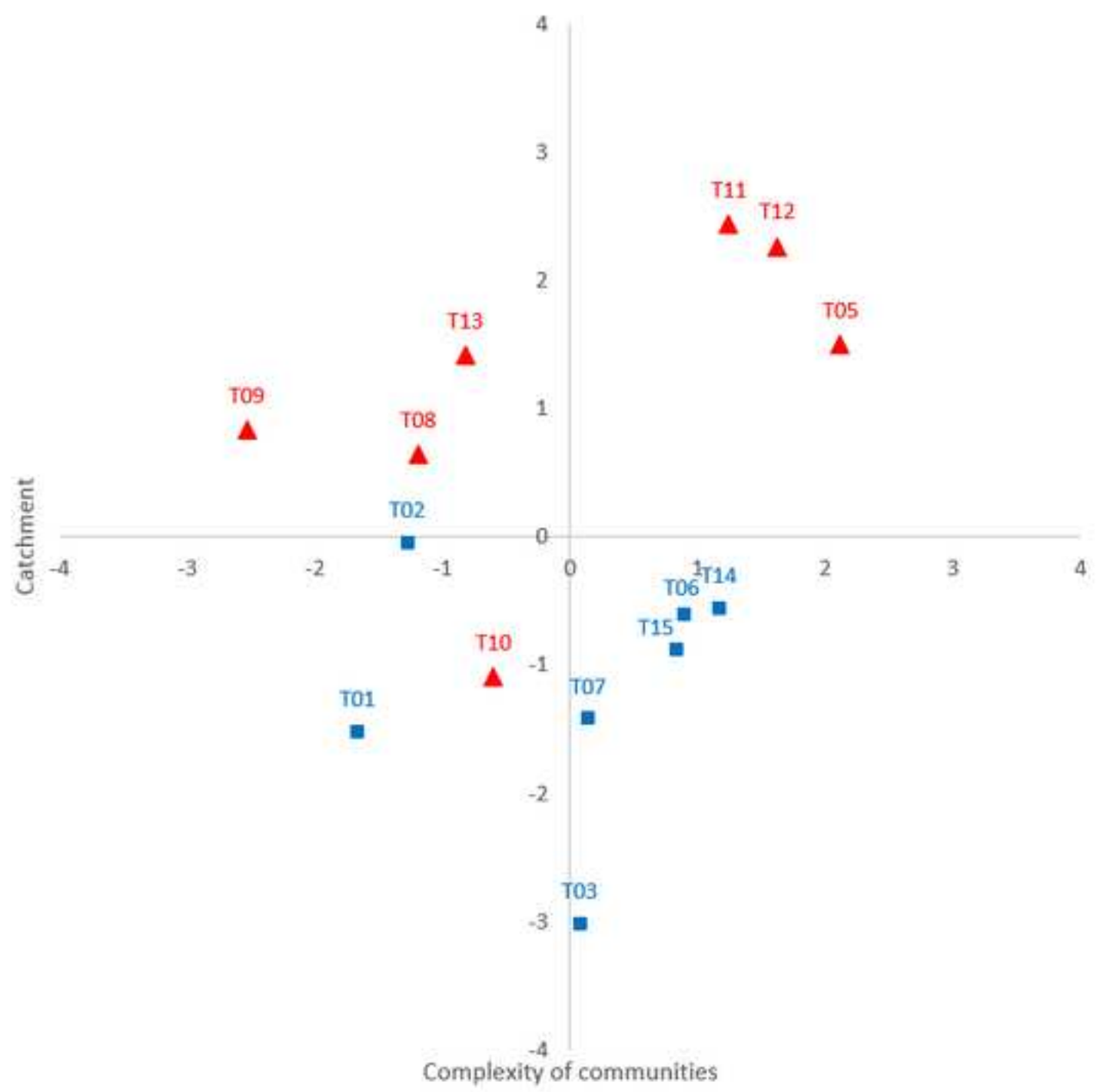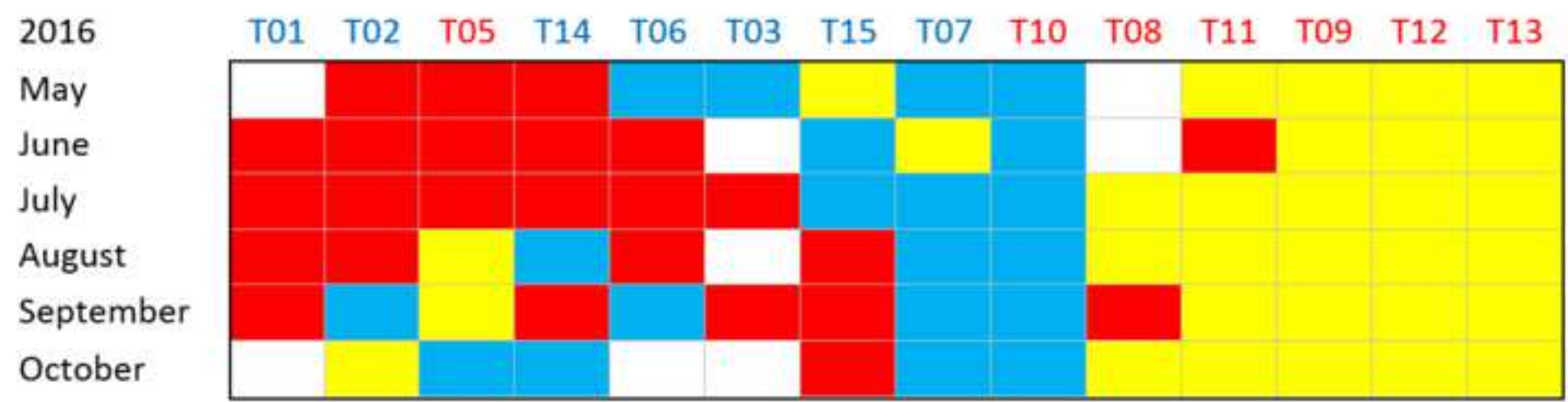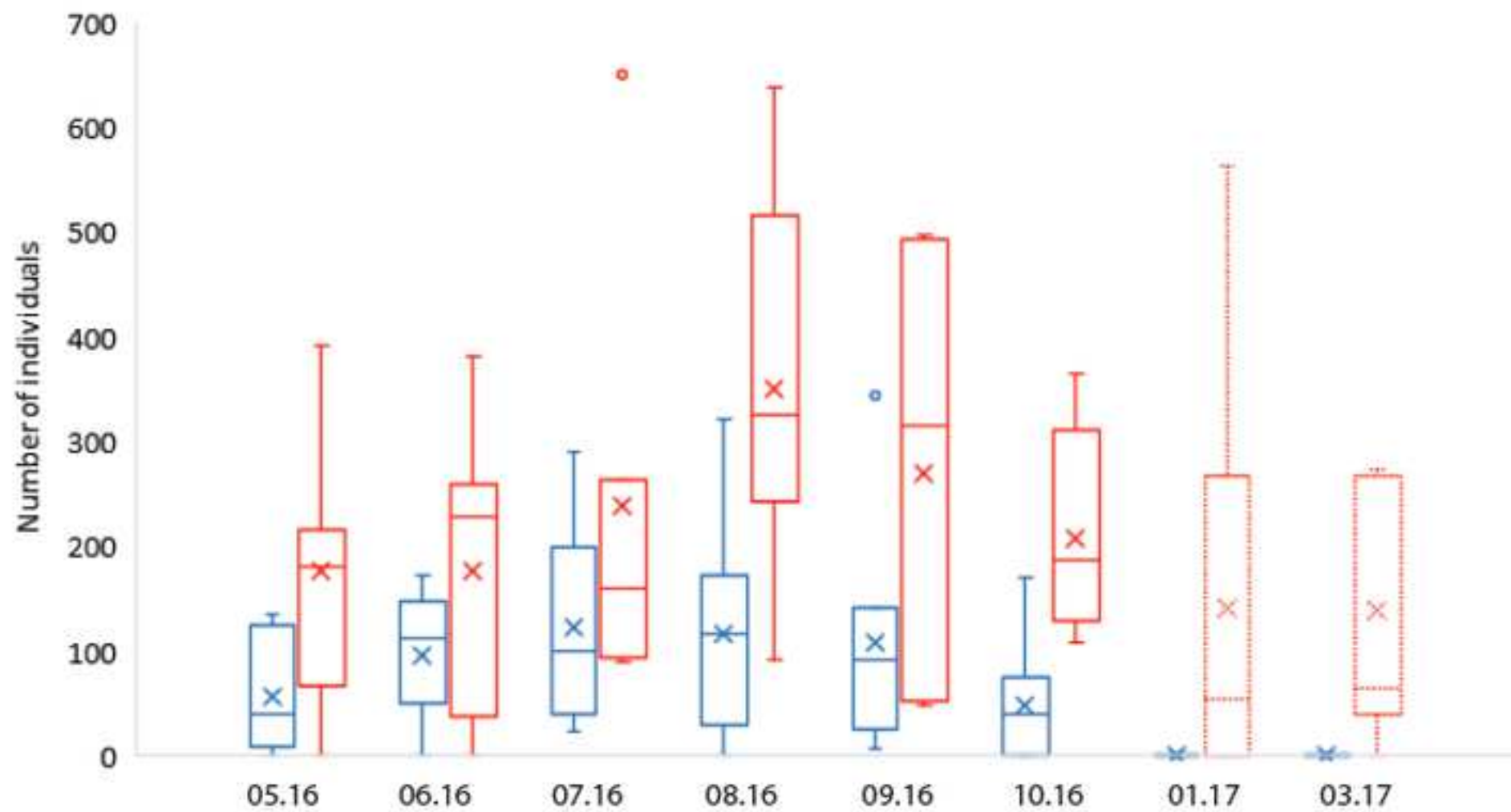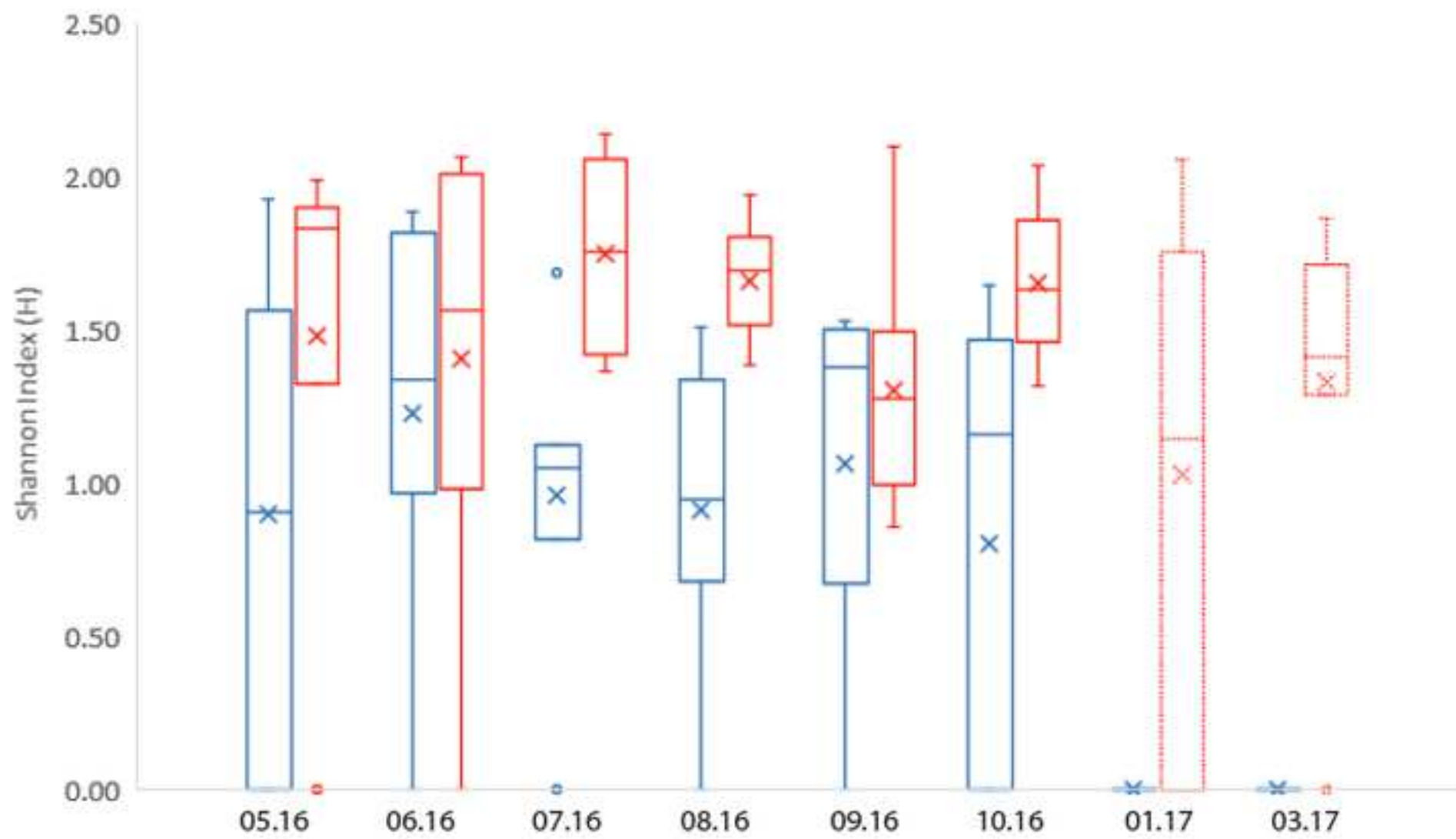
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