Cost-efficient sampling methodologies for lake littoral invertebrates in compliance with the European Water Framework Directive

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Abstract

Lake shores are characterized by a high natural variability, which is increasingly threatened by a multitude of anthropogenic disturbances including morphological alterations to the littoral zone. The European Water Framework Directive (EU WFD) calls for the assessment of lake ecological status by monitoring biological quality elements (BQEs) including benthic macroinvertebrates. To identify cost- and time-efficient sampling strategies for routine lake monitoring, we conducted sampling of littoral invertebrates in 32 lakes located across a European gradient. We compared the efficiency of two sampling methodologies, defined as habitat-specific and pooled composite sampling protocols. Benthic samples were collected from unmodified and morphologically altered shorelines. Variability within macroinvertebrate communities did not differ significantly between sampling protocols across alteration types, lake types and geographical regions. In addition, field composite samples and artificially computed composite samples did not show significant differences in their macroinvertebrate communities, and performed equally well in the calculation of various macroinvertebrate metrics, and in their correlation to a predefined morphological stressor index. We conclude that a benthic invertebrate sampling protocol involving proportional composite sampling represents a time- and cost-efficient method for routine lake monitoring as requested under the EU WFD, and may be applied across various European geographical regions.

Key word: morphological alteration; macroinvertebrates; lake monitoring; method comparison; littoral zone; EU Water Framework Directive.

Introduction

The constant increase of anthropogenic disturbances to freshwater ecosystems is threatening their ecological integrity strongly (Carpenter et al., 2007; Strayer & Findlay, 2010; Solimini & Sandin, 2012). While eutrophication and acidification continue to be major threats to European lakes, human modifications of lakeshore zones have only recently been acknowledged as an increasing pressure on their ecological status (Brauns et al., 2007b; Strayer & Findlay, 2010). Lake shores offer habitat for numerous species, dispersal corridors for aquatic fauna and flora, and a variety of ecosystem services such as opportunities for recreation, flood prevention, dissipation of wave energy and preservation of water quality (O'Connor, 1991; Taniguchi et al., 2003; Gabel et al., 2012). Morphological degradation of lakeshores caused *inter alia* by human settlement or industrial development is not only associated with considerable losses in habitat and physical complexity in the lake littoral (Solimini et al., 2006), but also in the above mentioned ecosystem services. Severe effects on lake biotic communities have been demonstrated in detail for littoral fish assemblages (Jennings et al., 1999; Scheuerell & Schindler, 2004) and recently also for benthic invertebrate communities (Brauns et al., 2007b; Porst et al., 2012, in press; Solimini & Sandin, 2012).

Littoral benthic invertebrates are a major component of lake ecosystems and their functioning (Wetzel, 2001; Vadeboncoeur et al., 2002) and can be found in their highest diversity in the eulittoral zone which is characterized by its high physical complexity and habitat diversity (Taniguchi et al., 2003; Strayer & Findlay, 2010). This natural habitat diversity offers macroinvertebrates a great variety of ecological niches, protection from foraging predators and refuge from physical disturbance such as wind- or ship-induced waves (O'Connor, 1991; Schneider & Winemiller, 2008; Brauns et al., 2011; Gabel et al., 2012). However, shoreline development is typically accompanied by the loss of important littoral habitats such as emergent or submerged macrophytes, submerged tree roots or coarse woody debris caused by clear cutting of littoral and riparian zones of lakes. Consequently, increasing intensities of shoreline development strongly affect littoral macroinvertebrate communities by reducing littoral invertebrate biodiversity and altering macroinvertebrate community structures at highly modified shorelines (Bänziger, 1995; Brauns et al., 2007b; Porst et al., 2012; McGoff et al., 2013; Pilotto et al., in press)

The European Water Framework Directive (EU WFD) (EC, 2000) has acknowledged the influence of increasing morphological alterations on the composition and abundance of biotic communities of European freshwaters. To be in compliance with the requirements of the EU WFD, ecological assessment methods need to be based on biological quality elements (BQEs) including phytoplankton, macrophytes, fish, phytobenthos and benthic invertebrates (EC, 2000). The development of assessment tools for the monitoring of ecological integrity of European lakes has so far focused mainly on quantifying the impacts of eutrophication on biotic communities based on phytoplankton(Phillips. et al., 2011; Søndergaard et al., 2011; Mischke et al., 2012), sublittoral and profundal invertebrate abundances and composition (Saether, 1979; Brodersen & Lindegaard, 1999; Langdon et al., 2006). Impacts of anthropogenic shoreline alterations on lake ecological status yet need to be quantified and adequate monitoring programmes developed (EC, 2000). With life-cycles spanning between several months and years and often sedentary aquatic life stages, benthic macroinvertebrate assemblages potentially reflect changes to their physical, chemical and ecological environment over time (Reice & Wohlenberg, 1993; Pinel-Alloul et al., 1996). Benthic macroinvertebrates generally exhibit a strong dependence on the lake littoral and its diversity and will consequently respond to habitat loss (Jurca et al., 2012; Porst et al., 2012; Solimini & Sandin, 2012; Timm & Möls, 2012). Thus, littoral invertebrates can be expected to form a suitable indicator group for the assessment of morphological pressures to lake ecological status as part of routine monitoring programmes (Porst et al., 2012; Solimini & Sandin, 2012; Urbanič et al., 2012).

While it has been argued that the high natural variability of littoral habitats and associated macroinvertebrate communities make this organism group unsuitable for assessment purposes (Rasmussen, 1988; Harrison & Hildrew, 1998; Moss et al., 2003), habitat stratification has been identified to overcome the problem of inherent variability of the littoral zone of lakes (Tolonen et al., 2001; Weatherhead & James, 2001; Tolonen & Hämäläinen, 2010). For standardised routine monitoring of lakes, time and cost efficiency are important components which can decide on a monitoring program's feasibility. Assessment methods based on littoral macroinvertebrates typically involve time- and cost-intensive processing and identification of macroinvertebrates in the laboratory, while a comparatively small amount of time and associated expenses have to be spent for collection of samples in the field (Ferraro et al., 1989; Haase et al., 2004; Tolonen & Hämäläinen, 2010; Porst et al., 2012). Habitat-specific sampling regimes, frequently applied for lake monitoring in the past, however, generate considerably higher numbers of macroinvertebrate samples compared to a 'pooled' multi-habitat sampling approach. Consequently, habitat-specific sampling involves a much greater working effort and, thus, potentially accounts for higher associated expenses when compared to a multi-habitat sampling programme. While the stratified sampling regime might improve signal precision by reducing variability within macroinvertebrate samples, the collection of pooled composite macroinvertebrate samples could, thus, offer an alternative time- and cost-effective sampling strategy for routine lake monitoring. So far only a limited number of studies focusing on only a few large oligotrophic and mesotrophic lakes in the Central Baltic region (Schreiber & Brauns, 2010; Porst et al., 2012) and one Mediterranean riverine lake (Mastrantuono et al., in press) have compared the efficiency of habitat-specific and composite sampling techniques for routine assessment of lakes. The suitability of the latter method for routine monitoring purposes has, however, not yet been quantified across a gradient of European lake types.

This study aimed at identifying the most suitable sampling methodology for routine monitoring of lake ecological status based on benthic macroinvertebrates in compliance with the requirements of the EU WFD. Based on results from a previous pilot study (Porst et al., 2012) we compared macroinvertebrate samples collected from morphologically altered and unmodified shorelines from a total of 32 lakes located in 3 European countries, with varying trophic status. We tested the adequacy of composite against habitat-specific macroinvertebrate sampling for routine lake monitoring by comparing macroinvertebrate diversity and community structures of unmodified with soft (recreational beaches, grassland) and hard (retaining walls, ripraps) altered shorelines across a trophic and European gradient. Composite sampling comprised pooled proportional sampling of available habitats at a site, while for habitat-specific sampling samples collected from different habitats were kept separate. We hypothesised that pooled composite macroinvertebrate samples would represent a littoral sampling site equally well compared to stratified habitat-specific samples independent of morphological status of a sampling site and are, thus, suitable for routine monitoring of ecological status of European lakes.

Methods

Invertebrate sampling

Benthic invertebrate samples were collected from 32 lakes in three European countries/geographical regions representing a north-south gradient (Map/Figure 1). In Ireland (North-Western Europe - climate: temperate maritime; topography: lowlands) benthic macroinvertebrates were sampled from 9 lakes in April/May 2009, in Germany (Central Europe - climate: temperate continental; topography: north-eastern lowlands) from 8 lakes in

May/April 2010 and in Italy (Southern Europe - Northern Italy: climate: temperate sub-continental; topography: subalpine; Southern Italy: climate: mediterranean; topography: volcanic) from 15 lakes in August-November 2009, with lakes comprising a gradient of total phosphorus (TP range Ireland/North-Western Europe: 8.8 – 80.7 µg/L; TP range Germany/Central Europe: 26.3 – 162.6 µg/L; TP range Italy/Southern Europe: 8 – 130 µg/L). Benthic macroinvertebrate samples were collected from three morphologically differing shoreline types, which were *a priori* classified as 'soft alteration' (recreational beaches or riparian clear-cutting/grassland), 'hard alteration' (retaining walls and ripraps) and unmodified shorelines. In each study lake three unmodified shoreline sites, three sites with soft alterations and three sites with hard alterations were sampled for benthic macroinvertebrates. Sampling sites comprised a shoreline section of minimum 25 m length and extended to the maximum wadable water depth, generally < 1.2 m. At each sampling site, three habitat-specific samples, ideally from sand, stones and macrophytes plus one composite sample were collected. In cases where not all three habitats were present at a sampling site, a second sample of the dominant habitat at this site was collected. In cases where only one habitat was present, i.e. only sand habitats at recreational beaches, three samples from the same habitat were collected. For habitat-specific samples, macroinvertebrates were collected from an area of 1 m for each habitat. Composite sampling comprised the collection of macroinvertebrates from different habitats proportional to habitat availability within each sampling site, generally following the method of the AQEM consortium (AQEM Consortium, 2002; STAR Consortium, 2003). Sampling of single habitats for habitat-specific and composite sampling generally followed the methods described in Brauns et al. (2007b). In short, samples from stones were collected by brushing off attached macroinvertebrates, while macrophyte and sand habitats were sampled using a hand net (500 µm mesh size). While single habitat samples were kept separate for habitat-specific sampling, macroinvertebrate samples from different habitats were subsequently pooled for the composite sampling approach. All macroinvertebrate samples were preserved in ethanol in the field and processed in the laboratory. Macroinvertebrates were identified to species level, whenever possible, except Chironomidae (subfamily), other Diptera (family), and Oligochaeta (class).

Statistical analysis

Based on findings by McGoff et al. (2013) and Miler et al. (2013), which identified macroinvertebrate communities to differ significantly among geographical regions, macroinvertebrate data were divided into geographical regions for statistical analysis. Initially, we tested whether the habitat configuration at the sampling sites systematically differ with alteration type or ecoregion. Therefore, we conducted a permutational analysis of variance (ANOVA) with number of habitats and proportional availability of habitats as the dependent and alteration type and ecoregion as the independent variables. Permutational ANOVA has the advantage over its classical counterpart that normality and homoscedasticity are not required (Gotelli & Ellison, 2004). The level of significance was calculated with 10.000 permutations and the analysis was conducted using the R software (R Core Team, 2013). Non-metric multidimensional scaling (NMDS) was used to display similarities in macroinvertebrate community structures between habitat-specific and composite macroinvertebrate samples within different alteration types in each country (PRIMER® version 6, PRIMER-E Ltd, Ivybridge) (Clarke & Warwick, 2001). A two-way nested analysis of similarities with factors ‘lake’ and ‘habitat’ (ANOSIM, PRIMER® version 6, PRIMER-E Ltd, Ivybridge) tested for significant differences in macroinvertebrate community structures among habitat and composite samples within alteration types in each country using 9999 permutations.

To test whether variability of macroinvertebrate community structures within composite samples was significantly different from variability within habitat-specific samples within different alteration types in each ecoregion/country, the homogeneity of dispersion of individual habitats sampled was tested using permutational analysis of multidimensional dispersion with 9999 permutations (PERMDISP, PRIMER® version 6 with PERMANOVA+, PRIMERE Ltd, Ivybridge) (Anderson et al., 2008). Owing to a low number of replicate samples (n<3) the habitat-specific samples from stones at unmodified shoreline sites and from macrophytes at soft alteration sampling sites in Germany could not be included in the ANOSIM or PERMDISP analyses. PERMDISP, furthermore, tested the adequacy of composite samples for monitoring of lake ecological status by comparing the composite samples collected in the field with artificially computed composite samples again within different alteration types and ecoregion/country. To assess the necessity of proportional sampling for the adequate representation of macroinvertebrate communities at a site, artificial composite samples were generated by accumulating single habitat samples once according to their proportional availability at respective sampling sites (proportional artificial composite sample) and again assigning equal weight to each single habitat sample collected at a site (unproportional artificial composite sample). ANOSIM and PERMDISP subsequently tested for differences in macroinvertebrate communities and associated homogeneities of dispersion among collected and proportional and unproportional artificially generated composite samples across different alteration types in each geographical region. NMDS ordinations, ANOSIM and PERMDISP analyses were based on a Bray-Curtis similarity matrix of arcsine-transformed proportional abundance data to account for differences in sampling methodologies.

Macroinvertebrate communities can be described for assessment purposes based on ‘metrics’. These are defined as summary measures of parts or processes of a biological system that should change in value along a gradient of anthropogenic impact, i.e. in this case morphological alteration. To test the efficiency of the composite sampling approach for lake assessment based on multimetric indices, 10 invertebrate metrics commonly used for morphological assessment purposes in lakes (Gabriels et al., 2010; Timm & Möls, 2012; Miler et al., 2013) were calculated exemplarily based on macroinvertebrate abundances from proportional and unproportional artificial composite and field composite samples (Table 1). The calculated metrics were subsequently correlated separately with a predefined morphological stressor index using Spearman-Rank correlations. The morphological stressor index was calculated as a mean of variables calculated from Lake Habitat Survey (LHS) parameters (Rowan et al., 2006; Rowan et al., 2008). The stressor index contained the variables 'Number of habitats'/'Habitat diversity', 'Total PVI'/'Sum of macrophyte types', 'Sum of vegetation cover types', 'Sum of Coarse Woody Debris/roots/overhanging vegetation' (CWD), 'Pressure index' and 'Natural/Artificial dominant land cover type' and its composition differed between the three geographical regions Germany, Ireland and Italy (Table 2). The development and structure of the morphological stressor index is described in more detail in Miler et al. (2014). Ranges of Spearman-Rank correlation coefficients computed for field composite, proportional and unproportional artificial composite samples were compared using a paired t-test. All metrics were calculated by means of the software program ASTERICS 3.1.1. (www.fliessgewaesserbewertung.de/en) and Spearman-Rank correlations and paired t-tests performed with SAS 9.2 (SAS Institute Inc., Cary, NC, USA.).

Results

Habitat availability

Habitat diversity as well as proportional availability of habitats varied significantly among alteration types (Permutational ANOVA: $F = 9.97$, $p < 0.001$; F 10.33, $p < 0.001$) but not among geographical regions (Permutational ANOVA: $F = 0.96$, $P > 0.05$; $F = 2.48$, $p > 0.05$). Similarly, there were no significant interactions between alteration type and ecoregion for habitat diversity ($F = 1.52$, $p > 0.05$) and proportional habitat availability ($F = 1.25$, $p > 0.05$).

In Germany most dominant habitats found at unmodified sampling sites were sand (n=38; median proportional availability/site 63%, range 14-94%) and macrophytes (n=32; median proportional availability/site 40%, range 30-94%). The only two stone samples collected from unmodified sampling sites in Germany had a median average proportional availability/site of 33% (range 6-60%). Soft alteration sampling sites in Germany were dominated by sand habitats with a median proportional availability of 100% (range 60-100%; n=63) while stones accounted for only 16% (range 10-40%) median proportional availability/site when present (n=7). Macrophyte habitats were found only at 2 soft alteration sites representing, however, 20 % (range 10-30%) median proportional availability/site. Hard alteration sites were characterized again by sand habitats (n=48) in German lakes with median proportional availability of 90 % (range 30-100%). Stone habitats were found at 7 hard alteration sampling sites and accounted for 30 % (range 5-70%) median proportional availability/site. The only 4 macrophyte habitats found at hard alteration sampling sites in German lakes accounted for 22.5% (range 5-30%) median proportional availability/site.

The most dominant habitat with highest median proportional availability/site at unmodified sampling sites in Ireland were stones (n=40; median proportional availability/site 100%, range 33.33-100%). Second highest proportional availability at unmodified sampling sites was found for sand habitats (n=17; median=66.67%, range 42-100%). While a comparatively higher number of macroinvertebrate samples were collected from macrophytes, median proportional availability/site of this habitat accounted for only 33.33% (16%-100%). Number of samples collected from different habitats at soft alteration sampling sites in Irish lakes was relatively equally distributed among habitats (macrophytes n=26; sand n=23; stones n=32) but highest median proportional availability/site was found for stone habitats (median = 100%; range 33.33-100%) followed by sand habitats (median = 94%, range 37-100%) and macrophyte habitats (median = 58.33%, range 12-100%). Hard alteration sampling sites were dominated, however, by stone habitats (n=65) which showed a median proportional availability/site of 100% (range 16-100%). Sand and macrophyte habitats were sampled for macroinvertebrates only from 8 (n=10) and 5 (n=6) sites, respectively and had a median proportional availability/site of 33.33% (range 26-84%) and 33.33% (range 33.33-66.66%), correspondingly at hard alteration sites in Ireland.

In Italy macrophytes were the dominant habitat found at unmodified sampling sites (n=80) with a median proportional availability/site of 60% (range 10-100%). Stone and sand habitats accounted for 40 and 30 macroinvertebrate samples, and median proportional availabilities/sites of 60% (range 5-80%) and 40% (range 5-70%), respectively. Soft alteration sampling sites in Italy were characterised by sand habitats (n=106) with median proportional availability/site of 100% (range 40-100%). Stone and macrophyte habitats were represented by 28 and 7 macroinvertebrate samples, respectively, with comparatively lower median proportional availability/site of 70% (range 10-100%) and 30% (range 20-60%), correspondingly. Highest number of samples collected at hard alteration sites in Italy were stone habitat samples (n=71; median proportional availability/site 80%, range10-100%). Sand habitats accounted for 28 macroinvertebrate samples with median proportional availability/site of 60% (range 30-100%) and macrophytes for 12 macroinvertebrate samples with comparatively low median proportional availability/site of 25% (range 10-100%).

Community composition

NMDS in combination with ANOSIM identified no differences among macroinvertebrate composite and habitat-specific samples at unmodified sampling sites in all countries (Figure 2, Table 3). Macroinvertebrate community structures at soft alteration sites varied between composite and stone habitat samples in Germany, and composite and macrophyte habitat samples in Italy (Table 3). No differences in macroinvertebrate community structures were identified among composite and habitat-specific samples in Ireland at soft alteration sampling sites (Figure 2; Table 3). At hard alteration sites NMDS together with ANOSIM identified significant differences in macroinvertebrate community structures only between composite and stone habitat samples in Germany (Table 3). All other habitat-specific samples did not differ from those collected using the composite sampling approach in all countries (Figure 2; Table 3).

PERMDISP identified no significant differences in homogeneity of spatial dispersion in macroinvertebrate community structures among composite and habitat-specific samples in all alteration types in Germany. In Ireland, homogeneity of dispersion of macroinvertebrate community structures within composite and habitat-specific samples did not vary significantly from each other at all alteration sites with the exception of composite and stone habitat samples at soft alteration sites (PERMDISP, $t = 2.61$, $P_{(perm)} < 0.05$). In Italy differences in variability in community structures were identified only between composite and sand habitat samples at unmodified and hard alteration sampling sites (PERMDISP, $t = 3.42$ and $t = 4.03$, both $P_{(perm)} < 0.05$ for composite/sand at unmodified and soft alteration sites, respectively).

ANOSIM and PERMDISP did not detect significant differences in macroinvertebrate community structures and associated homogeneities of variances between collected and proportional and unproportional artificially generated composite samples, respectively, in all countries and all alteration types (Table 4; PERMDISP, unmodified: $F = 1.305$, $F = 0.152$, $F = 2.788$, hard: $F = 0.1063$, F = 1.98, $F = 0.216$, soft: $F = 1.289$, $F = 2.134$, $F = 0.431$, Germany, Ireland and Italy, respectively, all $p > 0.05$).

Invertebrate metrics calculated from macroinvertebrate abundances of proportional and unproportional artificial composite and field composite samples performed equally well in correlating with the morphological stressor index (Table 1). Ranges in Spearman-Rank correlations did not differ significantly among different composite sample types (Table 1; paired t-tests, Germany: composite – proportional artificial, $t = 1.49$, $p = 0.1795$, composite –

unproportional artificial, $t = 1.60$, $p = 0.1533$; Ireland: composite – proportional artificial, $t = 0.01$, $p = 0.9941$, composite – unproportional artificial, $t = -0.76$, $p = 0.4681$; Italy: composite – proportional artificial, $t = -0.32$, $p = 0.7539$; composite – unproportional artificial, $t = -0.41$, $p = 0.6913$).

Time-effort

Time estimated for the collection and processing of macroinvertebrate samples was assessed in order to compare the efficiency of different sampling methodologies. Collection of German habitat-specific and composite samples in the field accounted for 30 minutes on average each sample. For the sorting of macroinvertebrate habitat-specific samples in the laboratory an experienced worker had to spend 8 h on average per sample. Sorting of German composite macroinvertebrate samples involved 10.3 h on average. Time-effort needed for macroinvertebrate identification, however, was not assessed quantitatively but accounted for the same amount of time on average irrespective of the sampling method used for German samples. In Ireland, field sampling using both sampling protocols also accounted on average for 30 minutes each sample. Sorting of habitat-specific samples in the laboratory involved on average 6 h for an experienced worker while about 10 h had to be spend for sorting of composite samples. Identification of habitat-specific macroinvertebrate samples took on average 4 h and 8 h for composite samples in Ireland. For the collection of macroinvertebrate habitat-specific and composite samples in Italian lakes, an average of 15 minutes was spent per sample in the field. Sorting and identification (no separate estimates available) of macroinvertebrate habitat-specific samples accounted for 7 hours on average each sample while sorting and identification of composite samples took about 11 h per sample. In summary, collection and sorting of macroinvertebrates accounted for 10.8 h using the composite sampling and 25.5 h using the habitat-specific approach in Germany. In Ireland, 18.5 h were spent for collection and processing of macroinvertebrates using the composite sampling and 31.5 h with the habitat-specific sampling approach. For the collection and processing of macroinvertebrate samples in Italy, 11.25 h were needed using the composite and 21.75 h with the habitat-specific sampling approach.

Discussion

This study aimed at identifying the most suitable method for routine monitoring of European lakes as required under the EU WFD. The complexity and heterogeneity of littoral habitats has often led to the recommendation of habitat-specific sampling for lake assessment purposes in order to reduce variability within littoral macroinvertebrate samples and consequently improve signal precision (Tolonen et al., 2001; Weatherhead & James, 2001; Brauns et al., 2007a). In accordance with our hypothesis we were able to show that pooled composite benthic macroinvertebrate samples when collected proportional to availability of individual habitats at a morphologically altered or unmodified sampling site, represent individual sampling locations effectively. We were able to corroborate the results from our pilot study (Porst et al., 2012) and to demonstrate that the results apply for a wide range of lake types across a gradient of morphological alterations and a north-south gradient of European geographical regions/countries. Macroinvertebrate community composition of pooled composite samples did not differ significantly from habitat-specific macroinvertebrate samples across differing shoreline types and countries with only a few minor exceptions. In Germany macroinvertebrate stone habitat samples showed significant differences in community composition when compared with composite samples from soft and hard alteration sites. Stone habitats made up only a comparatively small fraction of macroinvertebrate habitats at modified shorelines (both dominated by sand habitat) and consequently only a minor proportion of collected composite samples in Germany. Littoral invertebrate samples collected from macrophyte habitats at soft alteration sites in Italy also varied from composite samples from respective sampling sites. This once again is a result of the comparatively low proportional availability of this habitat at this alteration type in Italy. Macrophyte samples were collected from only few soft alteration sampling sites and represented the lowest proportional availability when compared to the other two habitats at respective morphologically altered sampling locations in Italy.

PERMDISP analysis generally revealed no significant differences in homogeneity of dispersion in macroinvertebrate community structures from individual habitats compared with those from pooled composite samples collected at morphologically differing shoreline types across geographical regions. This once again supports the suitability of the collection of pooled macroinvertebrate composite samples for routine lake monitoring as requested under the EU WFD and is in accordance with our preliminary study comparing different sampling methodologies at Lake Werbellin, Germany (Porst et al., 2012). In contrast, Schreiber and Brauns (2010) found variability within habitat-specific macroinvertebrate samples to differ considerably from that of pooled composite samples. The latter study, however, did not account for respective proportional availabilities of individual habitats at each macroinvertebrate sampling location giving each habitat sample equal weight in the computation of artificial pooled samples. This once more emphasizes the importance of the proportional sampling approach for the collection of representative littoral macroinvertebrate samples for the assessment of morphological shoreline alterations as applied in our study.

For the assessment of lakes, benthic macroinvertebrate communities collected from single littoral habitats are typically combined into pooled samples in order to obtain a single signal per site. These artificial composite samples also form the basis for the calculation of different macroinvertebrate metrics containing information about certain characteristics or traits of the macroinvertebrate community rather than individual abundances of single species. In our study, proportional and unproportional artificially computed littoral macroinvertebrate composite samples did neither differ significantly in their community structures nor homogeneity of variances in community structures when compared with those of composite samples collected in the field. While variability in macroinvertebrate community structures was generally slightly lower in artificially computed composite samples, the differences were never significant and support the adequacy of the collection of pooled composite macroinvertebrate samples for lake monitoring. Furthermore, proportional composite samples collected in the field proved suitable for use in lake monitoring programmes based on multimetric indices (Hering et al., 2004; Gabriels et al., 2010) for the assessment of lake ecological status. Field composite and proportional and unproportional artificial composite samples performed equally well in the correlation of 10 selected invertebrate metrics typically used for lake morphology assessments with a previously calculated stressor index (Miler et al., 2013). While both artificially computed composite samples showed similar results in the comparison with collected macroinvertebrate composite samples, it should not be concluded that proportional sampling of littoral habitats would not be necessary for obtaining meaningful results in lake assessment programs. In our study habitat proportions in the field generally showed relatively equal distributions among habitats across alteration types and lakes in all countries/geographical regions. We conclude, however, that higher variability in habitat proportions would result in a comparatively less accurate representation of sites using a non-proportional approach as demonstrated in the study by Schreiber and Brauns (2010).

Our study demonstrated the suitability of the proportional composite sampling methodology for regular lake monitoring for the generally dominant littoral habitats sand, stones and macrophytes. While these habitats showed highest proportional availabilities across all littoral sampling sites in all three European countries, other macroinvertebrate habitats such as woody debris or roots could also be considered to be included for monitoring purposes. These habitats, which usually account for a fraction of the area of a sampling site only and thus would make up only a small part of respective composite samples, are known to inhabit rare or sensitive macroinvertebrate taxa (Lorenz et al., 2004; Strayer & Findlay, 2010; Porst et al., 2012). The inclusion of disturbance sensitive taxa is required by the EU WFD and metrics describing the percentage or taxa number of disturbance sensitive taxonomic groups are a central part of many macroinvertebrate based multimetric assessment systems (Hering et al., 2004; Lorenz et al., 2004; Hering et al., 2006; Schartau et al., 2008; Gabriels et al., 2010; Timm & Möls, 2012). Our previous study assessing the suitability of the composite sampling method at Lake Werbellin (Porst et al., 2012) already demonstrated the adequacy of the latter sampling method also for the inclusion of these usually comparatively scarcely represented littoral habitats in contrast with the study by Schreiber and Brauns (2010). We recommend the inclusion of additional habitats only if those habitats cover a minimum of 5% area of the sampling site following the AQEM/STAR method for the assessment of streams using benthic invertebrates (AQEM Consortium, 2002; Timm & Möls, 2012) or if assessment is being carried out for conservation purposes rather than basic quality assessment.

Time- and cost-effectiveness are important factors for the design and implementation of regular lake monitoring programmes. While usually the largest fraction of time needed for assessment purposes using benthic macroinvertebrates is spent on the processing and identification of samples in the laboratory, the collection of macroinvertebrate samples in the field involves far less time and associated expenses (Ferraro et al., 1989; Haase et al., 2004; Tolonen & Hämäläinen, 2010). In our study the collection of benthic samples in the field using either of the two sampling methods accounted for approximately the same time and made up only a comparatively small amount of total time required for sample processing. Sorting and identification of macroinvertebrate samples in the laboratory was found to be more efficient for individual habitat samples. Total time needed for the collection, sorting and identification of benthic macroinvertebrate samples, however, was about twofold higher for collection, sorting and identification of all habitat samples representing a site. Thus, the working-effort required for the stratified habitat-specific sampling method is considerably higher and consequently accounts for undoubtedly higher associated costs when compared to the suggested composite sampling method.

Conclusions

This study demonstrated that pooled macroinvertebrate composite samples when collected proportionally to habitat availability at a littoral sampling site have the potential of being used in routine monitoring programs for the WFD compliant assessment of European lakes with respect to morphological alterations in the lake littoral. We were able to show that proportional composite samples represent both, morphologically altered as well as unmodified shorelines adequately in terms of macroinvertebrate community compositions across a range of lake types and a European gradient while their processing additionally accounts for considerably less time and associated costs. The results of this study emphasize the importance of applying the proportional sampling approach for the assessment of lake ecological status and support its use as a time and cost effective sampling strategy. While our sampling scheme focused on the three dominant habitats present across the European gradient, the inclusion of additional habitats which might account for only a fraction of the sampling site could be considered for the design of lake assessment programmes beyond the purposes of the EU WFD. In case lake littoral zones are sampled for other purposes, as for identifying effective restoration options for lake littoral habitats, or to survey rare and endangered invertebrate species, we recommend habitat-specific sampling, in order to record habitat specificities of target species.

Table 1: Spearman-Rank correlations of metrics calculated from macroinvertebrate abundances of field composite (CO), proportional (CO1) and unproportional artificial composite (CO2) samples with the morphological stressor index. Shown are 10 selected metrics that are typical for morphological assessment methods based on lake invertebrates and their respective Rho- and p-values.

	CO		*CO1*		*CO2*	
Metric	ρ	p	ρ	p	ρ	p
Germany						
ASPT	-0.21	0.084	-0.20	0.088	-0.20	0.088
Margalef Diversity	-0.51	<0.001	-0.48	<0.001	-0.49	<0.001
r/K relationship	0.38	<0.001	0.43	<0.001	0.44	<0.001
Type Lit %	-0.22	0.058	-0.21	0.076	-0.20	0.088
Odonata %	-0.54	<0.001	-0.46	<0.001	-0.46	<0.001
Trichoptera %	-0.40	0.001	-0.41	<0.001	-0.40	<0.004
Diptera %	0.26	0.025	0.11	0.354	0.15	0.199
No. Odonata Taxa	-0.52	<0.001	-0.40	<0.001	-0.40	<0.001
No. Trichoptera Taxa	-0.47	<0.001	-0.42	<0.001	-0.42	<0.001
No. ETO Taxa	-0.42	<0.001	-0.42	<0.001	-0.42	<0.001
Ireland						
ASPT	-0.26	0.020	-0.28	0.013	-0.29	0.009
Margalef Diversity	-0.18	0.118	-0.23	0.039	-0.23	0.043
r/K relationship	-0.11	0.333	0.04	0.738	0.10	0.363
Type Lit %	0.19	0.096	0.18	0.112	0.26	0.022
Odonata %	-0.00	1.000	-0.06	0.628	-0.05	0.658
Trichoptera %	-0.18	0.113	-0.14	0.206	-0.14	0.199
Diptera %	0.11	0.322	0.02	0.866	2	0.884
No. Odonata Taxa	-0.03	0.756	-0.09	0.406	-0.08	0.489
No. Trichoptera Taxa	-0.15	0.174	-0.19	0.094	-0.18	0.109
No. ETO Taxa	-0.25	0.026	-0.23	0.036	-0.23	0.037
Italy						
ASPT	-0.32	<0.001	-0.16	0.053	-0.16	0.053
Margalef Diversity	-0.38	<0.001	-0.42	<0.001	-0.41	<0.001
r/K relationship	0.37	<0.001	0.25	0.006	0.25	0.006
Type Lit %	0.05	0.614	0.17	0.058	0.18	0.042
Odonata %	-0.41	<0.001	-0.48	<0.001	-0.49	<0.001
Trichoptera %	-0.18	0.042	-0.30	<0.001	-0.31	<0.001
Diptera %	0.07	0.418	-0.03	0.731	-0.04	0.678
No. Odonata Taxa	-0.43	<0.001	-0.37	<0.001	-0.37	<0.001
No. Trichoptera Taxa	-0.20	0.028	-0.29	0.001	-0.29	<0.001
No. ETO Taxa	-0.38	<0.001	-0.42	<0.001	-0.42	<0.001

Table 2: Composition of the morphological stressor index developed for the three geographical regions Germany, Ireland and Italy.

	Geographical region		
Stressor Index Component	**Germany**	**Ireland**	**Italy**
Number of habitats	X		
Habitat diversity		X	X
Total PVI	X		X
Sum of macrophyte types		X	
Sum of vegetation cover types	X	X	
Sum of CWD/roots/overhanging vegetation	X		X
Pressure index	X	X	X
Natural/artificial dominant land cover type			X

Table 3: Results from two-way nested ANOSIM analysis comparing benthic macroinvertebrate communities from habitat-specific and composite samples from unmodified, hard and soft alteration sampling sites in different geographical regions/countries.

Germany			***Ireland***			***Italy***		
Unmodified			*Unmodified*			*Unmodified*		
Groups	R-statistic	P %	Groups	R-statistic	P %	Groups	R-statistic	P %
Composite, Macrophytes	-0.027	53.3	Composite, Macrophytes	-0,105	97	Composite, Macrophytes	-0.018	60.6
Composite, Sand	0.071	18.2	Composite, Stones	-0,037	64,5	Composite, Sand	0.087	7.6
			Composite, Sand	-0,054	61,7	Composite, Stones	0.034	24.1
Hard alteration			*Hard alteration*			*Hard alteration*		
Groups	R-statistic	P %	Groups	R-statistic	P %	Groups	R-statistic	P %
Composite, Sand	-0.118	94.9	Composite, Stones	-0,077	91,7	Composite, Sand	-0.045	62.1
Composite, Stones	0.562	0.06	Composite, Sand	0,045	30,1	Composite, Stones	-0.027	76
Composite, Macrophytes	-0.013	46.7	Composite, Macrophytes	0,225	11,8	Composite, Macrophytes	0.176	12.8
Soft alteration			*Soft alteration*			*Soft alteration*		
Groups	R-statistic	P %	Groups	R-statistic	P %	Groups	R-statistic	P %
Composite, Sand	-0.043	68.4	Composite, Macrophytes	-0,135	95,1	Composite, Macrophytes	0.559	2.2
Composite, Stones	0.73	0.08	Composite, Stones	-0,127	86,7	Composite, Sand	0.045	9.6
			Composite, Sand	0,096	30	Composite, Stones	0.037	32.2

Table 4: Results from two-way nested ANOSIM analysis comparing benthic macroinvertebrate communities from collected composite samples (CO) with proportional artificial composite (CO1) and unproportional artificial composite (CO2) samples from unmodified, hard and soft alteration sampling site in different geographical regions/countries.

Germany			***Ireland***			***Italy***		
Unmodified			*Unmodified*			*Unmodified*		
Groups	R-statistic	P %	Groups	R-statistic	P %	Groups	R-statistic	P %
CO, CO1	-0.087	87.4	CO, CO1	-0.09	97.2	CO, CO1	0.008	32.7
CO, CO2	-0.092	89.3	CO, CO2	-0.089	98	CO, CO2	0.007	33.3
CO1, CO2	-0.114	95.7	CO1, CO2	-0.097	99.4	CO1, CO2	-0.066	99.8
Hard alteration			*Hard alteration*			*Hard alteration*		
Groups	R-statistic	P %	Groups	R-statistic	P %	Groups	R-statistic	P %
CO, CO1	-0.068	75.4	CO, CO1	-0.063	84.1	CO, CO1	-0.02	66.4
CO, CO2	-0.028	55.3	CO, CO2	-0.066	87.1	CO, CO2	-0.02	63.8
CO1, CO2	-0.121	94.8	CO1, CO2	-0.095	98.6	CO1, CO2	-0.076	99.7
Soft alteration			*Soft alteration*			*Soft alteration*		
Groups	R-statistic	P %	Groups	R-statistic	P %	Groups	R-statistic	P %
CO, CO1	-0.062	78.5	CO, CO1	-0.063	81.8	CO, CO1	0.006	36.3
CO, CO2	-0.063	77.7	CO, CO2	-0.065	84.2	CO, CO2	0.003	36.1
CO1, CO2	-0.116	95.5	CO1, CO2	-0.11	99.2	CO1, CO2	-0.057	99.6

Figure 1: Map of Europe showing 32 lakes sampled in 3 different European geographical regions.

Figure 2: NMDS-plot of macroinvertebrate species arcsine-transformed proportional abundance data from unmodified, soft and hard alteration sampling sites in Germany, Ireland and Italy.

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Figure 1
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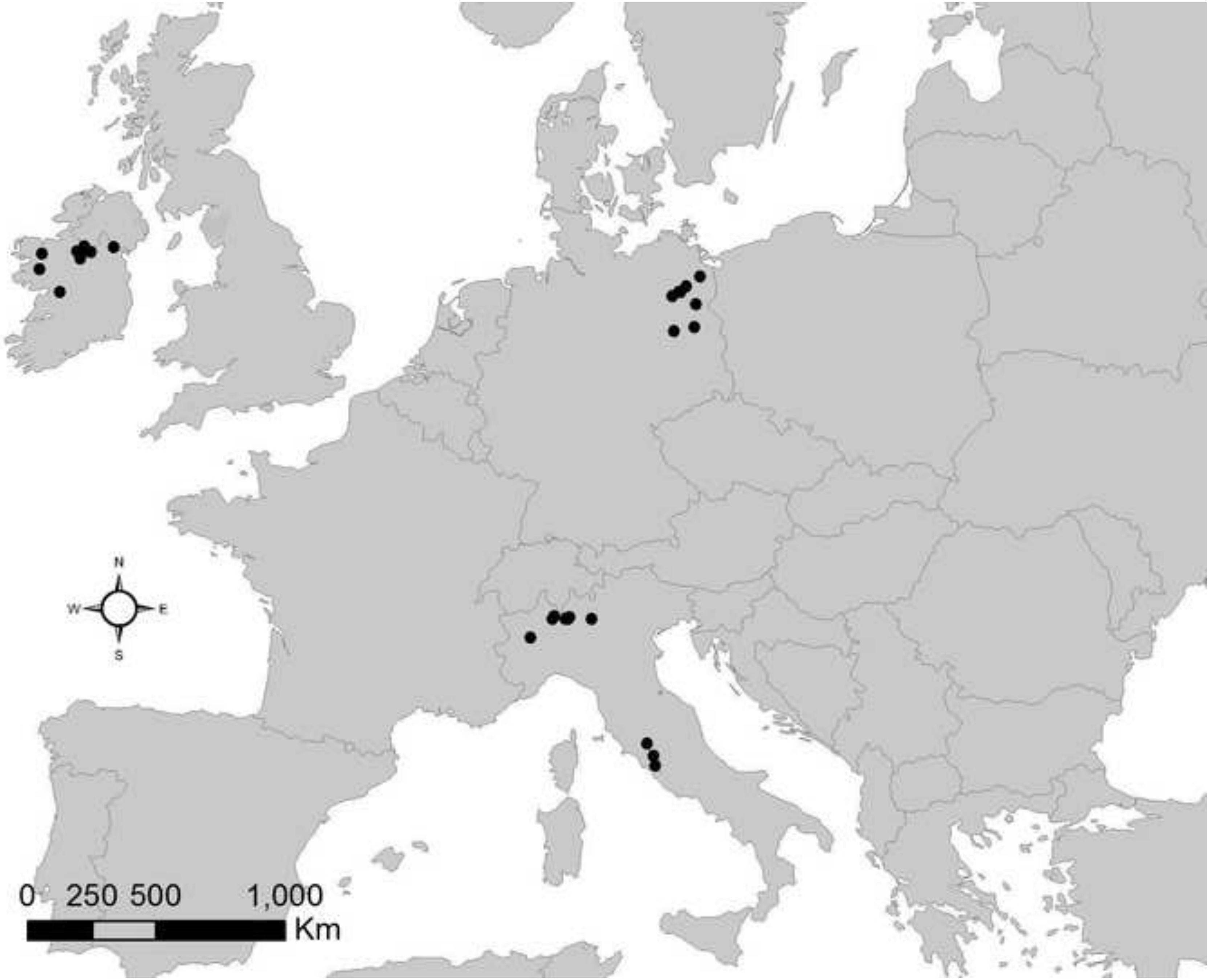

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