The challenges of long-term ecological research in springs in the northern and southern Alps: indicator groups, habitat diversity, and medium-term change

Reinhard GERECKE*, Marco CANTONATI1), Daniel SPITALE1), Elisabeth STUR2) and Sofia WIEDENBRUG3)
environments, documenting a number of factors and their impact on both fauna (e.g., Clarke et al. 2008; Heino et al. 2009) and flora (e.g., Johansson et al. 1998; Lindström et al. 2004; Barinova & Nevo 2010), for pristine and anthropogenically altered habitats, such data are completely lacking for springs. We see methodological problems as the main motive. If standardized methods for sampling stream zoobenthos are applied, much diversity is overlooked for the following reasons: (1) Marginal microhabitats with transitional conditions along a wet-dry gradient are not documented appropriately; (2) a high number of taxa only occur in submerged habitats as larval stages, which cannot be identified to species level (Franz et al. 2006). Also, specific methods may be necessary to sample some crenic habitats for benthic algae (cf. Cantonati et al. 2007).

Since ecological research into natural crenic habitats must cope with an extraordinary degree of diversification at various scales, investigations into four important areas are combined in this paper: meio-benthic assemblages, emerging insects, diatoms, and macroscopic benthic algae. These are treated separately, but discussed together to develop a comprehensive methodological approach for future studies on crenic habitats, focussing on the following:

1. Springs as ecotonal meeting points between terrestrial and aquatic fauna: Which spring types support the highest diversity?
2. Microhabitat/habitat differentiation. Examples of mosaic-like structures within springs and spring complexes: How do they influence the documentation of the fauna and algae?
3. Medium-term change: How stable is the faunal and microfloral composition under constant environmental conditions? How are environmental changes reflected by the animal and algal communities?

2. METHODS

2.1. Collecting sites

2.1.1. Berchtesgaden

In 1994, crenic habitats in the Berchtesgaden National Park (Upper Bavaria) were selected as a focus for long-term research. In contrast to the Adamello-Brenta (see below), the area is geologically rather homogeneous, characterized by dominant limestone rocks. Intense karst phenomena have a strong influence on the distribution and characteristics of the springs: (1) Stable crenic flows are mostly restricted to two "plateaus", one around 1300-1600, the other below 1000 m a.s.l. Only a few, often unstable, outflows are found above 1600 m, and areas above 2000 m lack any surface flow (Franz et al. 2006a). (2) Ionic content is generally rather high (lowered conductivity may indicate small-scale groundwater catchments exposed to surface water influence; Haseke & Pröll 2006).

Over the first ten years, a large-scale inventory of the whole area was undertaken, and more than 100 springs were documented from the geomorphological, physico-chemical, faunal, and, to a minor extent, floristic points of view (see Gerecke & Franz 2006 for more details of the area, study sites and fauna). In this period, several methods were developed and tested, with the principal aim of surveying the organismal diversity, and to plan how potential climate-change induced variation in communities could be observed. Most of the data here were obtained from two long-term study sites, Schapbach and Herrenroint. Information on other springs in the project are also occasionally included.

The **Schapbach** complex (sites: 300, 304, 308, 312, 314, 318) is surrounded by spruce forest and forms a stream whose waters disappear from the surface after about one km, reaching Lake Königssee along subterranean channels. It includes several weakly flowing, typologically diverse, crenic habitats. Particular attention was paid to an upper rheohelocrene, rich in mosses and travertine precipitation (site 308), and a helocrene, with water seeping through deep organic sediments with abundant herbaceous vegetation (site 312). Both sites have extended transitional zones between patchy aquatic and terrestrial areas and are strongly exposed to sunlight.

The two spring complexes also differ in the stability of their surroundings. The vegetation at Schapbach remained unchanged throughout the investigation, and had done so for a long time before. In contrast, extended areas of the spruce forest around Herrenroint collapsed before the investigation began, as a consequence of a bark beetle (Ipidae) invasion combined with heavy snow. Throughout the investigation period, the Herrenroint spring was influenced by the decay of broken wood, the expansion of herbaceous vegetation, and the slow growth of a new deciduous tree canopy.

2.1.2. Adamello-Brenta

An exploratory investigation of 30 springs (Cantonati 1998a) beginning at the start of the 1990's in the Adamello-Brenta Nature Park (south-eastern Alps,
Autonomous Province of Trento (the geographic area of the Alps called Trentino), studied several parts of the biota, but focussed on diatoms and the zoobenthos. Five springs were selected for a long-term ecological research programme (Cantonati et al. 2005), using standard sampling methods since 1998 (the first data for these sites are from 1993 or 1996). Over the years there were some changes concerning the sampling sites; one of the carbonate sites was replaced, and two sites on siliceous substrata were added (see Cantonati et al. 2009a for Cambiali). One of the latter is the helocrenic spring Nambino (AD1790), added to the long-term study in 2009. Four (Borzago AD1300, Fumo AD1853, Levade AD2153, Nambi BR1358) have been studied since the beginning of the investigation and form a core dataset. Five of the current seven springs are located on holocry stalline substrata (Adamello mountain range) and include hyperdilute, very-low alkalinity systems (Fumo, Levade) that are extremely sensitive to any airborne pollutants. Two springs are located on limestone and dolomitic rocks of the Brenta Dolomites, and have medium-low conductivities (150-220 µS cm⁻¹). The 7 springs lie within the nature reserve at 1300 to 2170 m a.s.l., and are all close-to-pristine (with the exception of Cambiali that has been canalised, details in Cantonati et al. 2009a). However, most of the sites are impacted by surrounding pastures or the vicinity of tourist paths and roads. Borzago, a medium-sized rheocrene on siliceous substratum, is occasionally (and illegally) dammed with boulders downstream, in order to rear fishes (trout and char) in the upper part of the spring-stream.

Between 2004 and 2007 this long-term investigation became part of the CRENODAT project (Biodiversity assessment and integrity evaluation of springs of Trentino - Italian Alps - and long-term ecological research), which allowed the study of more than 110 springs, distributed throughout the territory of the Autonomous Province of Trento, covering the altitudinal gradient and the variety of lithologies in the area (Cantonati et al. 2010). Since 2008 the south-eastern Alps long-term research programme has been part of a project funded by the Adamello-Brenta Nature Park ("ACQUATEST_PNAB") using selected aquatic habitats in the nature reserve to monitor environmental and climatic change.

The long-term research springs in the south-eastern Alps are sampled at least once each year between end of August and beginning of September. Seasonal studies (monthly sampling) were carried out in Borzago and Nambino in 1994-1995 (Cantonati 1999; Cantonati & Pipp 2000; Spitale et al., 2011 subm.a), and in Borzago, Nambi, Fumo, Levade, Cambiali, Valagola (BR1315) in 2005-2006, with one sampling per season.

This paper deals in detail with three springs:

1. The rheocrene Borzago, 1300 m a.s.l. on siliceous substratum in a Norway spruce wood has a constant flow and remarkable thermal stability, one of the most easily identifiable “spikes” in the otherwise rather flat diagram (see Fig. 15a) is the increase during the heatwave of 2003 (the warmest European summer in the last 500 y, with temperatures 2.3 °C above average; Stott et al. 2004). Temperatures also increase slightly in the final year (2009) but only data from subsequent years can confirm if this is a trend; pH is circumneutral or very slightly acidic, alkalinity is between 160 and 300 µeq L⁻¹ (Cantonati et al. 2005) which allows this spring to be classified as slightly sensitive to acid deposition/insufficiently buffered (cf Camarero et al. 1995). Discharge fluctuations occur seasonally, with additional peaks caused by heavy rains, and frequently accompanied by changes in concentrations of major nutrients (nitrates and total phosphorus, Cantonati 1999).

2. The (rheo)limnocrenic Fumo is relatively small (discharge: 0.2-10 L s⁻¹), at 1857 m a.s.l. on siliceous substratum on a slope with pastureland and mires (Cantonati et al. 2005). Its upper part is dammed by a small natural, somewhat unstable rim, generating the typical limnocrenic character (silt-debris as the dominant substratum, abundant liverworts etc.). It is a typical very-low conductivity (10-13 µS20°C cm⁻¹) and alkalinity spring (see Fig. 17) (see Camarero et al. 1995) and strictly oligotrophic (NO₃-N = 70-240 µg L⁻¹, TP = 2-7 µg L⁻¹); temperature shows seasonal cycles with a rather broad annual amplitude (6-7 °C, Fig. 15b); also discharge fluctuates seasonally, with marked peaks caused by major rain events.

3. The rheohelocrenic Nambino, 1790 m a.s.l., is permanent, oligotrophic (NO₃-N = 210-260 µg L⁻¹, TP = 7-9 µg L⁻¹), with low conductivity (27-28 µS20°C cm⁻¹) and alkalinity (200 µeq L⁻¹). It was added to the long-term monitoring in 2009, since this type of spring was previously lacking from the research programme.

2.2. Methods

The projects in the N and S Alps were initiated with different foci: a faunal inventory in Berchtesgaden, a more holistic approach with a distinct phycological focus in the Adamello-Brenta and converged over the first decade, in the course of countless discussions about sampling techniques. Thanks to the CRENODAT project in Trentino (Cantonati et al. 2007) and the start of phycological studies in Berchtesgaden (Cantonati & Lange-Bertalot 2010), a rationale was developed for methodological harmonisation (see below), but methodologies previously applied in the two areas will be presented separately. Apart from selected taxonomic groups which will be deposited in other Institutions, most of the material collected in the Berchtesgaden National Park is deposited in the Zoologische Staatsammlung München, and that from the south-eastern Alps in the collections of the Museo Tridentino di Scienze Naturali (Autonomous Province of Trento).
2.2.1. Zoobenthos sampling

After initial attempts to sample and analyze different microhabitats selectively, a more robust collecting method that integrates the whole eucrenal and all its characteristic substrata was adopted in Berchtesgaden. After defining the extent of the eucrenal (more branched flow channels and richer bryophyte vegetation, or in doubtful cases the uppermost 10 m of flow only), the relative cover of different substrata was estimated, and a composite sample taken by washing stones, gravel, detritus and plants from small, randomly selected areas. The aim was to obtain a sample that reflected the relative contribution of the different substrata. This sample was sorted into two fractions through sieves of 1000/250 µm mesh size. Sorting was done in the field from the living material (large fraction), or in the laboratory under a stereo microscope, after sub-sampling if necessary.

This procedure gave a semi-quantitative sample, allowing presence/absence documentation of species for macrozoobenthos from the large fraction, and for relative abundance estimates of smaller invertebrates (meiozoobenthos) from the fine fraction (Franz et al. 2006).

This methodology was used for benthic samples at all sites in the Berchtesgaden National Park, on an annual basis from 1994 onwards at the main monitoring sites. A much more selective sampling of small substratum patches near the emergence traps (360a-b, 308, 312a-c, see below) followed in 2005.

For the CRENODAT Project (Trentino), zoobenthos was sampled separately from the main substrata using a modified small Surber Sampler net (14.3 × 14 cm = 1/50 m², mesh size = 100 µm) on stone substratum and sampling three small patches of the same surface area as the net opening (1/50 m²) each for 5 s. The dominant submerged or marginal bryophyte species was sampled in three different places to obtain at least 20 g fresh weight to study different biotic components (10 g were used for the zoobenthos). Surface sediments, from two small patches of the same surface area as the net opening (1/50 m²), were sampled with a large bore syringe or a spoon and then filtered through the 100-µm zoobenthos net (5 s for each patch). The three substratum collections were complemented by qualitative collections (tweezers) for about 10 min. More details, including delimitation of the spring area sensu stricto (eucrenal), etc., can be found in Cantonati et al. (2007).

For the springs considered for the long-term project in the south-eastern Alps a specific procedure, slightly differing from and developed previously than the one outlined above adopted for the 110 CRENODAT springs, has always been followed. This consisted in the sampling (for an overall sampling duration of 30 s) of at least three specific patches (different substrata) indicated on a sketch of the spring area with a 100-µm zoobenthos net.

2.2.2. Berchtesgaden: Emergence traps

In Berchtesgaden, emergence traps were installed in 1996, over two springs (308, 312) and one crenic brook (318) at Herrenroint, and over one site in the large spring area Schapbach (360, about 50 m from the uppermost spring mouth). In 2001, the trap 312 was about 4 metres from the 1996 trap site. In 2005, to investigate the effect of microhabitat variety on emergence patterns, two traps were exposed at the Schapbach spring (360a-b, about 2 m apart over stones, gravel and bryophytes - no apparent differences in substratum composition between the two sites), and three at one of the Herrenroint springs (312a-c). Site 312a was identical with trap 312 in 2001, site 312b with that in 1996, and 312c was new. When the traps were mounted in early spring, flow was rather strong at site 312c, while in summer the highest substratum humidity was at site 312a. A benthos sample was taken near each trap from a substratum similar to that below the trap in July 2005.

Traps were exposed and emptied weekly as follows: 360, 308, 312, 318 - 17 May to 27 October 1996 (Schrankel unpubl.); 360, 308, 312 - June and September 2001; 360a-b, 308, 312a-c - 13 May to 2 November 2005, Square traps covering 0.64 m² were used in 1996 and 2001, and round traps covering 0.25 m² in 2005. The decision to select June and September for the 2001 and 2005 studies was taken after the analysis of diversity data from 1996, when chironomid emergence in these two months contributed the highest share of total annual diversity.

To calculate the habitat preference of emerging insects (see Fig. 3), not only the Heteroptera, Homoptera, Thysanoptera, Lepidoptera, Coleoptera, and dipteran families generally considered terrestrial were excluded, but also the Chironomidae. More than 50% of the latter remain unidentified, because identification to species level was only possible for males (Stur & Wiedenbrug 2006; Ekrem et al. 2010), and larval habitat preference is largely unclear for many species. On the other hand, studying this family, the most species-rich at the sites, produced a wealth of interesting data on habitat diversification and change (see below). Selected data from the 1996 collections have already been published (Gerecke et al. 2006: Trichoptera and psychodid midges; Stur & Wiedenbrug 2006: chironomid midges). Additional results, from 1996, 2001, and 2005, are presented and discussed below. Unless stated otherwise, species shown in the graphs occurred with a relative abundance >5%.

2.2.3. Trentino: Sampling diatoms and macroscopic benthic algae

Diatoms were sampled separately from the different substrata using specific methods. For epilithon, ten cobbles or small boulders were brushed. To study the epibryon, entire plants of the dominant submerged or marginal bryophyte species were collected in at least three
different parts of the spring head. Diatoms were digested with hydrogen peroxide, counting about 450 valves in the permanent mounts (Naphrax) to calculate relative abundances of individual species (for more detail see Cantonati et al. 2007).

Cover of macroscopic algae was assessed in the field using a scale of 1-to-5. Each was then sampled separately and analysed in the lab to obtain an abundance value. Combining the field cover and microscopical abundance estimate gave a final abundance value (see Cantonati et al. 2007).

2.3. Numerical analyses

To evaluate the cumulative number of species as a function of sample number, we used species accumulation curves (Magurran 2004). Because the order in which samples are included influences the shape of the curve, samples were added randomly to the species accumulation curve, repeating the procedure 99 times. Spearman correlations were calculated between the Bray and Curtis triangular matrix and years to evaluate temporal trends in species assemblages. We used the utility RELATE in the PRIMER 6 statistical program (Clarke & Warwick 2001). The Rho-value indicates the strength of the temporal trend, with 0 denoting no linear trend and 1 perfect positive correlation between time and assemblage change. Significance of Rho was tested by a permutation procedure which randomizes the order of the years (999 randomizations).

3. RESULTS AND DISCUSSION

3.1. Species richness

3.1.1. Invertebrate diversity and ecology

Analysis of species numbers from the two projects (Fig. 1, for the CRENODAT Project see Spitale et al. 2011, subm.a) underlines the enormous significance of crenic habitats for the total diversity of an area. There is also evidence that the Alpine crenic fauna is characterized by a high proportion of emergers, i.e., species that pass part of their life in a terrestrial environment, and flying to suitable habitats. From the 26,490 insect individuals captured in emergence traps at Berchtesgaden in 1996, 269 species could be identified (Schrankel unpubl.; Stur & Wiedenbrug 2006, unpubl.). As most of these species are unidentifiable at the larval stage, of the 745 species collected over ten years (Gerecke & Franz 2006), about a third could only be identified after emergence trap exposure. This percentage would be considerably increased if data became available for another 20, still unstudied, dipteran families (Anisopodidae, Ceratopogonidae, Mycetophilidae, Tabanidae, Phoridae, Pipunculidae, Sepsidae, Sciomyzidae, Sphaeroceridae, Tephritidae, Drosophilidae, Chloropidae, Agromyzidae, Heleomyzidae, Anthomyiidae, Muscidae, Fanniidae, Calliphoridae, Sarcophagidae, Tachinidae - Schrankel 2006). Furthermore, since these traps only operated at four sites in two crenic complexes, the resulting data must be considered a random sample, and the true insect diversity of Berchtesgaden springs may be two or more times higher. However, for water mites, which occur as adults in submerged habitats, accumulation curves for two springs over ten years level out, indicating that a representative taxonomic survey of this group is possible in a reasonable time (Fig. 2).

With respect to total emergence in 1996, the rheocrenic complex 360 has the highest numbers (11,517 emerging individuals m\(^{-2}\) season\(^{-1}\)), followed by the helocrene 312 (9375) and the rheohelocrene 308 (8165), while insect emergence from the spring-brook 318 is much less intense (4651). The particularly high numbers hatching at 360 is due to the very high contribution of chironomids + ceratopogonids (83% of the total fauna, ca 70% at the other sites).
The site with the highest number of emerging species was helocrene 312 (n = 129), followed by rheohelocrene 308 (n = 119) and spring-brook 318 (n = 73 - Chironomidae not treated here); 106 species were captured at rheocrene 360 (Schrankel unpubl.; Stur & Wiedenbrug 2006; Stur unpubl.). Diptera were dominant at all four sites (79% or more of the emerging individuals). At the two sites where chironomid emergence was completely analysed, this family was the most abundant (% of all Diptera: 308, 60%; 360, 78%). A similar result was obtained for site 312 from the June and September data and would also be expected for site 318, where chironomids + ceratopogonids comprised 72% of the dipteran fauna (Schrankel unpubl.).

Attributing emerging insects to three ecological categories - species with predominantly aquatic, hygrophilous, or terrestrial larvae - the four sites differ with respect to individual numbers, with generally higher percentages of hygrophilous and terrestrial taxa in the (rheo)helocrene sites, 308-318 (Fig. 3a). Species numbers similarly differ: the number of truly aquatic species is similar at all sites (41 at site 308 to 51 at site 360), while hygrophilous and terrestrial taxa are responsible for diversity differences (Fig. 3b). Maximum species numbers are reached in the most weakly seeping helocrene, 312, but the other two sites in this complex, 308 and 318, are also more species-rich than rheocrene 360.

Given our limited knowledge of the ecological requirements of most dipteran species, this analysis remains preliminary. It is possible, that more species than currently thought have aquatic pre-adult stages, or that many of the species documented here are strictly terrestrial and were only collected in the traps by chance. However, rather similar results were found for strictly aquatic meiofauna using different sampling methods: The highest species number (42) occurs in the helocrene 312, followed by the rheohelocrene 308 (38) and the spring-brook 318 (30), with only 26 species at the rheocrene 360. Helocrenes were the spring type supporting the highest species richness in a study in Luxembourg (Gerecke et al. 2005). This trend of increased species richness in helocrenes could be caused by the greater variety of microhabitats in helocrenes compared to rheocrenes, where the aquatic - terrestrial transition is more abrupt, leaving less space for species with an intermediate habitat preference.

Similar results are obtained for the mite community: First samples from 21 springs and crenic complexes in the Berchtesgaden area produced 37 oribatid species (Schatz & Gerecke 1996, Gerecke & Martin 2006). While oribatids and other generally "terrestrial" groups could not be further analyzed from subsequent Berchtesgaden material, particular attention was paid to them in the CRENODAT project. From 3391 mites from 99 springs in the Trentino region, 178 taxa were identified (since the species-rich Mesostigmata remain unstudied, a definitive species richness of 200 is possible - Gerecke et al. 2009b). Surprisingly, moss mites (Oribatida, 101 species), rather than true water mites
(Hydrachnidia and Halacaridae, 57 species) were the most species-rich group. Only half of the identified oribatids were known to be hygrophilous or tyrphobiontic and five species are truly aquatic, numerous species are arboricolous, lichenicolous or even xerophilous. Obviously, some of these species dwell along the edge of waterbodies or tolerate temporary flooding, others are present by chance, but they reflect the significance of crenic habitats to the development of particular, multi-structured terrestrial environments. A high percentage of terrestrial or hygrophilous mites occur in helocrenic habitats (57% in helocrenes, 62% in rheohelocrenes, 63% in limnohelocrenes, combined under "(rheo)helocrenes" in Fig. 4) and limnocrenes (60%), while it is very low in rheocrenes (25%, with or without hygropetric surfaces, Fig. 4).

Fig. 4. Relative abundance of mite species belonging to terrestrial (Trombidia, Mesostigmata, Oribatida, Astigmata) and aquatic taxa (Halacaridae, Hydrachnidia) in Trentino springs (calculated from data published by Gerecke et al. 2009a).

The analysis of the emergence data from Berchtesgaden also allowed a first assessment of the significance of parasitism by water mite larvae on aquatic insects in Alpine springs. Contradicting previous hypotheses that presume that the parasitic stage would be suppressed in crenic water mites, this study and work on collections from Luxembourg showed that most Hydrachnidia must be categorised as emergers (Stur et al. 2005, Martin & Stur 2006, Martin et al. 2010). With respect to the proportion of so called "terrestrial" larvae (i.e. larvae that actively leave water and seek for terrestrial hosts) versus "aquatic" larvae (seeking hosts in submerged habitats), the helocrenic complex Herrenroint harbours more species with "terrestrial" larvae than the Schapbach rheocrene (7 v. 3, 25% v. 15% respectively). At Schapbach, only two specimens of Partnunia steinmanni were found, whereas 338 specimens were recorded from Herrenroint.

3.1.2. Diatoms, cyanoprokaryotes, and macroscopic benthic algae

Due to practical, analytical problems, obtaining a precise estimate of the total number of diatom species thriving in the springs in an area can be difficult. When analyzing diatom assemblages, the total number of species counted (i.e., identified while counting 450 valves for the quantitative evaluations) is a more objective and comparable measure of richness (e.g., Cantonati & Lange-Bertalot 2010). Thus, a total of 205 and 299 taxa were identified in the epilithic and epiphytic (bryophytes) samples respectively from the 110 springs of the CRENODAT project (Cantonati et al., in prep.a). However, many more taxa were encountered and identified during qualitative scans of the permanent slides, and much more work is needed to produce a consistent estimate of such low abundant taxa that may nevertheless have highly informative ecological preferences. Thus, especially for large studies on oligotrophic habitats, significant diatom species richness remains hidden among taxa that only occur at very low relative abundances and are not recorded by standard quantification procedures. Some material from oligotrophic habitats can be studied for days without ceasing to find previously unrecorded taxa. The study by Lange-Bertalot & Metzeltin (1996) who found almost 800 diatom taxa in samples from three oligotrophic lakes is a pertinent example. This is also clearly shown by the diatom accumulation curves of the springs to be discussed in more detail below (Fig. 5). Despite studying the sites annually for many years (including some seasonal studies), accumulation curves for the Fumo, Levade, and Borzago springs continue to increase (Fig. 5a-c). In particular, the analysis of new seasonal samples would lead to the identification of new species for the sites. To demonstrate that this effect is mainly due to a large pool of species present at very low relative abundances, accumulation curves were calculated using only taxa with maximum relative abundances >5% (Fig. 6). It is easier to obtain a precise estimate of total diatom richness for smaller datasets. For example, Cantonati & Lange-Bertalot (2010) found 104 diatom taxa in nine springs of the carbonate Berchtesgaden National Park (Germany). Of these, 16% were not found during counts, but were identified from several hours’ qualitative scanning of the slides, on at least two different occasions.

With respect to the spring types, highest diatom richness is usually found in helocrenic and rheohelocrenic springs, on both siliceous (e.g., Cantonati 1998b) and carbonate substrata (e.g., Cantonati & Lange-Bertalot 2010). In the south-eastern Alps long-term programme, this topic cannot be addressed because, except for the recently added Nambino site, only rheocrenes and (rheo)limnocrenes were studied.
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With respect to the cyanoprokaryotes, a significant constraint to assessing total species richness is the lack of taxonomic and ecological studies of oligotrophic habitats; several taxa are barely identifiable (see Cantonati 2008). However, 40 cyanoprokaryote species were identified in 19 springs on two contrasting lithological substrata (Cantonati et al. 1996), and 41 in 16 carbonate springs of the Dolomiti Bellunesi National Park (Cantonati 2008). Since the majority of these species are epilithic, highest richness was found in a small, hygropetric rheocrene (Cantonati et al. 1996) and in a medium-sized rheocrene (Cantonati 2008).

Of macroscopic benthic algae lowest species numbers occur in helocrenic and rheohelocrenic springs, since most mats, colourings, crusts, etc. develop on stony substrata that are typically absent in such springs. When diatom and cyanoprokaryote data from the CRENODAT Project are considered with respect to their environmental moisture preferences, they mirror the invertebrate results discussed. Van Dam et al. (1994) classified diatom moisture preferences on a scale of 1-to-5, from "never, or only very rarely, occurring outside water bodies" to "nearly exclusively occurring outside water bodies". Applying this to the diatoms found in 110 springs (Cantonati et al. in prep.a) revealed that a quarter of the taxa (25% for the epibryon, 21% for the epilithon) belong to categories 4+5 ("mainly occurring on wet places" + "nearly exclusively occurring outside water bodies"). Following Cantonati & Lange-Bertalot (2010) 18% of taxa in nine springs of the Berchtesgaden National Park belonged to categories 4+5. A tentative application of an analogous classification to cyanoprokaryota from 70 springs revealed that more than one third (34%) belonged to categories 4+5 (Cantonati et al. in prep.b). This agrees well with the species-richness of cyanoprokaryotes in hygropetric habitats, including a number of taxa that adapted to periodic desiccation, while diatoms usually form species-poor assemblages of highly-specialized taxa in seasonally-flowing springs (cf. Cantonati et al. 2006). The diatom results were obtained by sampling mainly substrata inside the wetted perimeter (although marginal hygrophilous bryophytes were often considered in carbonate springs due to the absence of submerged bryophytes). The study of diatom assemblages along transverse transects across the land-water ecotone would emphasize the role of xerotolerant taxa.

3.2. Microhabitat differentiation and medium-term change

As documented above, crenic animal community composition variously characterizes habitat types: Not only specialists, which are often restricted to particular spring typologies, but also terrestrial and hygrophilous species attracted by certain, often largely unknown properties of a spring, may make a significant contribution to total diversity in the habitat and its surroundings, especially in helocrenes with extended transient shore areas. In fact, crenic faunas may be described as comprising three major fractions: (1) more or less acciden-
tal, terrestrial or hygrophilous species, attracted by conditions such as shade and humidity; (2) insect species with a life cycle including aquatic and terrestrial stages (usually with immature stages restricted to running waters, swamps or hygropetric shore areas and terrestrial adult stages); (3) species that complete their entire life cycle under water. We hypothesize that various types of environmental change will act differently on representatives of these three fractions. Indirect consequences of environmental change, most evident in the surrounding vegetation, with extensive change in shading, organic input and quality of shore substrata (e.g., Heino et al. 2009) will result in strong change in the hygrophilous and accidental terrestrial fauna (fraction 1). This is the component that most closely reflects the environmental conditions around a spring. However, we assume that insects emerging from the aquatic habitat (fraction 2) will also be very sensitive to such change. As adults, they experience the structure of the riparian vegetation as well as the surrounding forest canopy, where many of them feed, shelter from predators, or meet to swarm and reproduce. Invertebrates that complete their life cycle without leaving the water (fraction 3) are probably the component least influenced by changes in the surroundings of the spring. Any effect of changes in shading or input of organic material on this component will probably be delayed, by several years or decades.

We suggest that the opposite sequence would apply if consequences of climate change act directly on the crenic habitats. Such effects would be expected to be slow-acting changes in temperature and flow, possibly accompanied by physico-chemical changes, and followed by an extensive change in submerged habitats. Such change would probably have a negligible effect on fraction 1, partly affect fraction 2 (interfering with temperature preference and microhabitat selection of larval stages), but be of key importance to fraction 3, in which all developmental stages are equally influenced by the properties of the ground water. Against these theoretical considerations, results of a study on meiobenthos (Microcrustacea and Acari), Trichoptera and Chironomidae from Berchtesgaden are given below.

3.2.1. Invertebrate communities

In 2005, the ostracod fauna is dominated by *Psychrodonomus fontinalis* at both sites of Schapbach. Given the low individual numbers of rare species (360a: *Cypria lacustris*, *Potamocypris pallida*; 360b: *Cavernocypris subterranea*, *Cryptocandona vavrai* und *Eucypris pigra* - see Fig. 7a), there are no statistical differences. Also throughout the foregoing monitoring period (1994-2004), a similar pattern was found, *Psychrodromus fon-
tinalis was always dominant, with Potamocypris fallax and P. pallida as subdominant associates.

A similar situation is seen in the copepod fauna; one species, Attheyella wierzejskii, is dominant at both sites, but in this case the accompanying species are the same, with only slight differences in relative abundance (Fig. 7b). Again, over the monitoring period (1995-2004), only these four species were found with similar abundance patterns.

Acari were only recorded in low numbers (Fig. 7c), but there was a similar taxonomic composition at both sites, with Oribatids and the halacarid Soldanellonyx chappuisi having the highest numbers, along with Hydrovolzia placophora at site 360a and Feltlia minuta at site 360b. All these taxa belonged to the dominant species also over the long term (1995-2004), but with different dominance patterns (e.g., Protzia distincta was most frequent, Soldanellonyx chappuisi rather rare).

Caddisfly emergence was largely similar at sites 360a and 360b; only one species was restricted to one trap (Fig. 8). While this matches the substratum similarity of the two sites in 2005, there are striking differences from the 1996 investigation: Seven of the 11 species from 1996 were not found again (Rhacophila producta, Ptilocolepus granulatus, Tinodes dives, Drusus chrysotus, D. discolor, Lithax niger and Micrasema morosum). Rhacophila bonaparti was the only species found in 2005, which was absent in 1996. Because of the low numbers, only limited comparison can be made with the benthic larvae. In agreement with the emergence data, Pseudosilopteryx zimmeri is frequent at both sites, but the abundance of Lithax niger larvae and several limnephilids (A. uncatus, H. rubricollis, further species?) is rather different.

There are considerable seasonal differences in the chironomid emergence between June and September (Fig. 9). In both seasons, Corynoneura lobata and Krenosmittia borealpina were dominant (>5% of the total emergence in 1996-2005, in decreasing abundance order), but in June Thienemanniia libanica, Helienniella spp., Thienemanniella spp, and Microspectra seguyi were also present, and in September Microspectra pharetrophora, Parmatricrennus stylatus and Chaetocladius laminatus. The most striking differences in relative
abundance was not between years, but between the two traps in 2005 (360a/b). If data from these traps are pooled, they agree rather well with those of the preceding investigations. The general presence/absence pattern is rather stable over the decade. A medium-term change in relative abundance is only observed for *Micropsectra seguyi* and *Thienemannia libanica* (both decreasing), and *Chaetocladius laminatus* (increasing). *Paratrichocladius skirwithensis* is the only species that was dominant in 2005, but absent before that.

The ostracod fauna of the Herrenroint helocrene is dominated by *Eucypris pigra*, a species that does not seem to be influenced by potential differences between the sites (Fig. 10a). The other five species were only found as single individuals. The same dominance pattern was observed from 1995-2004.

The copepod fauna comprises six species (Fig. 10b), one species being absent from each site, but a quantitatively significant difference is found only for *B. cuspidatus* (absent from 312a and 312c, dominant at 312b). The dominance pattern is rather heterogeneous, with *A. wierzejskii* and *B. echinatus* reaching their maxima at 312c, while *B. tatrensis* has its maximum abundance at both 312a and 312b. Data from 1994-2005 show a 312c-like pattern, with *Bryocamptus cuspidatus* and *B. tatrensis* the third most frequent species. Also the pooled data for 312a-c show a similar pattern, except that *B. tatrensis* is more abundant than the other two *Bryocamptus* species.

Compared with the two microcrustacean groups, the acari fauna (Fig. 10c) is characterized by a distinctly higher number of species that only occur as single individuals, pooled as "rare species". The three sites show a pattern at least partly explicable by the humidity preference of the dominant taxa. At 312b, the site with lowest humidity, not only are terrestrial taxa the most abundant, but the halacarid *Soldanellonyx chappuisi*, which, due to its reduced size, is able to survive drought in tiny crevices (Gerecke & Martin 2006) is also found dominant. *Partnunia steinmanni* is represented in the highest numbers at 312c, where the flow is stronger. Comparing the pooled data for 312a-c and mean values from 1995-2004 (Fig. 11) shows that *Parathyas palustris* and *Partnunia steinmanni* were more frequent in the 2005 samples. Both have "terrestrial larvae", reflecting the fact that all three traps were positioned over rather dry areas.

Five caddisfly species were recorded in the emergence traps in 2005 (Fig. 12), *Crunoecia kempnyi* being most frequent, followed by *Pseudopsilopteryx zimmeri*. Only one specimen of *C. kempnyi* was found in trap 312a, while low numbers of four species were present at 312c. Only *Parachiona picicornis* was found in trap 312b, but in large numbers. In 1996, *C. kempnyi* was also the most frequent species, but with additional individuals of *Allogamus uncatus*, *Ernodes vicinus* and *Micrasema morosum*, while *Parachiona picicornis*, *Pseudopsilopteryx zimmeri* and *Ptilocolepus granulatus* were only found in 2005. Compared with the benthic
samples, the absence of emerging *Ernodes, Beraea* and *Sericostoma* is interesting (the undetermined limnephilid larvae, i.e. almost half the benthic caddisfly larvae, could include representatives of *A. uncatus, P. picicornis* and *P. zimmeri* which were found in the emergence traps). The dominance of *C. kempnyi* confirms the benthos data, although its maximum density occurred at 312c rather than 312b, where the strongest emergence of this species was observed.

When chironomid emergence for traps 312a-c is compared for dominant species (>5% abundance in at least one trap, Fig. 13a), *Parakieferiella gracillima*, a typical helocrene species (Thienemann 1954), shows a preference for the drier sites 312a-b, but *Corynoneura lobata* and *Rheocricotopus effusus*, considered lotic species by Lindegaard (1995), for site 312c. An analysis of the chironomid fauna as a whole reveals the individuality of each trap site. A total of 54 species were recorded from 312a-c, but each trap only caught between 52% (312c) and 74% (312b) of the species. In addition, 16 species were recorded in only one trap, with the lowest proportion of such exclusive species at 312a, the most at 312b (Fig. 13b). Most exclusive species were single records, but *Paratrichocladius skiritwithensis* (312b, n = 24) and *Stempellinella ciliaris* (312c, n = 26) were found in higher numbers at their respective sites.

Comparing results from 2001 and 2005 (Fig. 14) reveals potential faunal change at 312a. In both years, by far the most abundant species in June was *Parakieferiella gracillima* (2/3 or more of total emergence), with an increase in *Rheocricotopus effusus* (rare in 2001, >10% in 2005) (Fig. 14a). In September in both years *Limnophyes bidumus* dominated (more than 1/3 of total emergence). *Chaetocladius laminatus* and *Tvetenia bavarica* were absent in 2001, but dominant in 2005; on the other hand, *Micropsectra sofiae* was dominant in 2001, but absent in September 2005 (Fig. 14b).

Also at 312b, the site studied in 1996 and 2005, *Parakiefferiella gracillima* was dominant (about 50% abundance) in both years. *Thienemanniella* spp. and *Paraspectra mendli* decreased, the latter being completely absent in 2005, but *Tvetenia bavarica* showed a strong increase (Fig. 14a). For the September emergence (Fig. 14b), the five most frequent species, *Limnophyes bidumus, Chaetocladius laminatus, Tvetenia*
bavarica, Heleniella spp. and Corynoneura lobata, appeared in similar proportions in 1996 and 2005. There was a rather strong decrease in Micropsectra pharetrophora, Metriocnemus eurynotus and Micropsectra sofiae, the last being absent in September 2005.

Compared to Schapbach (360), the Herrenroint (312) data show stronger changes in density of species; a formerly dominant species disappears and two species appear over the investigated period (Schapbach: no disappearance, one new appearance).
3.2.3. Algal communities

In the Borzago rheocrene (AD 1300; Figs 15a, 16), diatom assemblages are relatively stable (Fig. 16), dominated by rheophilic taxa (Achnanthidium subatomus, Cocconeis placenta var. euglypta), and by members of the Achnanthidium minutissimum species group. Subdominant taxa are: Gomphonema pumilum var. elegans (rheophilic - Cantonati & Spitale 2009); Diadesmis perpusilla (xerotolerant - Cantonati et al. 2006), Amphora inariensis (Red List species category 3, i.e. threatened; Lange-Bertalot 1996); Amphora pediculus (promoted by higher nitrate - Rott et al. 1999). The following species may also be favoured by heavy shading: Amphora pediculus, Cocconeis placenta var. euglypta, Diadesmis perpusilla (Cantonati et al. 2006). There was a risk that shading could be drastically modified by extensive forest cuttings in 2004, but negotiation with the forest managers prevented harvesting of spruce trees around the spring. Some short-term fluctuations in nutrient concentrations and grazing pressure might be due to the sporadic use of the dammed upper part of the crenon for rearing young fishes.

In the Malga Val di Fumo (rheo)limnocrene (AD1853; Figs 15b, 17-18), diatom assemblages (Fig. 18) included typical acidobiontic and acidophilous taxa, both among the dominant (Eunotia exigua, E. intermedia, E. subarcauotides), and subdominant species (Psammothidium acidoclinatum, Psammothidium marginatum). The latter two have different pH optima (Cantonati et al. 2002) and research is underway to test this for E. intermedia, E. subarcauotides. In addition, A. acidoclinata always developed below 70 µeq L\(^{-1}\) (unpublished data of M.C. and D.S.). Accurate assessment of the relative abundances of these species pairs could allow detailed monitoring of the alkalinity and acid/base status changes in this extremely fragile system. Trends in relative abundances for the four species seem to suggest an increase in alkalinity in recent years, confirmed by the annual measured values (Fig. 17). This would be consistent with trends observed in high-mountain lakes of the region. Tait & Thaler (2000) hypothesised that
alkalinity increases might be due to climate-change-driven enhanced erosion and leaching in drainage basins in mountain areas, and Monteith & Evans (2005) suggested the same for low-alkalinity UK lakes. In the light of relatively recent findings that an increase in organic acid concentrations might be favoured by environmental change (elevated CO₂ levels, warming and increased rainfall; Freeman et al. 2004) peatland-influenced mountain sites, e.g. Fumo spring, could be particularly well suited to acidity monitoring.

Fig. 17. Changes in alkalinity (measured annually) in a (rheo)limnocrenic spring of the Adamello (Fumo) over a fifteen-year period.

The Nambino helocrenic spring (AD1790) hosts a distinctive microflora and meiofauna. Two new species were discovered here, the diatom *Navicula cantonatii* Lange-Bertalot (Lange-Bertalot 2001) and a cyclopoid copepod of the genus *Dyacyclops* (*languidus* group - F. Stoch & C. Bruno, personal comm.). The only recently described diatom species *Eunotia glacialispinosa* Lange-Bertalot and Cantonati was found in this site (Cantonati & Lange-Bertalot 2010). Its type locality is a spring of the same morphological type in Berchtesgaden (Herrenroint 312, and it was present also in Herrenroint 308, both considered for the present paper).

4.4. Medium-term change in the studied springs

Diatom and water mite data were analysed to identify whether significant temporal trends were detectable (Tab. 1). Temporal trends are significant in 50% of the sites (same situation in the northern and southern Alps).

**Tab. 1.** Analysis of temporal trends in long-term ecological research sites in the northern and southern Alps.

<table>
<thead>
<tr>
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<th>Rho</th>
<th>P level</th>
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</thead>
<tbody>
<tr>
<td>North-eastern Alps – Water mites (Berchtesgaden National Park)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schapbach</td>
<td>0.354</td>
<td>0.017**</td>
</tr>
<tr>
<td>Herrenroint</td>
<td>0.085</td>
<td>0.322</td>
</tr>
<tr>
<td>South-eastern Alps - Diatoms (Adamello-Brenta Nature Park)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumo</td>
<td>0.436</td>
<td>0.002**</td>
</tr>
<tr>
<td>Levade</td>
<td>-0.007</td>
<td>0.482</td>
</tr>
<tr>
<td>Borzago</td>
<td>0.380</td>
<td>0.001**</td>
</tr>
<tr>
<td>Nambino</td>
<td>0.039</td>
<td>0.256</td>
</tr>
</tbody>
</table>

Confirming this result, the two south-eastern Alps sites exhibiting significant temporal trends were also found to be negatively correlated (*R²* = 0.528). Thus, time (medium-term change) significantly influences species dynamics in Schapbach, Fumo, and Borzago, while other factors (e.g., internal regulatory processes, such as food and species interactions) appear to be more important in the other three springs. This suggests that
more dynamic and unstable sites (Fumo, Schapbach) or those occasionally affected by impacts (Borzago), show more signs of medium-term changes, whilst more complex, stable systems (Herrenroint, Nambi) are characterized by fluctuations driven by internal processes.

We believe that these sites are of particular importance for the documentation of long-term changes, with the proviso that seasonal changes are periodically closely assessed (e.g., every 5 y) to ensure that potential long-term trends are not filtered out.

4. CONCLUSIONS

The dilemma for ecological research in crenic habitats is that highly diverse communities live in a very narrow space and it is impossible to obtain representative data from large substratum surfaces without severely damaging the habitat, and thereby destroying the possibility of longer-term observations. In fact, only a few attempts have so far been made to obtain an insight into the total diversity of a crenic habitat, and thereby destroying the possibility of longer-term observations. In fact, only a few attempts have so far been made to obtain an insight into the total diversity of a crenic habitat, and hardly any historical data are available to verify or falsify the hypothesis that springs are places of low population change and long-term stability. Heinroth et al. (2009) emphasised the importance of improving our knowledge of the distribution and ecology of freshwater organisms as a prerequisite for developing distribution-climate models that can be used as predictive tools in research on climate-change. Our aim is to propose a combined sampling regime that allows representative organisms to be extracted and analysed at several scales, in medium- and long-term investigations.

4.1. Zoobenthos

Integrating patchy samples taken throughout the eucrenal is a robust method for documenting assemblages of non-emerging invertebrates (Franz et al. 2006). In addition to microcrustacea and acari, such samples can also be studied for Nematoda, Oligochaeta, and Mollusca, and, to a lesser extent, beetle communities. In general, a relatively small amount (200 mL) of fine sediments sieved from about 1 L combined substratum (bryophytes, gravel, detritus, organismal films from stones and rocks) produces a representative sample of meiozoobenthos, allowing characterization of the habitat. Such data is useful for observing long-term change in springs, and, together with periphyton samples (4.5), documenting the hygrophilous fauna around the margin.

Our results from the 2005 Berchtesgaden investigation show that small samples from very restricted surfaces also provide comparable results for detritus feeders, such as copepods and ostracods. On the other hand, densities of predatory water mites are too low (usually one order of magnitude less than microcrustaceans, Gerecke et al. 2005) for detailed interpretation. As the three groups differ in their sensitivity to environmental variables (our data suggest that Ostracoda are least sensitive, mites most, and copepods intermediate), using them together provides a more accurate picture of the ecological situation.

Analysis of macrozoobenthos from samples collected as above is generally problematic. Larval stages of many taxa (mainly dipterans, but also other insect orders), are poorly known or fail to exhibit those character combinations that allow species discrimination. This is particularly true for early instars, which are often found in particularly high numbers in springs. To obtain an accurate estimate of population densities, larger quantities of substratum must be sampled. Alternatively, selective collecting of mature instars may provide an adequate measure of the Ephemeroptera, Plecoptera, and Trichoptera, but not for the Diptera, usually the most diverse insect order in springs (4.3).

4.2. Diatoms vs macroscopic benthic algae

The use of diatoms for long-term studies in springs appears to be highly appropriate for the following reasons: (1) diatoms are very species rich in most habitats; (2) they have the potential to provide excellent information on both the hydrochemistry (mineral content, pH, nitrates, etc.) and on morphological and physical parameters (current velocity, shading conditions, dominant substratum, etc.); (3) the taxonomy and ecological preferences of the majority of the species have been, or are being, worked out; (4) since the majority of taxa do not produce clearly identifiable macroscopic structures, diatoms are sampled by removing a certain amount of the epilithic biofilm, or of the substrata they colonize. Diatom sampling is easily standardized and requires negligible technical skills.

Sampling and field assessment of macroscopic benthic algae is more time-consuming, and requires more taxonomic and ecological knowledge. According to Sandlund & Aagaard (2004) the scarcity of taxonomic expertise in periphyton is the main drawback to using this biotic component in monitoring programmes and water-body classification required to implement the Water Framework Directive. However, macroscopic benthic algae provide an immediate assessment of field conditions, and some groups, cyanoprokaryotes in particular, are particularly amenable for studying land-water ecotones in hygropetric environments. Lindström et al. (2004) argue, after some years of observation at a given location, that it should be possible to formulate a list of “expected species”, which can then be used as a reference for a relatively straightforward assessment of change.

4.3. Emerging insects

The present data represent a first attempt to document microhabitat differentiation and medium-term change in springs using emergence traps. Consequently, the data should be interpreted with caution and methodological problems must be considered.
As a general rule, an important (in terms of biological diversity and biomass) component of the fauna cannot be adequately investigated without the use of emergence traps. Our data show that this approach can also document spatial and temporal changes in community structure, contributing to a survey of the entire insect species spectrum, undetectable using spot-like, single collections. However, there are four problems:

1. Selectivity of traps is exemplified by our results for the Trichoptera: As pupae of most species leave the water and walk to protected sites before hatching, species are not adequately represented. Our data also suggest that caddisflies are collected in higher numbers in square than round traps, possibly because corers offer protected places for pupae to hatch. As adults of many species spend extended periods of their life far from the larval habitat, hand-netting in the surrounding vegetation is not sufficient to find them. In order to balance this, mature larvae and pupae must also be collected.

2. In contrast to the integrated meiobenthos collecting, traps inevitably represent an extremely restricted component of the site. As shown in chapter 3.2.1, data therefore reflect the degree of patchiness in the crenic habitat, and are less representative when collected from helocrenes (with high substratum variety) than from rheocrenes, an effect that increases with decreasing trap size. If the aim is to obtain as complete a species inventory as possible, several traps should be positioned over different substrata and flow regimes. If positioning more than one trap per spring is impossible, rather large traps should be used.

3. Traps can affect the physico-chemical conditions, vegetation and fauna of the surface they cover, mainly by shading, but also by removing an important proportion of the biomass and hampering allochthonous input of living and dead organic matter. This problem is probably less acute in rheocrenes than helocrenes; such effects could be reduced by alternating sampling (trap in place) with non-sampling (trap removed) weeks.

4. Putting up and maintaining traps requires considerable logistical and scientific effort. Stur & Wiedenbrug (2006) proposed that this problem can be partly overcome by limited setting up at particular times of the year (e.g., in the Berchtesgadener National Park, most of the total chironomid diversity can be documented from data collected in June and September). With respect to scientific effort, it is inevitably more time-consuming to document completely highly diverse communities than simpler ones. However, once any monitoring programme has been started, subsequent analyses are simplified and accelerated by the knowledge acquired during the pilot phase.

Our results suggest that, for studies of faunal change, traps are best established in larger rheocrenes with rather homogeneous substrata. In helocrenes, they are useful for documenting microhabitat diversity when several traps are distributed over the entire area. However, given the limited extent of crenic habitats, covering a large proportion of the area with traps in order to obtain statistical data is not an alternative; the traps themselves induce environmental change, e.g. by shading and extracting biomass.

One important aspect of the emerging fauna is that, in their immature stages, most insects are exposed to the same factors as the meiofauna communities, but as adults, to factors that operate outside the crenic habitat itself. Such factors, which may have considerable short- and medium term significance, are annually fluctuating climatic conditions or the state of the surrounding vegetation. The greater change in emergence at Herrenroint than Schapbach over the investigated decade (see 3.2.1.), could be a result of such factors. There were significant changes in the vegetation around Herrenroint, but negligible ones at Schapbach. Consequently, for documenting potential long-term-change in insect phenology, larger rheocrenes with stable conditions in the surrounding vegetation are more suitable.

4.5. Integrating methodologies

Due to the lack of appropriate studies on crenic communities globally, and especially in the tropics, there is still an immense gap in our knowledge of limnofauna and -flora. We presume that the lack of appropriate methodologies for comparative studies is an important reason for this. We therefore take this opportunity to propose an integrative approach that should allow large-scale comparisons and monitoring.

The investigation of meiobenthos and diatoms is crucial. Due to the generally steady population densities of meiofauna, single samples (preferably in early summer in temperate zones) are suitable for documenting species diversity and population density. For diatoms, the end of the summer low-discharge period would be the optimal time for temperate mountainous areas. In a long-term monitoring study in south-eastern Norway, Lindström et al. (2004) found that diversity increased from spring to autumn, but that inter-annual stability was particularly high in autumn, suggesting that the periphyton provided more reliable information at this time of year. If an investigation aims to document invertebrate diversity thoroughly, emerging insects should be sampled over an entire season. Trap size must be chosen in relation to the extent of the studied habitat. In a non-homogeneous crenon, it is important to place the trap over the most representative patch (with respect to vegetation and substratum), or to use 2-3 smaller traps. Based on a pilot investigation, the month(s) representing the maximum diversity should be selected for future monitoring projects.

With respect to photoautotrophs, an initial characterization of the macroscopic benthic algae would complement the diatom study. Once the operator is familiar
with the macroscopic photoautotrophs at a given site, change can be assessed in a reasonable period of time.

According to Heino et al. (2009), organisms capable of crossing terrestrial habitats between isolated water bodies, either mainly passively (e.g., diatoms) or actively by flying (e.g., aquatic insects) are likely to show the greatest potential for bioclimatic modelling; the responses of freshwater species distributions to present-day climatic conditions can be used to predict climate-induced shifts.

Sandlund & Aagaard (2004) noted that, despite the availability of well-established sampling methods for both aquatic insect larvae, and flying adults, even with reasonable sampling effort, 50-60% of species will be missed with a single annual sampling, but this proportion is reduced to approx. 20% if material from 3-4 consecutive years is combined.

Even in established, national monitoring networks (United Kingdom Acid Waters Monitoring Network, Monteith & Evans 2005) biological parameters are only monitored annually. However, other factors (e.g., physico-chemical variables) are measured more frequently. Monteith & Evans (2005) stress the importance of better understanding the seasonal dynamics of the biota, particularly with reference to phenology/climate interactions that might be important for annually sampled assemblage composition.

Continuous recording of some key parameters, such as discharge (in all core sites of the spring monitoring programme), and pH and alkalinity in low-alkalinity springs (Fumo, Levade, and Nambino) would be extremely valuable, but requires relevant funding (compare Monteith & Evans 2005).

Heino et al. (2009) strongly encourage the establishment of more long-term monitoring networks to examine temporal changes in biodiversity, recognizing the extremely limited number of monitoring programmes that address interannual variability at multiple freshwater sites. Such programmes would also be useful for determining how reference conditions for freshwater bioassessments are altered by climate change.

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