



Mayflies (Insecta: Ephemeroptera) and the assessment of ecological integrity : a methodological approach

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Abstract

Mayflies (Ephemeroptera) play an important role in almost all undisturbed freshwater communities and their larvae frequently form a considerable part of the material sampled during biomonitoring procedures. Mayfly taxa are widely accepted as bioindicators for water quality and form an integral part of standardized systems for the saprobic evaluation of running waters. Nevertheless, successful interpretation of data depends heavily on sampling strategies and the technical concepts adopted therein. The following paper discusses current problems affecting the correct assessment of mayfly community structure and proposes basic requirements to improve the meaningfulness of limnological routine sampling with regard to Ephemeroptera. The topics covered include: mayfly diversity in different types of freshwater communities in Europe; natural habitats and sampling sites / sampling strategies; life cycles and minimum sampling frequency; level of identification and interpretation of data.

Introduction

The assessment of ecological integrity provides information for environmental managers and decision-makers to take accurate and justifiable actions as well as to evaluate the effectiveness of legislative regulations already in force. While the political mandate to strive for ecological integrity has already been formally confirmed in legislation, the monitoring and assessment of the environmental status of surface water-resources has sometimes been hampered by a lack of conceptual clarity. For the benefit of both human society and nature itself, an effort must be made to ensure that national environmental policies are based upon the most comprehensive scientific information available.

Focussing on the insect order Ephemeroptera, this paper raises technical issues based on the present understanding of mayfly ecology, proposing an appropriate methodology for the establishment of standard operation procedures.

Technical questions addressed in this study include: (1) mayfly assemblages and diversity in differ-

ent types of Central European freshwater communities; (2) natural mayfly habitats and determination of habitats to be sampled (spatial sampling regime); (3) sampling equipment and sampling techniques; (4) life cycles and temporal sampling regime regarding minimum sampling frequency; (5) level of identification and identification problems in Europe, including a short review of the taxonomic situation in Ephemeroptera world-wide.

It is the main goal of this paper to walk the line between the needs of science and the financial and technical means of national monitoring projects. Applying the experience gained by taxonomic and faunistic research on Ephemeroptera will no doubt improve the results of limnological routine sampling and thereby contribute considerably towards a better understanding of the ecological integrity of aquatic systems.

Numerous studies demonstrate that mayfly community structure effectively reflects the environmental situation of watercourses. Since Kolkwitz & Marsson (1902) initially proposed the use of organismic indicators (including Ephemeroptera) to assess water pollu-

tion, their concept has proved highly successful, with mayflies forming an integral part of the taxonomic groups currently considered to be especially valuable for biomonitoring (Moog, 1993; Moog et al., 1997). When Illies & Botosaneanu established their river zonation concept in 1963, two species of Ephemeroptera (*Heptagenia sulphurea*, *Potamanthus luteus*) had been used to differentiate between the potamon and rhithron, together with Trichoptera (Lepneva, 1949), Plecoptera, and Coleoptera. Subsequent research led to a broader understanding of mayfly biocoenoses, revealing significant changes in diversity and community structure in connection with ecological changes along watercourses (e.g., Landa & Soldán, 1991; Krno, 1987, 1990; Sowa, 1975a). The application of modern theoretical concepts including trophic levels and functional feeding guilds (Cummins & Klug, 1979; Townsend, 1996; Vannote et al., 1980) is also supported by findings based on the distributional differentiation among Ephemeroptera.

Curiously, the significance of mayflies in indicating the ecological integrity of running waters has been widely neglected in the past (Karr, 1991), especially when compared with traditionally popular environmental indicators such as fish and bird communities (Errington, 1945; Kendeigh, 1934; Utschick, 1976). During the last decade, however, the so-called EPT-philosophy has successfully emphasized the outstanding importance of Ephemeroptera, Plecoptera, and Trichoptera in describing environmental conditions (Lenat & Barbour, 1994; Plafkin et al., 1989; Resh & Jackson, 1993). Besides water temperature, oxygenation and current, the availability of suitable mesohabitat structures (stratotopes, choriotopes) is clearly one of the most important factors influencing the occurrence and distribution of Ephemeroptera larvae (Cogerino et al., 1995; Krno, 1990, 1991). Ecological degradation of running waters caused by human impact (channelization, irrigation, impoundments) generally leads to deficiencies in or complete loss of characteristic natural structures, creating environmental uniformity and thereby significantly affecting mayfly diversity and abundance. Furthermore, interrelationships and possible cumulative effects between pollution and structural deterioration for mayflies are only poorly understood at present. The much higher drop in mayfly diversity in the potamon region is probably not due only to the (assumed) greater pollution in the lower courses of rivers or to technical problems in collecting. The availability of different mesohabitats in comparatively still natural (though

moderately polluted) rivers in eastern Europe versus in the heavily impaired watercourses in industrial Central Europe strongly suggests that channelization and impoundments are even more important than pollution. Under pristine conditions river dynamics create a mosaic of different, closely adjoining habitats. Changing currents and instable river banks allow for ramification, erosion, and gradual sedimentation. This in turn provides a rich variety of mesohabitats suitable for both rheophilic (riffle-dwelling) as well as stillwater forms inhabiting pools and stagnant backwaters.

Although the amount and frequency of monitoring studies have increased considerably within the last 20 years, great gaps still exist even in our basic knowledge. Autecological and faunistic studies of Ephemeroptera are rather limited at present and further co-operation between taxonomists and limnologists should prove especially fruitful. The urgent need for intensified research in the near future necessitates improving the meaningfulness of limnological routine sampling data. Although some of the problems dealt with in the present paper may appear rather trivial and have in fact already been discussed by Macan as early as 1957(a), experience shows that Ephemeroptera are still underrepresented in many monitoring programs. Understanding and considering group-specific requirements should perceptibly extend the basis for future data analysis.

Mayfly diversity in Central Europe

Under natural conditions mayfly diversity is directly correlated with habitat variety and therefore a modified 'Species-Deficit-Concept' ('Artenfehlbetrag'; Kothé, 1962) should effectively reflect the state of overall ecological integrity for a defined sampling area. The greatest impediment for practical application is the supposedly insufficient knowledge of the species inventory (as well as of the target list) for different sampling stations due to unknown distribution models and, subsequently, data incomparability. The outstanding importance of reliable faunistic baseline data and their value for future conservation projects is amply documented from long-term studies (Landa et al., 1997; Sartori & Landolt, 1998). In Europe at least, these difficulties may be overcome with regard to Ephemeroptera, providing that sampling standards are well defined and comparisons are restricted to the same river zone within the zoogeographical units defined by Illies (1978). The data

given in Bauernfeind et al. (1995) are a first step in that direction: they summarize records from Austrian watercourses in connection with limnosaprobity and zonation for all Ephemeroptera species. Table 1 shows as an example the zonal distribution of *Rhithrogena* species in ecologically intact streams and rivers in Austria. The average number of families and species in ecologically pristine to moderately disturbed conditions shows an increase of ephemeropteran taxa from the springs to the potamal sections (Figure 1).

The spring and springbrook zone (krenon)

No strictly steno–krenobiont taxa exist in Central Europe (Zollhöfer, 1997), but mayfly larvae may be numerous and at least some species are found in most types of springs. The occurrence of Ephemeroptera is apparently restricted to the meta- and hypokrenon, where species composition is similar to that in the upper courses of rivers (epirhithral), including euryzonal species as well. *Rhithrogena fonticola*, known so far only from the type locality, has been found in a small spring in southwestern France and may represent a krenophilic taxon. The suspected significance of *Acentrella sinaica** for the hypokrenal subzone (Zollhöfer, 1997) remains to be confirmed. Haybach & Fischer (1997) suggest that, along the northwestern border of its distribution area, *Ecdyonurus subalpinus* is restricted to springs. In Italy, *Electrogena gridellii* has also been found under similar conditions (Moog, 1995, unpubl.). These observations fit well into Kühnelt's (1943) concept of 'Regionale Stenözic', explaining the preference for different habitats under changing external conditions throughout the geographical distribution area of a species.

With regard to Ephemeroptera, diversity in the spring brook zone is comparatively low, seldom exceeding about five to seven species (one to three families) in Austrian karst springs (Weigand, 1998; Weigand & Tockner, 1996). In Switzerland a total of 19 mayfly species (five families) has been found in various types of springs, most of them rhithrobiontic or euryzonal (Zollhöfer, 1997). Whether all of the taxa recorded from the metakrenon finish their developmental cycle there, or whether they undergo a drift phase before emerging, is not known so far due to the lack of emergence trap data.

Species composition is mostly influenced by substratum structure (especially by the occurrence of leaf

litter and mosses), with minor human impact having little effect. More radical habitat changes like tapping of a spring or piping usually destroy the community altogether.

Streams and rivers (rhithron)

The mayfly community structure of Central European mountain torrents differs significantly above and below approximately 400 m NN (coinciding roughly with the lower border of the montane vegetation zone), presumably because it is dependent on characteristics of the surrounding terrestrial ecosystem as well as on current velocity/gradient. The autecological basis for this phenomenon is not well understood, but food resources may be a partial explanation. At present this interesting vertical faunistic cline cannot be explained convincingly with historical distribution patterns or the temperature regime alone (Moog & Wimmer, 1994), nor does it correspond exactly with the conventional understanding of the meta- and hyporhithron.

In Central Europe the upper zone is strictly characterized by *Baetis alpinus*, *Epeorus alpicola*, *Rhithrogena loyolaea*, *Rhithrogena alpestris*, and *Ecdyonurus helveticus*, the latter usually accompanied by at least one of the very closely allied taxa *E. austriacus*, *E. zelleri*, and *E. picteti*. *Electrogena lateralis* is also a typical member of the community throughout the Alps, whereas western populations occur at lower elevation. Species richness is highest in the genus *Rhithrogena*, of which five to eight species may be present at a single sampling station; unfortunately, species-specific ecological amplitude and distributional patterns remain poorly understood. Breitenmoser-Würsten & Sartori (1995) found a negative correlation between the number of species and the slope (and most probably altitude as well). Usually a total of about 15–20 mayfly species (five to six families) may be expected. Structural deficiencies are not always clearly reflected by reduced mayfly diversity, perhaps due to compensational immigration of azonal species, although the rhithron environment may be threatened by fragmentation in the future (Zwick, 1992).

Below 400 m NN in Central Europe, *Electrogena lateralis* is replaced by *E. ujhelyii*; *Ecdyonurus venosus* usually follows *E. helveticus* and its allies, sometimes forming a very short range of overlap. *Rhithrogena picteti* and *Ecdyonurus starmachi* clearly prefer streams, whereas *Rh. semicolorata* and *E. venosus* are usually found in rivers. Species diversity is

* Presumably based on a misidentification. Material received by Graf (pers. comm.) was identified as *Baetis alpinus*.

MAYFLY DIVERSITY (mean, range)

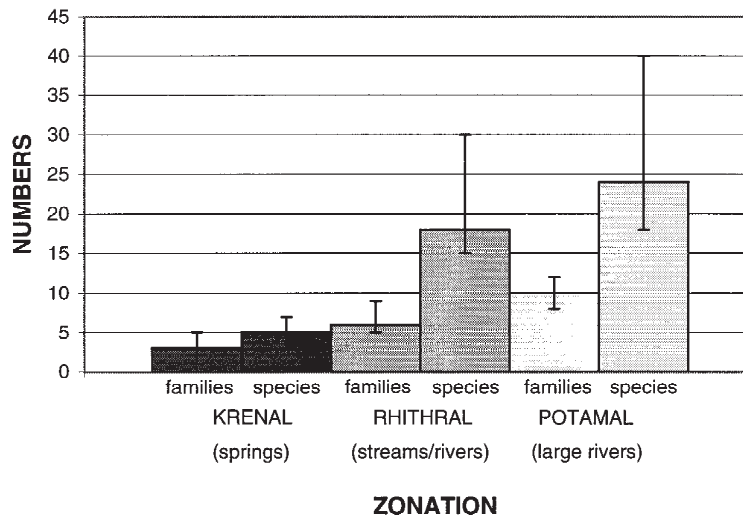


Figure 1. Average number of families and species in Austria. Ecologically pristine to moderately disturbed sampling stations

highest in the genera *Baetis* and *Ecdyonurus*. The occurrence of up to 30 species (seven to nine families) of mayflies reflects pristine environmental conditions or ecological integrity. Eutrophication, increasing water temperature due to extensive clearings of bank vegetation, the loss of riparian structures (roots, dead wood, sandy/silty back-currents, leaf litter) as well as a smaller variety of current/substratum types significantly reduce mayfly diversity, especially in the genera *Ecdyonurus*, *Rhithrogena*, and *Leptophlebiidae*. The phenomenon is also well reflected in a quantitative index (ratio Baetidae:Heptageniidae), which decreases progressively with decreasing altitude and/or increasing disturbance (Devan & Mucina, 1986).

The lower courses of rivers (potamon)

In ecologically intact potamalic rivers the extremely high diversity in Ephemeroptera is based on the variety of different habitat types, which provides a wide amplitude of temperature, current, oxygenation, and substratum conditions. More than 30 species (10–12 families) may be found in an undisturbed river section including connected backwaters and ramifications. Diversity is especially high on the genus and family level, the region being clearly characterized by the simultaneous occurrence of three or more species of *Heptagenia*. The replacement of *Ecdyonurus venosus*

by *E. dispar* (*E. aurantiacus* in southern and south-eastern Central Europe) is also significant. Structural losses are common, considerably affecting community structure and allowing for a quick and sound assessment even on a less precise taxonomic level. A sample containing members of *Rhithrogena* (*Rh. beskidensis*, *Rh. semicolorator*) and *Ecdyonurus* (*E. dispar*, *E. aurantiacus*, *E. ruffii*) together with at least two species of *Heptagenia* (or of the typical potamobiont families Isonychiidae, Oligoneuriidae, Potamanthidae, Polymitarcyidae, Prosopistomatidae, Palingeniidae) evidently indicates an ecologically intact environment. Loss of river dynamics disturbs riffle/pool sequences and is immediately reflected by the combined absence of *Rhithrogena* (riffle structures), *Ecdyonurus* (shingle banks) and *Leptophlebiidae* (pools/roots/leaf barriers). *Choroterpes picteti* is usually dependent on deep pools with macrophytic vegetation; its occurrence in backwaters may signalize the influx of cool groundwater, whereas *Paraleptophlebia weneri* (and *Siphonurus aestivalis*) prefer leaf barriers in riverine flood-ponds or dense vegetation in more stagnant waters. *Electrogena fasciocolata* and *E. affinis* are mostly found on dead wood or submerged roots, preferably along the edge of currents bordering still water. Steep clay banks were once colonized by the burrowing larvae of *Palingenia longicauda* (Sartori et al., 1995) and *P.*

Table 1. Species-specific distribution of the Austrian species of *Rhithrogena* according to biocoenotic region (Rhithral-Potamal-Concept), (10-point system; Moog, 1995)

	Spring	Springbrook	Epirhithral	Metarhithral	Hyporhithral	Epipotamal
<i>R. taurisca</i>	1	6	3	–	–	–
<i>R. loyolaea</i>	1	5	4	–	–	–
<i>R. nivata</i>	1	3	6	–	–	–
<i>R. alpestris</i>	–	3	7	+	–	–
<i>R. degrangei</i>	–	2	5	3	–	–
<i>R. rolandi</i>	–	1	7	2	–	–
<i>R. hybrida</i>	–	1	6	3	–	–
<i>R. endenensis</i>	–	1	5	4	+	–
<i>R. picteti</i>	–	+	7	3	–	–
<i>R. puytoraci</i>	–	+	5	5	–	–
<i>R. austriaca</i>	–	–	5	5	–	–
<i>R. puthzi</i>	–	–	5	5	–	–
<i>R. carpatoalpina</i>	–	+	4	5	1	–
<i>R. allobrogica</i>	–	–	4	5	1	–
<i>R. circumtatica</i>	–	–	2	5	3	–
<i>R. podhalensis</i>	–	–	2	4	4	–
<i>R. zelinkai</i>	–	–	4	4	2	–
<i>R. beskidensis</i>	–	+	+	2	5	3
<i>R. landai</i>	–	–	3	4	3	+
<i>R. savoienensis</i>	–	–	3	4	3	+
<i>R. vaillanti</i>	–	–	1	5	4	–
<i>R. gratianopolitana</i>	–	–	1	3	5	1
<i>R. iridina</i>	–	–	1	4	4	1
<i>R. semicolorata</i>	–	+	1	3	4	2
<i>R. hercynia</i>	–	–	+	4	4	2
<i>R. germanica</i>	–	–	–	2	5	3

fuliginosa, both species now threatened in Europe and restricted to a limited area in the east.

Special types of streams and rivers

In some cases low mayfly diversity is the result of extreme ecological conditions in the natural environment.

The alpine glacial brooks and streams

Such streams are typically inhabited by the highly specialized *Rhithrogena nivata*, almost invariably accompanied by *Baetis alpinus* and *Ecdyonurus picteti*. The joint occurrence of *Rh. loyolaea*, *Rh. alpestris*, *Rh. degrangei*, and *Epeorus alpicola* clearly indicates increasing pluvial influences. Most heptageniid species are absent in North European glacial streams. Human impacts such as minor eutrophication, artificial bank stabilization, or even channelization are

not reflected by immediate and obvious changes in the mayfly community, as long as key environment features (discharge regime, temperature, and bed-load situation) remain unimpaired.

Acidic streams and highland brooks

The high vulnerability of mayflies to acidification (compared with Trichoptera and some genera of Plecoptera) is well documented and commonly used for bioindication (Braukmann, 1992; Engblom & Lingdell, 1987; Fjellheim & Raddum, 1990; Guerold et al., 1991, 1993). The naturally low pH-value of brooks draining *Sphagnum* bogs and fens is reflected by an extremely species-poor Ephemeroptera community consisting mainly of *Leptophlebia vespertina* (in slow current only) and potentially *Ameletus inopinatus* in higher altitudes. *Leptophlebia marginata*, *Baetis niger*, and *B. buceratus* may also be found when conditions are favourable, but their ecological amp-

litude remains poorly understood. The same applies to *Heptagenia fuscogrisea*, which is common in some limestone lakes and slow-flowing brooks in Ireland as well as in some slightly acidic brooks in the Bohemian Forest and northern Europe.

Rivulets and ditches

The inconspicuous and very small watercourses draining wetlands and traditionally farmed meadows have rarely been included in biomonitoring programs. Dramatic changes in agricultural land use threaten these often neglected habitats and their characteristic mayfly assemblage, consisting typically of *Habrophlebia fusca* and sometimes *Paraleptophlebia cincta* (known among fly fishermen under the characterizing name 'Ditch Fly'). *Baetis niger* (replaced by *B. muticus* in more alkaline water) is also abundant, accompanied by more ubiquitous taxa (*B. rhodani*, *B. vernus*, *Centropetium luteolum*, *Cloeon simile*, *Caenis luctuosa* etc.). Eutrophication by agricultural sewage and channelization may significantly reduce diversity in favour of the tolerant ubiquitous taxa, whereas piping destroys the community altogether.

Intermittent streams

Lowland brooks and streams that dry up during summer are sometimes inhabited by mayfly species that are only rarely discovered elsewhere but are abundant where they do occur, e.g., *Metreletus balcanicus* and *Siphonurus armatus*. Suitable habitats are extremely scarce in Central Europe and should be of concern for nature conservation. Under similar conditions, species of *Caenis*, mostly *C. robusta* and *C. luctuosa*, may also be found along with the ubiquitous *Cloeon dip-terum*. All of them are very tolerant to eutrophication and relatively high water temperature and may quickly colonize slow-flowing or stagnant shallows that fall dry in autumn.

Stretches within karst streams that regularly fall dry during summer are recolonized by downstream drift and presumably from within the substrate; their vernal aspect in species usually does not differ significantly from their upper reaches, although only little systematic research has been conducted.

Large lowland rivers with sandy bottom

The river bed of large lowland rivers, consisting mainly of shifting sands, is extremely thinly inhabited by highly specialized mayfly species which are only rarely encountered by limnologists. Typical examples for eastern and southeastern Europe are *Behningia*

ulmeri, *Baetopus wartensis*, *Brachycercus harrisellus*, *B. minutus*, *Oligoneuriella pallida*, and *O. borysthen-ica*. *Ametropus fragilis* and *Electrogena affinis* are found almost exclusively among dead wood or rotting logs partly embedded in sand.

Different habitats and sampling sites

Due to high substrate specificity the mayfly diversity at a given sampling station is easily underestimated if certain (meso)habitats are unintentionally neglected; seasonal changes in habitat preference further complicate matters. In most *Ecdyonurus* species, nymphs accumulate in the mesolithal along shallows (Macan, 1957b), where they find suitable conditions for emergence in water less than 5 cm deep. Similar movements occur in most *Leptophlebiidae* (independent of water depth), whereas in *Oligoneuriella rhenana* emergence usually takes place in midstream (Reisinger, 1995). Nymphs of many species of *Baetis* and *Rhithrogena* inhabit the riffle section of streams and rivers, but may also emerge further downstream in relatively strong currents. Habitats with the same substrate composition but different water velocity or water depth often harbour different species or different developmental stages. Incomplete sampling will therefore seriously influence the results, not only with regard to the number of species found, but also by affecting the potential level of identification and by increasing the amount of time necessary for identification. Careful selection of sampling sites (including all visually different (meso)habitat types) is therefore crucial for correctly assessing ecological integrity by means of Ephemeroptera diversity. The comparison between different stations within the same river reach may be misleading or impossible if a different set of habitats has been sampled or if the samples have not been taken at approximately the same time of year. For longitudinal studies, sampling for all stations should be completed within 1 week.

The interpretation of limnological routine sampling data is often hampered by the incomplete record of mayfly taxa due to inadequate sampling strategies, frequently combined with poor taxonomic standards or insufficient level of determination. When Danecker (1992) analysed a 16-year water quality survey based on 1017 samples from more than 50 sampling stations in southeastern Austria (Burgenland), single samples on average contained 10–20% of the total species number found, but some samples covered as little

as 0.6% of the species later found at the same station. Clearly, the basic requirements for successfully monitoring mayfly diversity include a spatial and temporal sampling regime as well as suitable sampling equipment and techniques. Sampling strategies must be carefully adapted to the individual watercourses, as described for instance in the Austrian Standard ÖNORM M 6232 (1997). The final establishment of sampling sites and sampling locations requires at least one preliminary site survey, preferably during low water discharge in autumn.

Sampling equipment and sampling techniques

Although numerous sampling techniques and devices have been described and successfully used in the field (see review in Elliott & Tullett, 1978, 1983), only relatively few have proved to effectively collect representative mayfly larvae samples. The most versatile piece of equipment is a simple triangular handnet (ISO 7828) with a side length of approximately 20 cm that may be attached to a long handle for use in deeper water. A relatively short, shallow net bag (not drawn out acutely) about 20 cm long and made of stiff plastic or wire allows for quick and easy sorting out of live specimens, which is often preferable to fixing of the total net contents. The advantages of sorting live samples in the field include better direct control of collecting success, better preservation, less damaged specimens and a considerable saving of time. For monitoring purposes a mesh size between 0.5 and 1.0 mm is most suitable – young larvae usually cannot be identified with any reliability, and debris held back by a finer mesh unnecessarily complicates sorting. The same net should be used for kick-sampling (all substrates), sweeping through roots and submerged plants, scraping the moss-covered surface of boulders, and sieving rotten leaves. This method yields comparable semi-quantitative results when applied in a standardized manner, e.g., 10 ‘sweeps’, 10 ‘kicks’ (disturbing approx. 400 cm² of substrate) each for the different mesohabitats. Sorting must be done after every single kicking or sweeping action and therefore a fixed number of actions is preferable to given time standards for collecting. Especially in Ephemeroptera the equipment and techniques described above have many advantages over (supposedly) more quantitative sampling techniques (Pescador et al., 1997): the small net is fit for use even under restricted circumstances like very low water, along the broken edge of shingle

banks, among submerged roots, or in close conditions and patchily distributed small habitats. The timesaving qualities allow a greater number of samples to be taken per unit time, increase efficiency and comparability, and lower costs. Sampling large rivers efficiently is always difficult (Petto et al., 1991) and no special recommendations can be given for Ephemeroptera. Standard methods are described in Clifford et al. (1989), Humpesch & Elliott (1990) and Ofenböck (1998). Under extreme conditions, acceptable results can be obtained by scuba-diving. Even in the most frequently investigated rivers, some substrates/habitats are far more difficult to sample than others. Leptophlebiidae, Siphonuridae, Ephemeridae, and some Caenidae are often underrepresented or even missing in routine samples because they prefer habitats that are neglected or insufficiently/ineffectively treated. Big boulders as well as barriers of leaf litter, accumulated dead leaves, sand and mud are major obstacles for the collector, and a far greater number of samples must be taken to allow for these difficulties. When sampling among leaves, sand and mud, only very small quantities of substrate should be taken into the net, otherwise cryptic species are easily overlooked. Larvae of the potamobiont genus *Baetopus* inhabit the thin layer of silt or F-POM on boulders in the riprap section of channelized rivers, whereas *Ephemerella mesoleuca* may be found hidden in small crevices of the same habitat. Under more natural conditions both have been found among submerged vegetation and gravel in relatively swift current. Nymphs of *Oligoneuriella* and *Epeorus* may cling persistently to the rough surface of big boulders in deeper water and strong current, where they are very rarely obtained by mere ‘kicking’. The same applies to the small larvae of the nearly extinct potamobiont *Prosopistoma foliaceum*. Shifting sands are extremely thinly inhabited. They require taking a great number of substrate samples over a longer period as well as the use of artificial substrates, drift nets, and light traps. Uniform stretches of sandy bottom in smaller streams may be effectively sampled using a shovel sampler (Jazdzewska, 1997).

The aim of ultimately obtaining a complete species inventory for a given locality is greatly enhanced if light traps are used parallel to the more conventional bottom sampling techniques. This applies especially to large rivers or localities otherwise difficult to sample. The additional use of light traps is highly recommended for biomonitoring projects and is considered to be state-of-the-art for Trichoptera, Ephemeroptera and Plecoptera (Reusch, 1995; Usseglio-Polatera, 1997).

This technique considerably reduces costs by reducing sampling frequency, yielding an increased range of taxa, and simplifying determinations.

Life cycles and minimum sampling frequency

Life history parameters are part of the framework underlying 'ecological plasticity' in mayflies (Brittain, 1991) and basically limit the ability to withstand both natural and man-made ecological disturbances. The different life cycles of Ephemeroptera (Landa, 1968; Sowa, 1975b) clearly influence both the completeness and comparability of samples. Furthermore, among the Heptageniidae (which comprise about 45% of the mayfly fauna of Central Europe) only fully grown nymphs can be reliably identified on the species level. Investigations that continue to neglect these considerations impair ecological and faunistic interpretations (Bauernfeind, 1998). The expense of field work makes the issue of minimum sampling frequency crucial for any biomonitoring program. As Reusch (1985, 1995) and others have pointed out, monthly samples over at least 1 year are the only means to obtain objective data on insect community structure in streams and rivers. For economical reasons, restrictions towards two to four samples a year, depending on altitude or insect groups, have been proposed (Finck, 1998; Finck et al., 1992; Holm, 1989; Peissner, 1992). In the case of Ephemeroptera the minimum sampling frequency for compiling an accurate species inventory at a given locality should be determined individually by considering the range of theoretically expected taxa based on altitude, river zonation, and discharge dynamics. A rough guideline for choosing sampling dates is provided in Bauernfeind (1994, 1995) and Marrer (1992) for most Central European species and information on zonal distribution is listed in Moog (1995). A longitudinal survey covering more than one river zone requires a higher sampling frequency than a more restricted local investigation. A minimum sampling scheme suitable for many rivers in Central Europe should at least consider the following sampling dates:

Krenon

planar-submontane (0–500 m): April and July
montane-alpine (500–2000 m): May and August

Rhithron

planar-submontane: March, May, July
montane-alpine: May and August

Potamon

March, May, July, October

Nevertheless, a higher sampling frequency is desirable whenever possible. In the planning phase, monitoring projects should consult local faunistic and taxonomic papers for pertinent information on life cycles. For qualitative aspects a 3-year study period and monthly samples are almost indispensable.

Level of identification

From the scientific point of view indicators must be accurately identified to species (De Pauw & Vanhooren, 1983; Furse et al., 1984; Hilsenhoff, 1987; Marten & Reusch, 1992; Moog et al., 1997; Resh & Unzicker, 1975; Rosenberg et al., 1986). Most biological monitoring techniques are dependent on correct identifications. Better taxonomy produces more accurate results and clearly increases the precision of site classifications, improving the ability to detect subtle changes in environmental quality (Lenat & Barbour, 1994). Although higher taxonomic units have occasionally been proposed either for rapid assessment of water quality (Alba-Tercedor & Sanchez-Ortega, 1988; Hilsenhoff, 1988) or to overcome difficulties of identification ('morpho-taxonomic groups'; Buffagni, 1997), the loss of biological information is a serious drawback, even if the method itself is adequate for the purpose stated. The ready availability of published data on ecological and faunistic research is an additional argument for identification on the species level.

Species identification in Ephemeroptera does present certain difficulties, but this is balanced by the relatively small number of taxa and comparatively good knowledge of both larval and imaginal stages.

Requirements for species level identification

Modern regional keys and revisions are available for most of Europe (see detailed review in Studemann et al., 1992; Bauernfeind, 1994; Engblom, 1996), whereas the taxonomic basis for identifying Ephemeroptera from the Balkans and south-central to southern Europe remains unsatisfactory. For Russia and adjacent countries, the identification of most species was recently summarized by Kluge (1997). Keys and descriptions published before 1980 contain valuable information for the specialist but should be used with caution by the less experienced: the evaluation of

diagnostic characters has changed considerably and many species have since been newly described.

For North and Central America, regional keys on the species level were discussed by Edmunds et al. (1976) and the environmental requirements and pollution tolerance of Ephemeroptera have been dealt with in detail (Hubbard & Peters, 1978a). A more recent checklist for North America is now available (McCafferty, 1997), along with an online database described by McCafferty (1996). For Central and South America the taxonomic basis is still rather incomplete and keys exist only for small groups or geographically limited areas. Checklists including nominal species have already been prepared by Hubbard (1982) and McCafferty & Lugo-Ortiz (1996). A recent generic level key that also includes some species is now available for Argentina (Dominguez et al., 1994).

With the exception of Japan (Gose, 1979–1980) no modern keys on the species level are available for any major area in Asia, although checklists have been provided for India (Hubbard & Peters, 1978b), China (Gui Hong, 1985) and Korea (Bae et al., 1994).

Comprehensive information on mayfly species in Australia was fully summarized by Campbell (1988), and recently a key to genera for mayfly nymphs was provided by Dean & Suter (1997). Identification of Ephemeroptera remains rather difficult in Africa; since the annotated list by Demoulin (1970), no comprehensive or regional keys for more general use have been published. Revisional work, however, is progressing rapidly and a provisional checklist of South African Ephemeroptera has recently been published (McCafferty & de Moor, 1995).

High species diversity in some genera and close morphological similarities between certain species still impede the more general use of Ephemeroptera as indicators for ecological integrity, even in Central Europe. Nevertheless, experience shows that many identification problems actually arise from inadequate sampling strategies, inefficient collecting, or poor preservation. All of these can be easily avoided.

Larval identifications require much care because most key characters only apply to fully grown mature nymphs. No reliable keys are available at present for early instars, and misidentifications even at the genus or family level are possible.

Basic technical requirements for preservation and preparation are discussed in Edmunds et al. (1976) and Bauernfeind (1994). For documentation and future research the material collected should be preserved permanently to allow for comparison and re-evaluation.

A sufficient number of undamaged voucher specimens should be housed in an institution where curatorial care is guaranteed.

Selected groups and identification pitfalls (Central Europe)

Heptageniidae probably represent the most difficult family and instruction by trained taxonomists is recommended. Especially in *Rhithrogena*, problems are numerous and benthos samples should be accompanied by light trap catches. The use of reference collections is often necessary and keys should be used with discretion. Egg chorionic structures provide valuable additional information for identifying mature female nymphs and most of the discriminating characters are already visible at a magnification of about 400×, provided a suitable mounting medium is used. In *Ecdyonurus*, mature nymphs and male imagines can usually be correctly identified (Bauernfeind, 1997; Hefti et al., 1989), but some training is essential. To acquire the necessary experience the use of reference specimens is advisable. *Electrogena* comprises only a few species in Central Europe, most of them easily recognisable in the nymphal and imaginal stage; the separation of *E. fasciocolata* and *E. affinis* may prove more difficult (Malzacher, 1996*; Sowa, 1984). The genus *Heptagenia* should provide no difficulties and even half-grown larvae are readily identifiable with the aid of current keys.

Oligoneuriidae are represented by the single species *O. rhenana* in Central Europe, but the geographic range of closely allied taxa from eastern and south-eastern Europe may show some overlap. For dubious specimens, consult Mol (1984). Discriminating characters are difficult to interpret and comparison with reference material is advisable.

In Siphonuridae, only fully grown mature nymphs and imagines of both sexes are identifiable with certainty and slide preparation may be necessary. Distinct genital characters are discernible in male nymphs.

Most Baetidae can be identified in the larval stage, whereas male imagines require more experience. Although very time consuming, careful slide preparation is a prerequisite to become sufficiently acquainted with variations in the characteristic pronotum pattern in *Baetis*, which usually allows for quick separation of most of the material. Larvae of *Centroptilum* s.l.

* *Electrogena rivuscellana* Sartori & Landolt is currently considered a junior synonym of *E. ujhelyii* Sowa. See Belfiore & Desio (1995).

(usually included in *Procloeon* Bengtsson or *Pseudocentropilum* Bogoescu, see McCafferty & Waltz, 1990) are poorly characterized morphologically and only incompletely dealt with in most keys; the descriptions in Keffermüller & Sowa (1984) should be consulted. Egg chorionic structures are usually distinctive. Fully grown nymphs and imagines of Leptophlebiidae are readily identifiable to species, but the interpretation of gills and abdominal colour pattern requires some experience and well-preserved, undamaged specimens. The shape of spines on the surface and border of femora provide valuable diagnostic characters, but correct interpretation is dependent on adequate slide preparation and reference material is desirable for the less experienced.

Identification of most Ephemerellidae species poses no difficulties; the gill arrangement and the shape of gill II are characteristic features. Note, however, that the well-known ventral abdominal markings in *E. notata* are not exclusively distinctive and variation in colour patterns is generally extremely high in this family.

Ephemeridae include only five species in Central Europe, but differentiating morphological characters are poorly developed; also, abdominal markings in nymphs are less distinctive and more blurred in nymphs than in most imagines. Larvae of the rare potamobiont *Ephemera lineata*, for example, may easily be confused with the common rhithrobiont *E. danica*.

Although most Caenidae can be readily identified in the larval, subimaginal, and imaginal stage, some experience and microscopical preparations are necessary to avoid misidentifications. Morphological characters for separating nymphs of the more rhithrobiont *Caenis beskidensis* from the potamobiont *C. pseudorivulorum* are sometimes misleading and identification of single specimens may remain doubtful.

The remaining families Isonychiidae, Potamanthidae, Polymitarcyidae, Palingeniidae and Prosopistomatidae are each represented only by a single species in Central Europe and identification of nymphs as well as of half-grown larvae should cause no special problems with current keys. Their status, however, is precarious in Europe, and any records would be of especial interest. Forthcoming specimens should at any rate be permanently preserved.

Summary

Successful assessment of ecological integrity by means of Ephemeroptera diversity data, obtained by biomonitoring or more specialized studies, depends on a careful evaluation of the basic criteria influencing the spatial and temporal distribution as well as the abundance of mayfly larvae.

A thorough study design should include the following aspects:

- (1) Evaluation of the potential species inventory of a given river section with regard to zoogeographic distribution, longitudinal zonation, and typological aspects which affect the regional patterns of species assemblages. Major river types and their characteristic mayfly communities are discussed for Central Europe, concentrating on longitudinal and altitudinal typologies.
- (2) The distribution and abundance of mayfly communities is strongly dependent on habitat composition and mesohabitat structure. Compiling a nearly complete species list therefore requires sampling all habitat types available.
- (3) Seasonal variations are reflected not only in different abundance and distribution but may also influence identification considerably. Problems may arise especially when comparing samples with a theoretically derived target list or with samples taken at different seasons or stations. Guidelines for a suitable temporal sampling scheme are discussed.
- (4) Successful sampling depends on suitable equipment and sampling techniques, adapted to allow for the patchy distribution of mayfly nymphs as well as for the different structure in the various habitats. A simple handnet is cost effective and can be used with maximum versatility in medium-sized streams and rivers.
- (5) The correct use of ephemeropteran assemblages to assess ecological integrity must be based on identification on the species level. The taxonomic situation world-wide and possible identification pitfalls in Central Europe are discussed in brief.

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