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THE USE OF CHIRONOMID PUPAL EXUVIAL TECHNIQUE (CPET) IN FRESHWATER BIOMONITORING: APPLICATIONS FOR BOREAL RIVERS AND LAKES

FACULTY OF SCIENCE,
DEPARTMENT OF BIOLOGY,
UNIVERSITY OF OULU

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Academic dissertation to be presented, with the assent of the Faculty of Science of the University of Oulu, for public defence in the Auditorium of Kouvola-talo (Varuskuntakatu 11, 45100 Kouvola), on January 11th, 2008, at 12 noon

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Supervised by
Professor Timo Muotka
Doctor Riku Paavola

Reviewed by
Professor Peter S. Cranston
Professor Willem Goedkoop

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Faculty of Science, Department of Biology, University of Oulu, P.O.Box 3000, FI-90014 University of Oulu, Finland
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Abstract

In this thesis, I used the Chironomid Pupal Exuvial Technique (CPET) to detect anthropogenic impacts and to determine chironomid species composition in boreal rivers and lakes. The main objectives of the thesis research were i) to evaluate the importance of timing of sampling in the use of the CPET method (I, II), ii) to identify chironomid indicators of different environmental conditions (II, III, IV), and iii) to compare performance of the CPET method and more traditional sampling techniques in detecting anthropogenic impacts and chironomid species composition (III, V). I also determined emergence patterns of lotic chironomids in southern Finland (II, IV).

Timing of sampling was found to be a critical design factor in the application of the CPET, especially if the trophic gradient between study sites was short. Sampling occasions need to match with the emergence periods of indicator chironomid taxa to ensure the maximum likelyhood of detecting human impacts, if any exist. However, the optimal timing of sampling varies spatially and is dependend on several environmental factors, such as latitude, altitude and trophic gradient. The shift in taxonomic composition of emerging chironomids was found to be especially rapid in spring, and tended to decrease towards autumn. This was probably due to the short emergence periods of some spring-emerging univoltine species, with their annual emergence taking only a few weeks. In contrast to whole genera, the detection of a certain species may require accurate timing of sampling. Thus, among-site differences observed at species level may reflect spatially varying emergence patterns rather than true differences in community composition. On the other hand, because of the among-species variation in species' tolerances towards, for example eutrophication, genus level identification may mask subtle differences between study sites. Nevertheless, for most monitoring purposes genus level identification seems practical and adequate, although species level resolution is desirable.

Comparisons of the CPET method and more traditional grab sampling showed that pupal exuvial samples provided a more complete picture of the chironomid fauna, and that this information was obtained cost-effectively. Further, the integrative nature of the CPET was found to be critically important in the assessment of both lotic and lentic habitats. Sampling only a single macrohabitat type may result in biased estimates of the ecological condition of the whole water body. Further, in comparison to profundal grab samples, integrating species from various habitats using the CPET method appeared to have only a minor negative influence on the signal strength.

Determination of emergence patterns of lotic chironomids showed that nearly 200 chironomid species occurred frequently in rivers of southern Finland. A major proportion of species richness was accounted for the sub-families Chironominae (emerging mainly during the summer months) and Orthocladiinae (spring and autumn). Overall, these studies demonstrated that the CPET is a cost-effective and sensitive method for the assessment and monitoring of freshwaters, and should be considered as an alternative and/or supplementary tool to more traditional sampling methods.

Keywords: benthic macroinvertebrates, biomonitoring, CPET, lakes, rivers

Joki

Kymi äitinä seutua hyväilee
tämän kaupungin kohtuna kosket,
se nähnyt on lastensa kyyneleet
myöskin riemusta hehkuvat posket.

Se nähnyt on ihmisten uurastavan
suven lämmössä kypsyvät sadot,
ja piippujen pilviin kohoavan
nyt kahlivat kuohuja padot

Sen syvissä uineet on miljoonat
ikimetsien kuuset ja hongat,
sen suvantopinnoilla kuvastuvat
sinitaivaalta valkeat longat.

Ja sen vaellusreittiä reunustaa
niityt, lehdot ja kalliokuilut,
työn äänet, tuotannon humina,
kesäiltoina lintujen huilut.
Kymi äitinä seutua hyväilee
ja vaieten kuormaansa kantaa,
monet katsoo nyt inhoten vanhukseen
joka saanut on kaikkensa antaa.

Paavo Smeds

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Valkeala, November 2007 Janne Raunio

List of original papers

The thesis is based on the following original papers, which are referred to the text by their Roman numerals:

I Raunio J & Muotka T (2005) The use of chironomid pupal exuvia in river biomonitoring: the importance of sampling strategy. Archiv für Hydrobiologie 164: 529-545.

II Raunio J, Paavola R & Muotka T (2007) The effects of emergence phenology, taxa tolerances and taxonomic resolution on the use of Chironomid Pupal Exuvial Technique in river biomonitoring. Freshwater Biology 52: 165-176.

III Raunio J, Ihaksi T, Haapala A & Muotka T (2007) Within- and among-lake variation in the benthic macroinvertebrate communities – comparison of profundal grab sampling and chironomid pupal exuvial technique. Journal of the North American Benthological Society 26: 708-718.

IV Raunio J & Paasivirta L (2007) Emergence patterns of lotic Chironomidae (Diptera: Nematocera) in southern Finland and the use of their pupal exuviae in river biomonitoring. Fundamental and Applied Limnology (Archiv für Hydrobiologie) (in press).

V Raunio J & Anttila-Huhtinen M (2007) Sample size determination for soft-bottom sampling in large rivers and comparison with the Chironomid Pupal Exuvial Technique (CPET). River Research and Applications (in press).

Contents

1 Introduction

Surface water monitoring in Finland started in the 1960s. The so-called “polluter pays”-principle was included into the Water Act, set in 1961, which formed the basis for the national obligatory surface-water monitoring. Monitoring of lakes, rivers and coastal areas were first based on water chemistry alone, but more emphasis was soon placed on monitoring also the biomass and community structure of the biota. Research efforts were primarily focused on lakes and coastal areas, and studies of lotic ecosystems have remained relatively scarce. Regardless of their obvious importance for the society (e.g. as an energy and freshwater resource), large rivers in Finland have remained rather poorly studied. Thus, similar reseach tradition as for lakes has never formed for large river research in Finland. Until very recently, biomonitoring methods designed for lentic environments have been applied, mainly without criticism, to also lotic environments.

During the early phase of national surface water monitoring, numerous Finnish rivers and lakes were drastically polluted and even relatively robust methods were adequate for detecting anthropogenic impacts then. River Kymi (SE Finland), for instance, suffered from severe impacts from industrial and domestic waste waters, and during the 1960s biomassess and densities of benthic macroinvertebrates at the most polluted areas of the river well exceeded the levels documented for other boreal rivers before and ever since; 1.8 million individuals and 7.2 kg m^{-2} (Pankakoski 1968)! Since that, however, water quality of even the most polluted rivers has substantially improved (e.g. Niemi *et al*. 1997), while routine monitoring practices have experienced little progress. In River Kymi the impacted areas have recovered from highly polluted to a good/satisfactory ecological condition (Raunio & Soininen 2007), although toxic compounds associated with the past wood-processing industry still remain in river sediments. With improving water quality, however, more sensitive methods are now required to reliably assess human impacts. It is therefore likely that at least some of the present-day monitoring methods may not meet the new challenges set for surface water biomonitoring by the European Water Framework Directive (EC Parliament and Council 2000). Thus, alternative and/or supplementary assessment methods may be required and comparative studies of potential methods are necessary before preference for any method in any environment is given. Unfortunately, comparative studies on the performance of different biomonitoring methods have remained relatively scarce thus far.

Benthic macroinvertebrates are the most commonly used biological group for freshwater monitoring (Hellawell 1986, Rosenberg & Resh 1993). Assessment of lakes has traditionally relied on profundal communities. In streams and rivers assessments are usually based on benthic macroinvertebrate communities in riffles, although in large rivers benthic samples are often collected from pools (due e.g. to high current speed and/or low availability of riffles). Accordingly, benthos of lake profundals have been thoroughly studied and community composition in lakes with different trophic status is rather well documented (e.g. Sæther 1979). Further, predictive assessment systems for riffle communities, such as the RIVPACS approach (Wright *et al*. 1998), have been developed and are widely applied. Reference condition approach developed in the RIVPACS has also been adopted in the Water Framework Directive. Although sampling methodology and assessment systems for lakes and smaller rivers appear to be well established, large rivers still remain as a "shadow region" in this regard. A different kind of sampling approach for their monitoring may be required, as many traditional sampling techniques are often difficult to use in deep, unwadeable rivers (Ofenböck & Moog 2000, Downes *et al*. 2002).

Alternative or supplementary sampling and assessment systems may also be required to assess lakes and smaller rivers properly. For example, the profundal of humic lakes may suffer from oxygen depletion (Fulthorpe & Paloheimo 1985, Crisman *et al*. 1998) and thus may not represent environmental conditions of the whole lake. Furthermore, compared to littoral habitats, profundal may provide less robust estimates of some anthropogenic stressors such as acidification (Johnson 1998) or regulation (Palomäki & Koskenniemi 1993). In addition, some studies have demonstrated that benthic macroinvertebrate communities in riffles may be rather tolerant toward changes in river water quality (Silveira *et al*. 2005), being more significantly related to geographical constrains and neighbourhood dispersal processes, and local environmental factors such as current speed, moss cover, sediment quality and riparian vegetation (Heino & Mykrä 2006, Mykrä *et al*. 2006). Therefore, it might be desirable to base assessments on benthic communities from multiple depths and habitats (Bonada *et al*. 2006). However, traditional sampling methods (e.g., grabs, corers and handnets) are often inefficient and difficult to apply in some sediment types, and large numbers of sample replicates are required to adequately represent even a single macrohabitat type (Veijola *et al*. 1996, V).

The insect family Chironomidae (Insecta: Diptera) occupy a key position among benthic macroinvertebrates, as the family is typically the most abundant,

species rich and widely distributed macroinvertebrate taxon in freshwaters (Pinder 1986, Ashe *et al*. 1987). Diversity of chironomid species is exceptional with an estimated number of species worldwide as high as 15 000 (Cranston 1995). Chironomidae inhabit virtually every kind of aquatic habitat: the profundals of the deepest lakes to high altitude streams, freshwater and saline environments, tropical and arctic regions, temporary and permanent waters, and severely acidic as well as pristine waters (Pinder 1986, Ashe *et al*. 1987, Kelly 1988, Cranston 1995). In Finland, more than 750 taxa have been recorded (for more information see www.saunalahti.fi/jailmon), and new species for the country have recently been discovered and yet some remain to be found. Chironomid species richness in Finland is higher than that of mayflies, stoneflies and caddisflies put together (see Nilsson 1997). As a species-rich family, chironomids offer a whole spectrum of responses to environmental stress (Rosenberg 1992), and inclusion of chironomids would be essential in any assessment or biomonitoring program. In river assessments, however, and particularly in those focusing on riffle habitats, chironomids are often ignored or identified only to a coarse taxonomic level. Instead, more emphasis is placed on studying other common groups, such as mayflies and caddisflies, presumably because of easier species identification. Thus, a significant part of the aquatic biodiversity and vital information for environmental assessment is ignored if Chironomidae are excluded or identified only to a high taxonomic level.

Due to the difficulties in chironomid larval identification and sampling of benthic fauna in large rivers, alternative approaches such as the Chironomid Pupal Exuvial Technique, CPET (Coffman 1973, Wilson & Bright 1973, Wilson & Ruse 2005), have been suggested. The CPET method has shown great promise for the biomonitoring and assessment of both lotic and lentic ecosystems (e.g. Ferrington *et al*. 1991, Ruse 2002, Calle-Martínez & Casas 2006). Although suggested first by Thienemann (1910) almost a century ago, the method has been used rather rarely in Finland or in other countries of the boreal region. Wilson & Ruse (2005) list the major advantages offered by the CPET method as follows:

1. Comparable samples may be taken throughout a complete catchment, from source to mouth of a river system, including any interventing lakes.
2. Samples may be taken easily from deep muddy rivers, canals and lakes, as well as from riffles in shallow rivers.
3. CPET provides an integrated sample of chironomids from all of the aquatic microhabitats.

4. Field sampling is free from operational bias, and can be carried out quickly by semi-skilled personnel. It causes minimal disturbance to the water-body during sampling.
5. The identification of chironomid pupal exuviae to genus level is relatively easy using available keys
6. CPET analysis is straightforward, and the data may be represented as graphs or joint plots, or may be further analysed by multivariate techniques. The results may be compared to chemical and physical data, and can be dove-tailed into other systems of biological assessment.

The advantages listed above are evident and appealing, but the disadvantages, drawbacks and reservations related to the CPET method may ultimately determine whether the method should be used or not. These factors, however, have received relatively little attention thus far in the scarce scientific litterature concerning the method. Perhaps the most common argument against the use of the CPET method in monitoring and assessment is the fact that pupal exuviae in a sample are derived from an ill-defined area upstream or upwind of the site. The distance drifted by pupal exuviae can be relatively long, i.e. up to 1.0-1.5 km in large rivers, but is usually considerably less in smaller streams and rivers (i.e. <250 m) (Wilson & Ruse 2005). Therefore, pupal exuviae may have drifted to the sampling site from upstream of the monitored point source of pollution. Thus, a collection of pupal exuviae at least few hundred meters downstream of the monitored site will most likely represent species capable of completing their aquatic life stage in the area. It is also unlikely that the distance between sampling sites in rivers is shorter than maximum distances drifted by pupal exuviae. In contrast, a common argument supporting the use of benthic macroinvertebrates in biomonitoring is that they are sedentary (Rosenberg & Resh 1993). In practice, it is often forgotten that their larvae, too, drift downstream (Williams 1989). Drift of aquatic insects is a well described phenomenon (e.g. Allan 1995), and drifting may be either active (e.g. predator avoidance and colonisation of new habitats) or passive displacement via current or flood. Larvae found in benthos samples thus may represent species recently colonized the area or displaced from upstream reaches. Although the distance drifted by larvae in a day is usually calculated to be <50 m (e.g. Hemsworth & Brooker 1979), Hershey *et al*. (1993) estimated that minimum drift distance by *Baetis* was 2.1 km for one-third to one-half of a nymph population during the three summer months of June to August.

Another argument against the use of the CPET is that several samples need to be collected within a year to obtain a reliable picture of chironomid communities. However, seasonality in emergence patterns affects larval sampling as well, with the exception that the effect of timing of sampling is often the opposite to when using the CPET. For instance, in order to detect some of the important indicator species in lakes, pupal exuviae should be collected in spring (III), but the fourth-instar larvae of these species can best be found in autumn (Johnson *et al*. 1990). Although early-instar larvae of at least multivoltine chironomid species may be found throughout the open water season, these usually cannot be identified to a low taxonomic level. Thus, more than one sampling period within a year would be desirable in any benthos survey, but this is rarely the case. Further, the total number of pupal exuvial samples will also be lower than in more traditional sampling methods because replicate samples are not usually collected.

Finally, CPET can be critizised of focusing on a sole group of organisms, when many other more familiar groups of macroinvertebrates exist. Many chironomid species also have shorter life cycles than other benthic macroinvertebrate taxa. Since long life cycles of benthic macroinvertebrates is considered advantageous for monitoring purposes (Rosenberg & Resh 1993), the use of chironomids as a sole group can be challenged. Although some small, multivoltine species may have rapid life cycles, chironomids also include species with long lifecycles, that is, one generation per year or less (Tokeshi 1995). Further, small multivoltine species tend to be poor indicators of water quality (IV) and their value in assessments is often low. Chironomid pupal exuvial samples also provide information of (indicator) species, such as *Glyptotendipes cauliginellus* (Langton 1991), that have not been found in benthos samples. Ultimately, the justification for using chironomids lies in their ubiquity, species richness, high ecological diversity, and the very high number of individuals (Coffman 1995).

In my thesis, I have used the CPET method in lakes and rivers and tested its performance in relation to other sampling techniques, with special emphasis on its application to freshwater biomonitoring. I also aimed to test if the CPET method could solve some of the common problems related to sampling of benthos and use of Chironomidae in freshwater biomonitoring. With these studies I aimed to provide information of chironomid species and their behaviour that would facilitate the use of Chironomidae in biomonitoring and would be essential in designing effective monitoring programs, based on the CPET method.

2 Objectives of the thesis

The main objectives of the thesis were to evaluate the importance of the timing of sampling in the use of the CPET method (I, II), to identify indicator chironomid taxa for different environmental conditions (II, III, IV), and to compare performance of the CPET method with more traditional sampling techniques in detecting anthropogenic impacts and chironomid species composition (III, V). We also aimed to determine the emergence patterns of lotic chironomids in southern Finland (II, IV) and to estimate the taxonomic coverage of monthly CPET samples (I). Finally, we estimated the adequate sample size for the use of grab sampling in large boreal rivers (V).

3 Material and methods

3.1 Study area

Material for this thesis was collected from southern Finland (Fig. 1). Medium-to-large sized rivers were particularly sampled (I, II, IV and V), but for one study, lakes were included as well (III). Since the focus of the thesis was on large rivers, R. Kymi (SE Finland), was sampled in all river studies (I, II, IV, V).

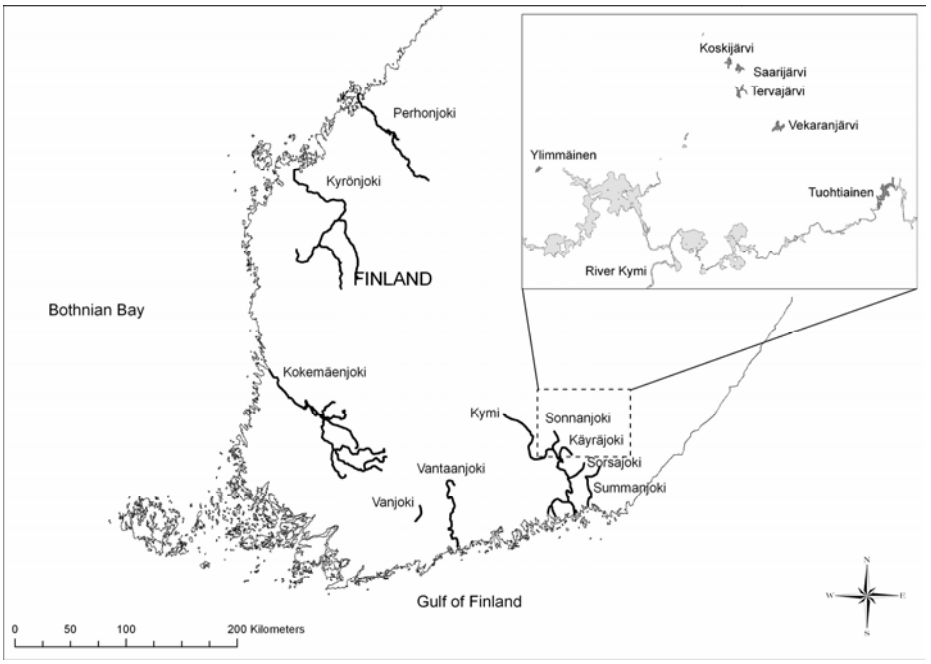

Fig. 1. Location of the study rivers and lakes in southern Finland.

3.2 Sampling and sorting chironomid pupal exuviae and benthic macroinvertebrates

Chironomid pupal exuvial samples were collected by scooping floating debris with a handnet (frame diameter: 30 cm, mesh size 250 µm) along river and lake margins, especially in accumulation areas (e.g. leeward shores in lakes) indicated by foam and floating material, and behind obstacles (see Ferrington *et al*. 1991,

Wilson & Ruse 2005, SFS-EN 2006). It should be noted, however, that alternative sampling techniques for chironomid pupal exuviae exist, such as drift-netting flowing waters (e.g. Hardwick *et al*. 1995). A collection period of 15-30 min was usually adequate to reach the recommended sample size of at least 200 exuviae (Wilson & Ruse 2005). At some sites and occasions, however, the recommended number of exuviae was not reached with the standard sampling effort, primarily reflecting the low densities of emerging taxa and/or low retention of exuviae at these sites. It should be noted that the number of exuviae collected cannot be determined in the field as many exuviae are small, transparent and indistinguishable by naked eye from the foam and debris. By sampling accumulation areas the potential bias caused by variable diel emergence patterns can be avoided, and samples thus represent a "rolling average" of chironomid taxa emerged during the past 48 hours (Coffman 1973, Wilson & Ruse, 2005). In addition, samples collected from accumulation areas can also be expected to represent species from varying habitats and depths. All collected material was sieved (sieve mesh size 200 µm) in the field and preserved in ethanol. When the number of exuviae in a sample was high, a random subsample of at least 200 exuviae was separated in the laboratory (Ruse 1993). Chironomid pupal exuviae were identified using the keys of Wiederholm (1986) and Wilson & Ruse (2005) (I), Langton (1991), Langton & Visser (2003) and Stur & Ekrem (2006) (II-V).

Benthic macroinvertebrates (III and V) were sampled with an Ekman-Birge grab, following the Finnish standard procedure (SFS 1989). Samples were sieved (mesh size: 500 µm) in the field and the residual was preserved in 70% ethanol. In the laboratory, benthic macroinvertebrates were sorted and identified to the lowest possible taxonomic level using the keys by Lafont (1983), Wiederholm (1983), Webb & Scholl (1985), Nilsson (1997) and Timm (1999).

3.3 Statistical methods

Differences in chironomid and benthic macroinvertebrate community composition was visualized with Non-Metric Multidimensional Scaling (NMDS) (I-V). NMDS is a nonparametric ordination method highly applicable to ecological data sets with unknown data distribution and numerous zero values (McCune & Grace 2002). Sørensen (Bray-Curtis) distance measure was used in the analysis, as recommended by McCune and Grace (2002). Prior to the analysis arcsine-squareroot transformation (arcsine$\sqrt{[x]}$) was performed. Most biological data sets benefit from one or more transformations, which e.g. make the distance

function work better (McCune & Grace 2002). NMDS analysis were run using an autopilot-mode, letting the program choose the best ordination solution (i.e., lowest stress) from 40 separate runs of the real data.

The strength of concordance between ordinations based on the generic and species levels (II) and profundal sampling and CPET ordinations (III) was assessed using Procrustes analysis (Jackson 1995, Peres-Neto & Jackson 2001). Site scores from comparable ordinations (the first two NMDS axes) served as the input data. Procrustes analysis works by scaling, rotating and dilating one ordination solution (i.e. the rotated ordination), and superimposing it on a second ordination (i.e. the reference or target ordination), maximising the fit between corresponding observations of the two ordination configurations. The squared residuals (m^2) between the two configurations were used as the measure of association, with low values of m^2 indicating strong concordance (Gower 1971, Digby & Kempton 1987). PROTEST extends Procrustes analysis by providing a permutation procedure to assess the significance of the Procrustean fit (Jackson 1995). The observed Procrustean fit was compared with 999 random permutations of the original observations of one matrix, the proportion of the statistic smaller than, or equal to, the observed value of m^2 providing the significance level of the test.

Multi-Response Permutation Procedure (MRPP) was used to examine differences among macroinvertebrate communities (III and V). MRPP is a nonparametric method designed to test for community differences between a priori defined groups (e.g. sampling sites). Sørensen's (Bray-Curtis) distance measure was used in the analysis. Blocked variant of MRPP (MRBP) was used to test for differences in chironomid assemblages in the CPET samples (I, II and V) because the consecutive samples are not true replicates but repeated measures. MRBP is especially suited for repeated measures and randomized-block designs (McCune & Grace 2002). Euclidean distance function and median alignment within blocks were used in the analysis as recommended by Zimmerman *et al*. (1985) (see McCune & Grace 2002). The observed delta values (mean within-group distance) of the MBPP was applied to evaluate within-group variability at the generic and species levels of identification (II). For a given mean overall distance, low observed delta values indicate tighter within-group clustering of samples. In addition to MRBP, repeated measures ANOVA was used to test for differences in benthic index values (CPET indices), calculated from the CPET data sets (I). Regression analysis was then used to assess the relationship between the index values and water quality (total phosphorus) (III).

Chironomidae and other benthic macroinvertebrate taxa indicative of impacted vs near-pristine conditions (II and III) were identified using indicator species analysis (IndVal; Dufrêne and Legendre 1997). The indicator value of a taxon varies from 0 to 100, and it attains the maximum value when all individuals of a taxon occur at all sites of a single group (in this case, impacted or near-pristine lakes or rivers). The method selects indicator species based on both high specificity for, and high fidelity to, a specific group. IndVal is considered superior to more traditional methods of identifying indicators (e.g. TWINSPAN) on both statistical and practical grounds (Legendre & Legendre 1998, McGeoch & Chown 1998). For example, it is robust to differences in within-group sample sizes and abundances across species. The significance of each species' indicator value was tested with a Monte-Carlo randomization test with 1000 permutations.

Signal-to-noise (S/N) ratios (e.g., Long and Wang 1994) of the CPET and the lake profundal grab sample data sets (III) were evaluated with Sørensen distance as a measure of dissimilarity in community composition (low values indicate similar communities). The distance measure was calculated for all possible pairs of sites (six lakes, 12 sampling sites, 66 possible site pairs) in both data sets. Then, we determined S/Ns by using among-group dissimilarity (mean of all near-pristine vs impacted site distances, based on 6 × 6 distances) as the signal, and within-lake dissimilarity (mean of all within-lake site distances, based on six distances) as the noise. A high S/N is obtained when mean among-group dissimilarity is high and mean within-lake dissimilarity is low. We compared the observed S/N values to 9999 random S/Ns that were obtained using a bootstrap-type Monte-Carlo randomization test. We drew the random samples from among the 66 pairwise distances. For each randomized trial, six random S/N pairs were drawn from the original distance measures and their mean value was calculated. Significance was judged as mean S/N $\geq$ observed S/N.

NMDS, MRPP, MRBP and IndVal analysis were done with PC-ORD (version 4.25; McCune & Mefford 1999). Repeated measures ANOVA and regression analysis were conducted using SYSTAT version 10-program. Procrustes analysis used in this thesis is available at Internet (www.zoo.utoronto.ca/jackson/pro1). The Monte-Carlo randomization tests were performed using a custom test (the source code available upon request).

4 Results and discussion

4.1 Emergence patterns of Chironomidae should be known when applying the CPET method

In showing temporally differing emergence patterns, Chironomidae are no different from other aquatic insects. Emergence of chironomids varies both diurnally and seasonally (Wilson & Ruse 2005). Synchronous emergence appears to be characteristic feature of Arctic chironomids. In temperate areas synchronicity of chironomid emergence is much less evident. Further, in the tropics and southern hemisphere, chironomid emergence takes place throughout the year (Armitage 1995). Nevertheless, for boreal rivers and lakes, at least, a single snap-shot sample at any time during the open water season may detect only a proportion of the species present in the area of interest. This proportion further depends on several factors, such as altitude, temperature regime, persistence of exuviae etc. Thus, in warm temperate areas, for instance, where chironomid emergence is distributed over a longer period, and species may have several generations within a year, the taxonomic coverage of a single CPET sample will be different from that in boreal areas. In addition, due to the variation in diurnal emergence patterns, collecting drifting pupal exuviae at any time of the day will detect only those exuviae from which the adults have emerged recently. Therefore, "drift-netting" pupal exuviae for, say, a few hours per day will show biased results regarding species composition (Wilson & Ruse 2005). To overcome these problems, it has been recommended that chironomid pupal exuvial samples should be collected from accumulation areas (i.e. behind obstacles in rivers and at leewards shores in lakes). Accumulation areas should represent a "rolling average" of the species emerging during the past 24-48 hours prior to the sampling (Coffman 1973). Early work by Ruse & Wilson (1984) also demonstrated that any combination of three monthly samples within a period from spring to autumn will detect about 80% of the chironomid genera present. Later, Gendron & Laville (1995) recommended seven or eight monthly samples to be collected in river biomonitoring in southern Europe.

Chironomids are known to vary in their tolerances towards anthropogenic stressors, such as organic pollution, eutrophication and acidification (IV). Some species are highly sensitive, others indifferent or tolerant towards a particular impact and some may even favour impaired conditions. It follows then that

different combinations of monthly CPET samples differ in their species composition and may actually differ in their ability to detect human impacts. A poor match of sampling schedule with the emergence periods of chironomid species indicative of either unimpacted or impacted conditions may results in the false conclusion of no differences between the studied sites (type II error) (I). In slightly impacted conditions or when the trophic gradient between the sampled sites is short, as was the case in River Kymi (I), chironomid species sensitive of pollution or indicative of oligotrophic conditions are of special importance. This is probably because tolerant species or indicators of eutrophy are not present at these sites or are represented in low numbers. In more heavily impacted conditions and with increasing trophic gradient, tolerant species might become equally, or even more important than sensitive taxa in detecting among-site differences (Calle-Martinès & Casas 2006). Our results showed that the optimal sampling strategy (the best discriminatory efficiency) may also vary from one river system to another (I). Although some other factors may have been involved (e.g. river types and faunal differences related to stream order), the results most likely reflected the varying trophic gradients between the studied sites. Therefore, the optimal sampling strategy should be determined for the river or rivers of interest prior to the start of a monitoring program, to ensure the maximum likelihood of detecting true human-induced impacts, if any exist. Depending on, for example, spatial variation in species distributions and their emergence patterns at any given site, the optimal sampling strategy probably varies spatially, and possibly at multiple scales. Although the timing of sampling, emergence periods of aquatic insects and discrimination efficiency of study designs have rarely been discussed, Johnson *et al*. (1990) suggested that in the boreal region lake profundal samples should be collected in autumn for the reasons discussed above.

Our estimation of the taxonomic coverage of three monthly CPET samples also differed slightly from that reported by Ruse & Wilson (1984). Although these discrepancies were most likely related to estimation methods, the expectation of 80% taxonomic coverage with three monthly samples (Ruse & Wilson 1984) is probably too optimistic for boreal rivers. Our results and recent biomonitoring data suggest, that for chironomid genera and species, 60-70% taxonomic coverage is a more realistic figure (Raunio 2007, I). Apparently, similar kind of variation as observed for the discrimination efficiency was not found for the taxonomic coverage of different sample combinations (I). This was probably related to the fact that several chironomid species, not to mention genera, may be found in low numbers outside their emergence peaks. Some species also may have another

generation or even several generations, within a year. However, their occurence can only be estimated reliably during the peak of their emergence.

4.2 Generic-level identification of Chironomidae is often adequate for biomonitoring, but species-level is desirable

One of the key features of the CPET method is that it relies primarily on genus-level identification. However, the adequacy of genus level identification of Chironomidae has rather rarely been tested rigorously (but see King & Richardson 2002). Further, chironomids classified as sensitive are especially important, because the relative proportion of sensitive individuals and taxa are the recommended metrics in the CPET method (Wilson & Ruse 2005). In paper II, we studied the importance of taxonomic resolution and emergence patterns of lotic chironomids in southern Finland (e.g. timing and extend of emergence and patterns in species richness of consecutive samples) for the use of CPET in river biomonitoring. The emergence of chironomids started in late April, but the number of taxa remained low until mid May. This was later found to be true for lakes as well (III). Total number of species, genera and taxa classified as sensitive reached the maximum in mid-to-late July. If only one sample can be collected per river, then this period might be the best choice, because a high proportion of all species (41%) and genera (49-58%) were detected in a single sample then. However, this proposition emphasizes the taxonomic coverage. The maximum likelihood for detecting anthropogenic impacts with a single CPET sample may be obtained with a different sampling strategy (III). Our results gave further evidence that a single snap-shot sample at any one time of the year will not summarize reliably the chironomid fauna of boreal rivers (but see Lindegaard 1995), although this may well be possible at lower latitudes with different climate and chironomid emergence patterns. The number of emerging taxa and individuals started to decrease in mid August and, in southern Finland at least, it may be advisable to collect the last monthly sample no later than early September.

Our results also showed that many chironomids classified as sensitive emerged only during a certain season, and the shift in the composition of consecutive samples was particularly pronounced in spring, decreasing towards autumn (II). This was probably related to spring emergence of some univoltine species in which case the intensive emergence occur within a period of 1-2 weeks, thus maximizing the chance of succesful reproduction (Tokeshi 1995). The strong concordance between ordinations based on generic vs species level data also

suggested that the CPET method could use primarily generic level identification. This finding is in accordance with Ruse (2002) who compared classifications of British lakes based on chironomid genera and species. PROTEST also showed that the similarity between the two taxonomic levels was generally weakest in spring and autumn. In addition, the ordination residuals were most strongly, and negatively, correlated with species richness, diversity and evenness, indicating that samples with low taxonomic richness and few dominating species were more effectively separated from the other samples at the species than generic level. Conversely, when species richness was high and taxa abundances evenly distributed, ordinations using species vs. genus-level data were strongly concordant. Therefore, species level resolution may become important when species with a short emergence period appear in great numbers, especially if these belong to species-rich genera with different species responding differently to anthropogenic impacts (see also Rossaro & Mietto 1998).

However, shorter emergence periods of individual species compared to whole genera resulted in shorter average within-group distances, in terms of compositional similarity, in generic than species level data. Thus, the detection of a particular species may require accurate timing of sampling, whereas a species-rich genus may be detected throughout a season. Given that the emergence of chironomids may vary from year-to-year and between sampling sites due, for example, to variation in temperature regime or larval food supply (Dansk 1978, Welsh *et al*. 1988), community differences detected at the species level may reflect between-site variation in species' emergence patterns rather than true differences in species composition, thus increasing the chance of committing a type I error. This may be especially true for large-scale studies with simultaneous sampling of, for example, small headwater streams and large lowland rivers, high- and low-altitude streams, or lakes with different water volume and surface area. Nevertheless, because the detection of patterns is related to the signal-to-noise ratio (McCune & Grace 2002, III), generic level identification may decrease the undesired noise, thus enhancing test effectiveness. Thus, the question of taxonomic resolution is a complex matter, which depends not only on trophic gradient (Resh & McElravy 1993), inclusion or exclusion of rare species (Cao *et al*. 2001), study objectives (Furse *et al*. 1984) and statistical analysis (Jackson 1995), but also on sampling method.

Overall, it seems that even one well-timed sample may include a relatively high proportion of the chironomid taxa present in a river. To obtain a reliable summary of the distribution of the taxa, however, knowledge of their emergence

patterns and allocation of sampling effort to different seasons is essential. Genus level identification seems sufficient for most monitoring purposes but should be used with some caution, because a genus may include species with different tolerances to a particular impact. Species composition within a genus is also expected to show spatial variation, which means that genus-level classifications have rather limited spatial applicability (IV). Finally, although multivariate description of community patterns might only be slightly affected by the taxonomic level (Bailey *et al*. 2001), species level resolution and related tolerance classification of chironomids is desirable for reliable and sensitive biomonitoring of boreal rivers (IV).

4.3 Knowledge of chironomid emergence patterns enables effective biomonitoring of boreal rivers

In paper IV we surveyed medium-to-large sized rivers in southern Finland to describe emergence patterns of 195 lotic chironomid taxa, using pupal exuvial and adult chironomid samples. The number of common species alone highlight the importance of chironomids in monitoring and assessment, and a significant proportion of aquatic biodiversity is lost if Chironomidae are ignored or identified only to a high taxonomic level. A major proportion of species richness was accounted for the sub-families Chironominae (emerging mainly during the summer months, June-August) and Orthocladiinae (emerging mainly in spring and autumn). The common lotic species constituted approximately 26% of the chironomid fauna recorded from Finland. By comparison, Seire & Pall (2000) recorded 184 taxa of chironomid larvae from Estonian running waters. It should be noted, however, that our data included only the most common species in southern Finland and the total number of chironomid species in the studied rivers is higher. For instance, 173 species were identified from the adult material collected from R. Kymi.

The timing of sampling is a critical design factor in river biomonitoring (I, II). Determination of species emergence patterns showed that many species indicative of oligotrophic conditions (some *Orthocladius* and *Micropsectra*) emerged mainly in spring, particularly during the first two or three weeks of May. In contrast, indicators of eutrophy emerged during an extended period in summer (e.g. *Chironomus* and *Glyptotendipes* species). Of the 195 common lotic species, we proposed a total of 98 indicator taxa (IV). Although the use of genus-level tolerance classifications has its obvious weaknesses (e.g. among-species variation,

see above) the use of the lowest possible taxonomic level and related tolerance groupings are not entirely free from similar pitfals. Recent studies have shown that some chironomids and other benthic macroinvertebrates previously considered as species are in fact aggregates of several species, which may differ in their ecological characteristics and geographical distributions (Stur & Ekrem 2006, Williams *et al*. 2006). Further, species may have locally adapted populations, as evidenced by varying behavioural patterns and spatially differing ecological characteristics (Neumann 1967, Jernelöv *et al*. 1981, Groenendijk *et al*. 1999). Thus, species-level classifications may have rather limited spatial applicability, or at the very least, they should be used with some caution for other regions. Further studies are needed to test the proposed tolerance classification for biomonitoring and assessment of boreal rivers.

4.4 Multihabitat sampling may be required for reliable monitoring of benthos in lakes and large rivers

The majority of previous research on sampling design in bioassessment studies in rivers has recommended sampling in the single most productive habitat, usually the riffle habitat (e.g. Parsons & Norris 1996). However, with regard to lakes and large rivers, the sampling approach has traditionally been the opposite, i.e. focus has been on the least productive habitats such as deep river pools and lake profundals. The primary reason given for single-habitat sampling is that sampling in multiple habitats may produce redundant results and serves only to increase sampling costs. Collections from more than one habitat type may also introduce undesired variation that can potentially mask water quality differences among sites (Parsons & Norris 1996). In contrast, by stratifying sampling according to habitat or by sampling only a single habitat, some of the undesired noise can be avoided (Johnson 1998). Accordingly, some river studies have found sampling a single habitat sufficient for monitoring purposes (Hewlett 2000). Johnson & Goedkoop (2002) also showed that variation in the benthic communities of lake littorals was largely accounted for by variation in habitat type.

The multihabitat approach to sampling in bioassessment is not a new concept (Hering *et al*. 2004), but is rarely applied in routine biomonitoring. The advantage of the multihabitat approach is to sample representative habitats that will address altered systems and provide an indication of impairment from both chemical and non-chemical stressors (Barbour *et al*. 2006). Although there are some variations on the original multihabitat sampling design, the basic approach is to sample the

major aquatic habitats in proportion to their representation in the river. However, lake profundals or deep river pools are species poor and individual densities may be several orders of magnitude lower than in other habitats. The proportion of such habitats may be rather small, and thus their fauna will be overrepresented in sampling in relation to other habitat types. Although sampling a single habitat alone may be logistically effective and in some cases adequate for water quality assessment, benthic communities in a single habitat may not represent the environmental condition of the whole water body (Kerans & Karr 1992, III, V). This may lead to biased estimates of impairment. Thus, there is increasing evidence that multihabitat sampling is desirable for a reliable assessment of lakes and rivers (Bonada *et al*. 2006, III). Our results also showed that sampling multiple habitats did not lead to increased noise in data and, in contrast to profundal grab sampling, the CPET data had signal-to-noise ratio significantly higher than expected by chance. Thus, low noise as well as additional information obtained from chironomid species from multiple depths and habitats clearly compensated for a slight loss in signal strength. Most contemporary bioassessments involve analysing the benthic macroinvertebrate assemblages by means of metrics, either individually or combined into a multimetric index. The likely explanation for why multiple habitat sampling may be more effective than single habitat sampling is that more taxa with a greater range of ecological requirements are encountered, and metrics based on such data may be more robust (III). The occurence of different habitats at different sites are also linked to effects of certain types of pollution or environmental stress, especially non-point sources that cause eutrophication, increased turbidity, and sedimentation. Thus, the best way to include the effects of habitat alteration in bioassessment studies is probably to use a multiple-habitat sampling design.

It is worth noting that multihabitat sampling was designed originally for smaller rivers in which different habitat types can be identified and their representation in the river is possible to quantify. Because sampled pupal exuviae derive from an ill-defined area, CPET may not be considered as a multihabitat sampling technique in a strict sense. However, species appear to be represented in the CPET samples in relation to their individual densities in various habitats (V), as they do in the multihabitat sampling design. Some deep pool or profundal species might go undetected in the CPET method (V), but this probably is not a serious bias in environmental assessment if these taxa occur in low numbers and represent a minor habitat type. However, our results also showed that the CPET method may in some cases detect rare profundal species more effectively than

profundal grab sampling (III). High species richness often associated with the CPET samples (II) is also a strong indication that the method clearly integrates species from multiple habitats. It should be noted, however, that some profundal species sensitive to low oxygen concentrations may be found from multiple depth zones and their pupal exuviae may originate from littoral or sublittoral habitats where these species may find refuge. In the worst case, this may lead to false negative results (type II error). Further, due to the method's integrative nature, separation and quantification of various impact forms (i.e. point-source vs. non-point source of pollution) may be difficult or even impossible. In biomonitoring it is often the ecosystem health or integrity that is monitored, and ideally the metrics (and the data which they are based on) should reflect various impacts rather than be impact-specific (Yuan & Norton 2003).

Finally, as far as the CPET method is concerned, the multihabitat sampling may not even increase the monitoring costs. Adequate number of sample replicates for a single-habitat approach have been found to be rather high, i.e. nearly ten sample replicates (Veijola *et al*. 1996, V). We found that the time and effort required for sampling, sorting and identifying grab samples was higher than for three seasonal CPET samples (III, V). Similar results have been found Ferrington *et al*. (1991), when comparing hand-net sampling and the CPET method in rivers. Therefore, sampling multiple habitats appeared to result in a higher number of species and yielded more reliable results concerning the ecological status of lakes and large rivers, and yet this information was obtained more cost-effectively than focusing on a single habitat.

5 Concluding remarks

Despite of its longer application for biomonitoring and assessment studies in e.g. Central Europe and North America, the CPET method is a new and rarely applied method in the Nordic countries. In Finland, it is thus far only applied for biomonitoring of R. Kymi (SE Finland), and it will probably take years for the method to be included in other monitoring programs. However, based on the results of this thesis, the method seems to be highly valuable for various monitoring and assessment purposes. In practice, however, it is not always chosen for monitoring or assessment of freshwaters. Instead, local research and monitoring traditions tend to dominate, and new methods may be ignored due to lack of personal experience. Often the lack of required knowledge (e.g. taxonomic expertise) can also exclude the use of an efficient monitoring tool even though experts would be available. In addition, money may have an overwhelming influence on the content of monitoring programs and on the quality of obtained results, as the cheapest bid usually wins the job. All these factors (and several others) affect monitoring practices which sometimes differ from what they should be, or what was desired. In the worst case, expensive and uninformative programs may have been conducted for years, yet the results have little value for the primary objective of the monitoring program. Therefore, scientific research is essential to promote knowledge and awareness of potential methods, including their advantages and restrictions. Monitoring, as opposed to “pure” science, is sometimes considered to have different methods, practices and requirements with regard to biological data quality. Now that most of the sampling methods used in Europe have been standardized, or are currently being standardized, this should not be the case in the future. Well conducted monitoring provides valuable information of the environment that is difficult to obtain otherwise (e.g. time-series data).

Until now, the CPET method has been used primarily for monitoring and assessment of freshwaters, but it could be of high value in biodiversity inventories as well. The integrative nature of the pupal exuvial sampling provides insight into chironomid fauna that are difficult or even impossible to detect with more traditional sampling methods. In addition to biodiversity inventories, the pupal exuvial technique could be a useful monitoring tool for coastal areas, where large areas need to be assessed effectively. With the traditional sampling methods, the number of samples rapidly inceases to unpractical levels, since sampling sites should cover various depths distributed over a large area, and multiple replicate

samples are required at each site. By using the CPET method coastal areas could be sampled rapidly and assessment would be based on fewer samples, yet composed of species from multiple habitats and depths (provided that samples are collected from the leeward shores).

Overall, these studies indicate that the CPET method bears great promise for monitoring and assessment of rivers and lakes. The method's greatest advantages are probably its ease of sampling, suitability for almost all aquatic habitats and a high number of chironomid species in samples, which provides a whole spectrum of responses to various stresses. On the other hand, the CPET method does not provide information from benthic macroinvertebrates other than chironomids. Thus, depending on how the European Water Framework Directive (WFD) is interpreted, the CPET method may not by itself, be adequate for the surveillance and monitoring of freshwaters. Further, challenges still remain in WFD-based assessment of rivers using benthic macroinvertebrates, especially with regard to large rivers, as determination of ecological status of streams and rivers will be based on riffle communities only. This means that large biomonitoring data sets collected from river pools will be ignored, despite of the fact that pools may be the dominant habitat type in large rivers.

Hopefully, my studies have contributed to our knowledge regarding the CPET method and its performance in relation to other sampling methods. At this stage, it seems that the main obstacle for its wide-spread use in Finland and other Nordic countries is the lack of taxonomic expertise and/or low number of experts. Whether this will suffice to exclude the method from biomonitoring and assessment programs remains to be seen.

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