EVEN MORE CHIRONOMID SPECIES FOR CLASSIFYING LAKE NUTRIENT STATUS

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ABSTRACT

The European Union Water Framework Directive (WFD) classifies ecological status of a waterbody by the determination of its natural reference state to provide a measure of perturbation by human impacts based on taxonomic composition and abundance of aquatic species. Ruse (2010; 2011) has provided methods of assessing anthropogenic perturbations to lake ecological status, in terms of nutrient enrichment and acidification, by analysing collections of floating pupal exuviae discarded by emerging adult Chironomidae. The previous nutrient assessment method was derived from chironomid and environmental data collected during 178 lake surveys of all WFD types found in Britain. Canonical Correspondence Analysis provided species optima in relation to phosphate and nitrogen concentrations. Species found in less than three surveys were excluded from analysis in case of spurious association with environmental values. Since Ruse (2010) an additional 72 lakes have been surveyed adding 31 more species for use in nutrient status assessment. These additional scoring species are reported here. The practical application of the Chironomid Pupal Exuvial Technique (CPET) to classify WFD lake nutrient status is demonstrated using CPET survey data from lakes in Poland.

Keywords: Pupal exuviae, ecological status, species optimum score, worked example, Water Framework Directive

Introduction

From 1998 the author has gathered chironomid species and environmental data from inland waterbodies across England, Wales and Scotland for the purpose of developing methods to assess ecological status for the Water Framework Directive (Council of the European Union 1999). The WFD requires European member states to achieve good ecological status of all water bodies (rivers, lakes, transitional waters, and coastal waters) by 2015. Ecological quality is being assessed in terms of a waterbody's biota, hydromorphology and physical-chemical condition. Lakes in their natural condition can be ordered along a gradient of increasing nutrient content with correlated increased productivity and changes in biota. The WFD requires the current ecological condition of lakes to be classified in relation to their reference condition prior to human perturbations.

CPET (Wilson and Ruse 2005) was used to obtain representative biotic samples from large waterbodies. CPET exploits the easy collection and identification of pupal exuviae (skins) discarded by emerging adults. CPET proved to be a simple and effective sampling method for implementing WFD ecological assessment of lake anthropogenic nutrient impact (Ruse 2010) and acidification (Ruse 2011). The collection of floating chironomid pupal exuviae at the leeward shore of standing water bodies provides a simple and safe means of obtaining abundant macroinvertebrate data representative of at least a large part of the lake. The sample is passively collected by wind and water currents, integrating adult chironomid emergence over the previous day or two. There is a European Standard guidance on sampling and processing chironomid pupal exuviae for ecological assessment (CEN 2006).

Chironomid and environmental data from 178 lake surveys were used to calculate the sensitivity of chironomid species to anthropogenic nutrient enrichment (Ruse 2010). The observed sensitivity score of a lake was compared with the lake's reference score modelled from impact-independent characteristics of the lake. A modified observed to reference ratio provided the Ecological Quality Ratio (EQR) required by the WFD (Anonymous 2003). The EQR was used to classify the ecological status of each lake as defined by the WFD (Council of the European Union 1999, Annex V), boundaries were calculated from the relative proportion of sensitive to tolerant species (Ruse 2010). Variation in EQR, due to contingencies of sampling time and place, were assessed from repeated sampling to determine the confidence of a lake classification based on its observed EQR. Since the publication of these methods by Ruse (2010) further lake surveys have added species available for inclusion within a multivariate ordination constrained by nutrient data. The derivation of new species optima, EQR and WFD class will be explained using a worked example of two Polish lakes and reference to Ruse (2010) is recommended for full details. The current study further improves our understanding of species distribution and ecology while refining the WFD CPET tool for measuring nutrient impact on lakes.

Methods

Full details of Methods are provided in Ruse (2010) since which 72 lake surveys have been added for reanalysis. In addition to surveys in the UK (Fig. 1) five surveys of Polish lakes have been included in the latest analysis (Fig. 2). All five are located in the Warmian-Masurian province of north-eastern Poland. Szwałk Wielki, Piłwąg, Łękuk

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Fig. 1 UK lakes fully-surveyed using CPET since Ruse (2010).

Fig. 2 CPET fully-surveyed lakes in Poland.

and Krzywa Kuta are located north-east of the town of Giżycko in the Pojezierze Ełckie region. Jegocin is located 2 km south of the largest lake in Poland, Sniardwy. In every case a full survey constituted 4 samples taken among the months of April through to October. These additional surveys increased the total for analysis to 250. Pupal exuviae were identified using the key of Langton and Visser (2003) and followed its nomenclature unless subsequently changed and recorded in Fauna Europaea (2013).

Sampling

Floating debris containing chironomid pupal skins was collected at the lake shore, to which the wind blew, using a 250 mm mesh net attached to an extendable lightweight pole. The netted sample was passed through a 4 mm and a 250 mm mesh sieve, then the residue of the coarse sieve was refloated and passed again through the sieve stack before repeating at least once more. Approximately 200 skins were subsampled randomly from each collection.

Data analysis

Chironomid data for each survey were amalgamated and species abundance recorded as a percentage of the total number of skins collected. Species found in less than three surveys were excluded from analysis in case of spurious association with an extreme environmental value. Chironomid data were constrained by one environmental vector, log-transformed Total Phosphorus*Total Nitrogen/mean lake depth, in a Canonical Correspondence Analysis (CCA) using biplot-scaling with emphasis on inter-species distances using CANOCO for Windows 4.56 (ter Braak and Šmilauer 1998). Ruse (2010) found TP*TN/mean depth was the forwardly-selected variable with the highest significant correlation between observed mean nutrient values and species optima scores when compared with other nutrient measures. Correlation coefficients between species-derived lake nutrient impact scores and log TP*TN/mean depth were as good with qualitative data as with weighted-average data using species abundance and/or niche breadth. Each species maximum abundance (estimated by CCA) is its optimum score in relation to the primary, nutrient axis. The mean optimum score for each lake survey is the sum of all species optima divided by the number of contributing species; its average species optimum or observed score.

The EQR of observed average species optimum (observed score) of a lake survey is divided by the reference average optimum (reference score) for that lake, modelled from a reference set of lakes with a wide range of physical characteristics. Ruse (2010) found the most efficient model predicting average species optimum for a lake's reference condition was −1.42–0.483 log surface area (ha) −0.57 log mean depth (m) +0.485 log retention time (days) +0.476 log catchment surface area (ha). EQR of observed score divided by the modelled reference score has to take account of negative optima of sensitive species and ascend with increasing ecological quality. This is achieved by adding 1.0 to both observed and reference scores and then subtracting the score from 2.0, as all optima within the data set ranged within –2 and +2 SD units. The calculation of EQR was thus:

$$EQR = 2 - (\text{observed score} + 1) / 2 - (\text{reference score} + 1)$$

After calculating EQR for all lakes surveyed (Ruse 2010) it was divided by the maximum EQR recorded (1.24) so as to range between 0 and 1. Boundaries between five ecological status classes were based on the proportions of sensitive and tolerant species, as preferred for the WFD (Schartau et al. 2008). The crossover point in a plot of relative frequencies of sensitive to tolerant species was used to objectively define class boundaries from EQR. The High/Good boundary was found at an EQR of 0.725, Good/Moderate at 0.56, Moderate/Poor at 0.37 and Poor/Bad at 0.21. CPET largely overcomes spatial and operator sampling error due to the passive collection of material from a large area of the lake. Error statistics of the contingency of seasonal sample collection were used to determine the percentage uncertainty of classifying lakes into the five WFD classes following the procedures of Ellis and Adriaenssens (2006).

Results

The distribution of 454 species was provided by the data set of 250 lake surveys and over 224,000 pupal exuviae. After removal of species occurring in less than 3 surveys, there remained 365 species for ordination constrained by the nutrient measure TP*TN/mean depth. This provided

Table 1 Additional species since Ruse (2010) with nutrient optima

Species arranged by taxonomic order	TP*TN/mnDp optima		Species arranged by optima	Frequency
Macropelopia notata (Meigen)	0.01	–1.01	*Diamesa tonsa* (Haliday)	3
Conchapelopia pallidula (Meigen)	–0.48	–0.98	*Rheotanytarsus rioensis* Langton & Armitage	3
Krenopelopia nigropunctata (Staeger)	–0.70	–0.97	*Tanytarsus* Pe 5a	3
Zavrelimyia barbatipes (Kieffer)	–0.86	–0.87	*Thienemannia gracilis* Kieffer	3
Diamesa tonsa (Haliday)	–1.01	–0.86	*Zavrelimyia barbatipes* (Kieffer)	3
Monodiamesa bathyphila (Kieffer)	–0.60	–0.84	*Rheotanytarsus pentapoda* Kieffer	8
Cardiocladius capucinus (Zetterstedt)	–0.06	–0.75	*Cricotopus similis* Goetghebuer	5
Cricotopus similis Goetghebuer	–0.75	–0.70	*Orthocladius ashei* Soponis	5
Cricotopus iso-spec2	0.26	–0.70	*Krenopelopia nigropunctata* (Staeger)	4
Eukiefferiella tirolensis Goetghbuer	–0.19	–0.60	*Monodiamesa bathyphila* (Kieffer)	3
Orthocladius ashei Soponis	–0.70	–0.50	*Rheocricotopus atripes* (Kieffer)	3
Orthocladius rivicola Kieffer	–0.16	–0.48	*Conchapelopia pallidula* (Meigen)	4
Orthocladius ruffoi Rossaro & Prato	–0.39	–0.42	*Polypedilum cultellatum* Goetghebuer	4
Rheocricotopus atripes (Kieffer)	–0.50	–0.39	*Orthocladius ruffoi* Rossaro & Prato	4
Bryophaenocladius muscicola (Kieffer)	0.52	–0.26	*Pseudosmittia* Pe2	3
Metriocnemus tristellus Edwards	0.02	–0.19	*Eukiefferiella tirolensis* Goetghbuer	5
Paratrissocladius excerptus (Walker)	0.07	–0.16	*Orthocladius rivicola* Kieffer	4
Pseudosmittia Pe2	–0.26	–0.11	*Micropsectra aristata* Pinder	6
Thienemannia gracilis Kieffer	–0.87	–0.06	*Cardiocladius capucinus* (Zetterstedt)	3
Chironomus acerbiphilus Tokunaga	0.48	–0.05	*Zavrelia pentatoma* Kieffer & Bause	3
Cladopelma goetghebueri Spies & Saether	0.64	0.01	*Macropelopia notata* (Meigen)	3
Chironomus carbonaria (Meigen)	0.45	0.02	*Metriocnemus tristellus* Edwards	4
Parachironomus danicus Lehmann	0.80	0.07	*Paratrissocladius excerptus* (Walker)	6
Polypedilum cultellatum Goetghebuer	–0.42	0.26	*Cricotopus* iso_spec2	5
Micropsectra aristata Pinder	–0.11	0.45	*Tanytarsus nemorosus* Edwards	3
Paratanytarsus grimmii (Schneider)	0.79	0.45	*Chironomus carbonaria* (Meigen)	5
Rheotanytarsus pentapoda Kieffer	–0.84	0.48	*Chironomus acerbiphilus* Tokunaga	10
Rheotanytarsus rioensis Langton & Armitage	–0.98	0.52	*Bryophaenocladius muscicola* (Kieffer)	3
Tanytarsus nemorosus Edwards	0.45	0.64	*Cladopelma goetghebueri* Spies & Saether	3
Tanytarsus Pe 5a	–0.97	0.79	*Paratanytarsus grimmii* (Schneider)	3
Zavrelia pentatoma Kieffer & Bause	–0.05	0.80	*Parachironomus danicus* Lehmann	3

Table 2 Nutrient classification of two Polish lakes based on species collected by CPET survey

Species	Optima	Krzywa	Łękuk
Procladius choreus (Meigen)	–0.06	1	1
Procladius sagittalis (Kieffer)	0.72	1	
Procladius Pe4	1.21	1	
Procladius rufovittatus (van de Wulp)	0.74	1	
Ablabesmyia longistyla Fittkau	–0.23	1	
Ablabesmyia monilis (Linnaeus)	–0.34	1	
Tanypus punctipennis Meigen	1.35	1	1
Cricotopus albiforceps (Kieffer)	–0.51		1
Cricotopus festivellus (Kieffer)	0.15	1	
Cricotopus flavocinctus (Kieffer)	0.02	1	
Cricotopus intersectus (Staeger)	0.97		1
Cricotopus reversus Hirvenoja	1.01		1
Cricotopus sylvestris (Fabricius)	0.97	1	1
Cricotopus trifasciatus (Meigen)	–0.29	1	
Nanocladius dichromus (Kieffer)	0.36	1	1
Psectrocladius bisetus Goetghebuer	–0.95	1	
Psectrocladius psilopterus Kieffer	–0.39	1	
Psectrocladius sordidellus (Zetterstedt)	0.45	1	
Psectrocladius oxyura Langton	0.01	1	
Corynoneura gratias Schlee	–0.45		1
Corynoneura lacustris Edwards	–0.71	1	
Corynoneura lobata Edwards	–0.17		1
Corynoneura scutellata Winnertz	0.68		1
Parakiefferiella bathophila (Kieffer)	–0.53	1	
Parakiefferiella Pe3	–0.99	1	
Chironomus anthracinus Zetterstedt	0.06	1	
Chironomus cingulatus Meigen	0.65	1	1
Chironomus Pe 24	0.92		1
Chironomus carbonaria (Meigen)	0.45	1	
Cladopelma virescens (Meigen)	0.80		1
Cladopelma viridulum (Linnaeus)	0.14	1	
Cryptochironomus defectus (Kieffer)	0.22	1	1
Dicrotendipes lobiger (Kieffer)	0.18	1	1
Dicrotendipes nervosus (Staeger)	0.58		1
Dicrotendipes pulsus (Walker)	0.16		1
Endochironomus albipennis (Meigen)	0.82		1
Endochironomus tendens (Fabricius)	0.76		1
Glyptotendipes cauliginellus (Kieffer)	0.21	1	
Glyptotendipes pallens (Meigen)	1.05		1
Lauterborniella agrayloides (Kieffer)	–0.71	1	
Microtendipes chloris (Meigen)	0.19	1	1
Parachironomus arcuatus (Goetghebuer)	0.57		1
Parachironomus tenuicaudatus (Malloch)	0.35	1	1
Paratendipes albimanus (Meigen)	0.04		1
Phaenopsectra „Pe f. Bala“ .	–0.29	1	1
Polypedilum sordens (van de Wulp)	0.74	1	1
Polypedilum nubeculosum (Meigen)	0.82		1
Sergentia coracina (Zetterstedt)	–0.64	1	
Stenochironomus gibbus (Fabricius)	0.09	1	1
Stictochironomus pictulus (Meigen)	–0.45	1	
Tribelos intextus (Walker)	0.16		1
Pseudochironomus prasinatus (Staeger)	–0.33	1	
Cladotanytarsus pallidus Kieffer	0.34	1	1
Cladotanytarsus lepidocalcar Krueger	0.86	1	
Cladotanytarsus mancus (Walker)	0.26	1	
Cladotanytarsus nigrovittatus Goetghebuer	0.02	1	1
Micropsectra junci (Meigen)	–0.07		1
Paratanytarsus bituberculatus Edwards	0.36		1
Paratanytarsus inopertus (Walker)	0.83		1
Paratanytarsus laetipes (Zetterstedt)	0.56		1
Paratanytarsus tenuis (Meigen)	–0.35	1	1
Stempellinella edwardsi (Edwards)	–0.36	1	
Tanytarsus bathophilus Kieffer	0.52		1
Tanytarsus ejuncidus (Walker)	0.72	1	1
Tanytarsus eminulus (Walker)	–0.30	1	
Tanytarsus excavatus Edwards	0.50	1	
Tanytarsus gregarius Kieffer	–0.44	1	
Tanytarsus inaequalis Goetghebuer	–0.29	1	
Tanytarsus longitarsis Kieffer	0.33	1	
Tanytarsus mendax Kieffer	0.90	1	1
Tanytarsus nemorosus Edwards	0.45	1	
Tanytarsus pallidicornis (Walker)	0.21		1
Tanytarsus quadridentatus Brundin	–0.43	1	
Tanytarsus recurvatus Brundin	–0.81	1	
Tanytarsus sylvaticus (van de Wulp)	0.88	1	1
Tanytarsus usmaensis Pagast	0.06	1	1
Tanytarsus verralli Goetghebuer	0.45	1	
		54	42
Observed score Σoptima/ taxa		**0.096**	**0.427**

Lake	Log Area	Log MnDp	Log Retn	Log Catc	Observed score	Reference score
Łękuk	1.332	0.716	2.386	3.130	0.427	0.180
Krzywa	2.118	0.778	2.665	3.699	0.096	0.170
Reference score = – 1.42–0.483 Area – 0.570 MnDp + 0.485 Retn + 0.476 Catc						

Boundary	EQR
HIGH/GOOD	0.725
GOOD/MOD	0.560
MOD/POOR	0.370
POOR/BAD	0.210

Lake	EQR	Class	% Confidence of classification			
Łękuk	0.564	GOOD	61.5	MOD	38.5	
Krzywa	0.878	HIGH	100			
EQR = ((2–(obs+1)/(2–(Ref+1))/1.24						

nutrient optima for an additional 31 chironomid species (Table 1) that previously could not be included in the WFD CPET lake nutrient classification. Several of these species occurred in only three surveys among the data set although the acid-tolerant *Chironomus acerbiphilus* Tokunaga was collected from ten lakes that were mostly surveyed since the WFD nutrient classification was developed. In a second ordering within Table 1 species are arranged from the most nutrient-sensitive (negative) species *Diamesa tonsa* (Haliday) to the most nutrient-tolerant of the newly-added species *Parachironomus danicus* Lehmann and the parthenogenetic coloniser of water distribution systems *Paratanytarsus grimmii* (Schneider).

Table 2 reports species collected from two of the Polish lake surveys and provides details of observed score, lake characteristics required to model the reference score, calculation of EQR, classification of lake nutrient status and the confidence of each classification. There were two species *Chironomus carbonaria* (Meigen) and *Tanytarsus nemorosus* Edwards now have optima used to calculate observed score in this table that were not available before the present analysis. Both *Chironomus carbonaria* (Meigen) and *Tanytarsus nemorosus* Edwards now have optima calculated because of the additional 72 surveys and were found at Krzywa. All taxa collected from Łękuk already had optima from the original WFD method development. Only one species, from Krzywa, did not contribute to the observed score and this was an uncertain determination of *Chironomus esai* Wuelker. If a taxon is found which does not have a calculated optimum then for the purpose of the WFD classification it is ignored. Table 2 reiterates the formula provided under Methods for calculating EQR. The EQR for Krzywa after including the two additional species was 0.878 and classified with 100% confidence of High ecological status because the EQR was well above the High/Good boundary. Before these new species were included the EQR was higher at 0.892 as both additional species had above average nutrient-tolerant optima compared with other contributing species. For all other species in Table 2 the original optima developed for the WFD classification by Ruse (2010) were used to calculate observed scores. The EQR of Łękuk was 0.564 which is very close to the boundary of Good and Moderate ecological status. From a plot of risk of misclassifying ecological status (Ruse 2010, Fig. 4) there is 61.5% confidence in Łękuk being of Good status and 38.5% of being Moderate status. Hypothetically, if the two newly-scoring species had been found at Łękuk the observed score would have changed very little, from 0.427 to 0.428 as both new species had optima close to the average of the other contributing species. The EQR would also have lowered by one point at the third decimal place to 0.563. However, because the actual EQR of Łękuk is so close to the class boundary, the hypothetical EQR would have reduced the % confidence of Good status to 41.3% with 58.7% probability of Moderate status. The EQR and class of the other three Polish lakes were; Jegocin 0.824 High status (100% confidence), Szwałk Wielki 0.428 Moderate (100% confidence) and Piłwąg 0.509 Moderate (100% confidence).

Discussion

The usefulness and relevance of a biometric for classifying ecological status is improved by its applicability to the habitats being classified. By using the Chironomidae, ubiquitous in freshwater habitats, CPET is already suitable and accepted for assessing the full range of standing water habitats being classified by the WFD. The addition of 31 species optima by the current study provides even better coverage and relevance to lake classification. Developed within the UK, the CPET European WFD protocol has also been used successfully in Ireland, France, Finland and Poland. Very few species from these Polish lake surveys were unavailable (no estimated optima) for calculating observed scores and in terms of relative abundance these species were negligible.

The 31 additional species available to the CPET lake classification are, by nature of their late inclusion, infrequently encountered among UK lakes being assessed for WFD classification. These species may be infrequent because they are strong indicators of rare habitats. *Chironomus acerbiphilus* was previously known as *Chironomus crassimanus* Strenzke and reported as new to the UK by Langton and Ruse (2005) after it was sampled from a Scottish loch and a Shropshire Mere during the original development of the CPET classification. Among the additional surveys of the present study *Chironomus acerbiphilus* has been collected in several humic, acid lakes of Galloway, south-west Scotland and from north-west Wales. Another additional species, *Chironomus carbonaria*, has also been reported by Langton and Ruse (2005) as new to the UK. This study reveals *Chironomus carbonaria* to be relatively nutrient-tolerant and it was collected from Jegocin and Krzywa as well as from England. Some species are infrequent in the lake data set because they are normally found in other habitats such as running-water, springs or lake marginal vegetation. As its generic name suggests *Krenopelopia nigropunctata* is associated with springs and in this study it was collected from upland lakes and reservoirs in Scottish Galloway, the English Lake District and Powys, Wales. According to Vallenduuk and Moller Pillot (2007) *Macropelopia notata* covers most of Europe where springs can be found, it is rare in the Netherlands but larvae were collected from a reed marsh. In this data set it was collected from small, spring-fed mountain lakes in North Wales, Cwm Bychan and Garnedd (Fig. 2) and from the lily-covered Tabley Mere in Shropshire (Ruse 2013). The three occurrences of the nutrient-sensitive species *Rheotanytarsus rioensis* are the first records within the UK; besides the published findings from Llyn Padarn in Gwynedd, North Wales and from Windermere in Cumbria, England (Langton and Ruse 2005) it has since been collected from Llyn Cowlyd, Gwynedd. *Conchapelopia pallidula* inhabits flowing water and rarely, according to Vallenduuk and Moller Pillot (2007), the lake littoral. Here it has been collected from three upland reservoirs and Coniston Water in the English Lake District. Vallenduuk and Moller Pillot (2007) also suggest that among *Zavrelimyia* species *barbatipes* is an inhabitant of running water and hardly ever found in springs. Here it was found in some of the highest altitude tarns in the Lake District as well as Caban Coch, one of the Elan Valley reservoirs of mid-Wales. *Cardiocladius* is a fast-running water genus but Langton and Visser (2003) report that *C. capucinus* is occasionally collected from mountain lakes. The three occurrences here were all from upland English reservoirs which would have had incoming streams or high spillways upstream of the collecting points. Pupal exuviae of other newly-scoring species could have originated from larvae inhabiting extraneous habitats but the author, who has sampled the majority of these lakes himself, took care not to sample close to inflowing streams. Marginal, overhanging vegetation would be a source of semi-terrestrial species. Early in the development of the CPET WFD tool it was decided to consider all species collected from lakes so as to maximise ecological evidence of habitats and conditions. For example, if a species inhabits sphagnum on acidic peat then its inclusion within the taxa list is considered relevant to the classification of the waterbody under study.

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