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Research Article

**PRELIMINARY RESULTS FROM THE INVESTIGATION OF
BENTHIC DIATOMS FROM POTENTIAL REFERENCE RIVER SITES
IN IRELAND***

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Abstract

Benthic diatom samples were collected from 45 potential reference river sites in Ireland. Diatom samples were also collected from 24 lowland eutrophic sites. The main objective for sampling the potential reference sites was to determine the type-specific biological typologies represented by these sites. The lowland sites were sampled as part of another project and are used here for comparative purposes. A larger number of taxa was identified from the reference sites (175 species) in comparison with the lowland sites. (71 species). Distinct differences in diatom distribution and diversity between the reference sites and the lowland sites were revealed by DCA analysis. DCA analysis also showed that the original typological classification of the reference sites was not reflected to any extent in the distribution of benthic diatoms between these sites. However, there was some differentiation between the reference river sites with respect to diatom distribution along axis 1 of the DCA ordination and this was strongly correlated with water conductivity.

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INTRODUCTION

The Water Framework Directive (WFD) requires that EU member states identify a range of type-specific biological reference conditions and monitor a selection of sites demonstrating high ecological quality for each waterbody type. As a result, the Environmental Protection Agency of Ireland (EPA) has funded a project titled 'Characterisation of reference conditions and testing of typology of rivers' (RIVTYPE). Work began on RIVTYPE in November 2002. A provisional typology of rivers was derived by the EPA using the physical descriptors from Annex II of the WFD. Fifty potential reference sites were selected and inventories of the phytobenthos, macroinvertebrates and macrophytes are now being compiled. Phytobenthos (benthic diatoms and other algae) have been surveyed at the designated sites during two seasons (winter 2002 and spring 2003).

This paper will focus on the diatom assemblage and will present preliminary results on diatom distribution at 45 out of the 50 designated sites. Analysis of the reference sites based on the diatom assemblage is presented and compared with the original typological classification. Most of the selected sites are found in the north-west, west, south-west, and south eastern regions of Ireland. No Q5 river sites are found in the calcareous lowland central plain of Ireland where agriculture is generally more intensive and where rivers are naturally more eutrophic. This presents a problem in terms producing a complete typology for Irish rivers, and reference sites may therefore have to be derived by modelling, hindcasting or other methods. For comparative purposes, results from the distribution of the diatom assemblage from the RIVTYPE sites is compared with the diatom distribution from twenty-four calcareous, lowland, and eutrophic rivers from the midlands which were sampled in 2002 as part of a biodiversity survey of diatoms in central Ireland (Ní Chatháin, unpublished). To date little work has been carried out with respect to algal assemblages in Irish rivers although some studies on single rivers have been conducted (Ní Chatháin 2002, Heuff and Horkan 1984, McDonnell and Fahy 1978).

MATERIALS AND METHODS

Rivers

A provisional typology of rivers has been derived using the physical descriptors from Annex II of the WFD. These include soil type (peat and non-peat), geology (siliceous and calcareous) and slope categories. Eight provisional 'types' were recognized by the EPA (Table 1). Potential reference sites have been selected within each type. Fifty sites with Q5 scores (EPA Quality Rating

Table 1
Provisional typology for the 50 potential reference river (RIVTYPE) sites in Ireland

Type	Type description	Number of rivers
1	Small catchment ^a , mid altitude, siliceous, non peat	3
2	Small catchment, lowland, siliceous, peat	2
3	Small catchment, lowland, Siliceous, non-peat	16
4	Small catchment, lowland, calcareous, non-peat	10
5	Medium catchment, lowland, calcareous, peat	2
6	Medium catchment, lowland, calcareous, non-peat	5
7	Medium catchment, lowland, siliceous, non-peat	11
8	Large catchment, lowland, siliceous, non-peat	1

^aSmall catchment = 10-100 km²; Medium catchment = 100-1000 km²; Large catchment = 1000-10,000 km²; Mid altitude >200m; Lowland <200m.

indicating high diversity and good water quality) have been nominated as potential reference sites. These sites are considered to be minimally impacted by human activities and meet most of the recognized criteria for reference condition. Results for 45 of the selected 50 sites will be presented in this paper. Five sites were excluded as no count data was available for these sites for either season

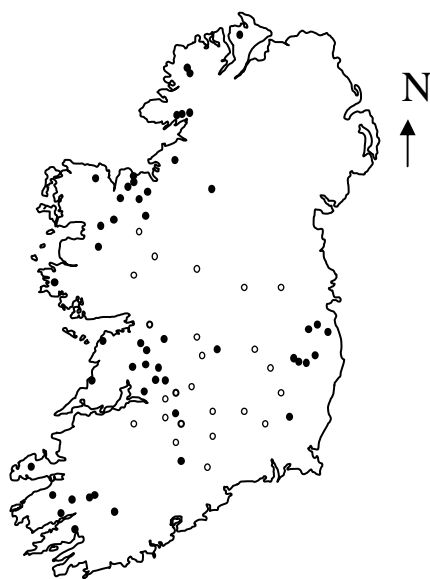


Fig. 1. RIVTYPE (Q5) (•) and lowland (Q3-4) (o) river sites.

sampled. The majority of the 50 sites are located in the west, south-west and north-west of the country (Figure 1). Diatom distribution at these sites is compared with the distribution in twenty-four lowland sites with predominantly calcareous substrata sampled in summer 2002 (Figure 1). These twenty-four sites show some degree of human impact in contrast with the RIVTYPE sites, and have been designated Q3, 3-4 or 4 by the EPA (moderately polluted, slightly polluted and unpolluted).

Diatom sampling, identification and enumeration

The sampling, preparation, identification and enumeration of benthic diatom samples were

Table 2 Frequency of occurrence (%) of principal diatom taxa in the 45 RIV-TYPE sites investigated

	% Frequency
<i>Achnantheidium minutissimum</i>	100
<i>Gomphonema parvulum</i>	98
<i>Fragilaria capucina</i>	89
<i>Achnanthes oblongella</i>	82
<i>Fragilaria capucina</i> var. <i>vaucherie</i>	82
<i>Cymbella minuta</i>	80
<i>Cymbella silesiaca</i>	78
<i>Nitzschia dissipata</i>	78
<i>Cocconeis placentula</i>	73
<i>Gomphonema olivaceum</i>	71
<i>Nitzschia palea</i>	71
<i>Navicula lanceolata</i>	67
<i>Navicula gregaria</i>	62
<i>Tabellaria flocculosa</i>	62
<i>Gomphonema angustum</i>	60
<i>Synedra ulna</i>	60
<i>Reimeria sinuata</i>	58
<i>Meridion circulare</i>	56
<i>Diatoma moniliformis</i>	44
<i>Achnanthes lanceolata</i>	42
<i>Hanannea arcus</i>	42
<i>Gomphonema truncatum</i>	40
<i>Surirella brebissonii</i>	38
<i>Brachysira vitrea</i>	36
<i>Gomphonema minutum</i>	36
<i>Navicula cryptotenella</i>	36
<i>Navicula halophila</i>	36
<i>Cocconeis pediculus</i>	29
<i>Amphora pediculus</i>	27
<i>Fragilaria pulchella</i>	24
<i>Meridion circulare</i> var. <i>constrictum</i>	24
<i>Rhoicosphenia abbreviata</i>	24
<i>Cymbella gracilis</i>	22
<i>Navicula tripunctata</i>	22
<i>Eunotia bilunaris</i>	21
<i>Achnanthes flexella</i>	20
<i>Diatoma tenuis</i>	20
<i>Gomphonema acuminatum</i>	20
<i>Gomphonema clavatum</i>	20
<i>Nitzschia linearis</i>	20
<i>Frustulia rhomboides</i>	18
<i>Eunotia</i> sp.1	16
<i>Frustulia rhomboides</i> var. <i>viridula</i>	16
<i>Cymbella microcephala</i>	13

carried out following CEN standards (prEN 13946 2000, prEN T230 2002). Diatoms were sampled from cobble substrata as the preferred substratum (epilithic diatoms). Identifications of prepared diatoms were made primarily with the monographs of Krammer and Lange-Bertalot (1986-1991). As the first samples collected (winter 02/03) were as part of a pilot survey, a count of 300 valves was performed on these samples. However, due to the dominance of *Achnantheidium minutissimum*, this count was increased to 500 valves per slide for the spring samples. A further scan of each slide was also made to ensure all taxa present were accounted for.

Statistical analysis

Diatom counts were converted to percent abundant data and subsequently log-transformed. Diatom distribution was investigated using detrended correspondence analysis (DCA) using the CANOCO 4 package of ter Braak and Šmilauer (1998).

RESULTS

Epilithic diatom diversity

A total of 175 taxa were identified from the RIVTYPE sites sampled (Table 2). Both the winter and spring dataset were dominated by *A. minutissimum*. This taxon consistently reached high abundances at the majority of river sites in both seasons and was the only species common to 90% of all sites. Nineteen species were common to 50% of the sites, and these were *A. minutissimum*,

Table 3
Frequency of occurrence (%) of principal diatom taxa in the 24 lowland sites

	% Frequency
<i>Achnantheidium minutissimum</i>	96
<i>Cocconeis placentula</i>	96
<i>Amphora pediculus</i>	96
<i>Gomphonema minutum</i>	88
<i>Navicula cryptotenella</i>	88
<i>Navicula lanceolata</i>	83
<i>Synedra ulna</i>	79
<i>Cymbella minuta</i>	75
<i>Gomphonema olivaceum</i>	75
<i>Rhoicosphenia abbreviata</i>	75
<i>Navicula tripunctata</i>	75
<i>Fragilaria capucina</i> var. <i>vaucherie</i>	71
<i>Achnanthes lanceolata</i>	71
<i>Gomphonema parvulum</i>	63
<i>Navicula gregaria</i>	63
<i>Cocconeis pediculus</i>	63
<i>Caloneis bacillum</i>	63
<i>Nitzschia dissipata</i>	58
<i>Nitzschia palea</i>	54
<i>Diatoma vulgare</i>	54
<i>Reimeria sinuata</i>	50
<i>Fragilaria capucina</i>	42
<i>Gomphonema angustum</i>	29
<i>Cyclotella meneghiana</i>	29
<i>Melosira varians</i>	29
<i>Cymbella silesiaca</i>	25
<i>Nitzschia recta</i>	25
<i>Navicula menisculus</i>	21
<i>Meridion circulare</i>	17
<i>Surirella brebissonii</i>	17
<i>Nitzschia fonticola</i>	13
<i>Diatoma mesodon</i>	13
<i>Gyrosigma acuminatum</i>	13
<i>Navicula atomus</i>	13
<i>Navicula cryptocephala</i>	13
<i>Navicula halophila</i>	8
<i>Diatoma tenuis</i>	8
<i>Surirella angusta</i>	8
<i>Achnanthes biasoletiana</i>	8
<i>Amphora montana</i>	8
<i>Cyclotella radiosa</i>	8
<i>Navicula capitata</i>	8
<i>Amphora inariensis</i>	8
<i>Aulacoseira ambigua</i>	8

Achnanthes oblongella, *Brachysira vitrea*, *Cocconeis placentula*, *Cymbella minuta*, *C. silesiaca*, *Fragilaria capucina*, *F. capucina* var. *vaucherieae*, *Gomphonema angustum*, *G. minutum*, *G. olivaceum*, *G. parvulum*, *Navicula gregaria*, *N. lanceolata*, *Nitzschia dissipata*, *N. palea*, *Reimeria sinuata*, *Synedra ulna* and *Tabellaria flocculosa*. A large number of taxa were rare in their occurrence and therefore exhibited a limited and patchy distribution. Seventy-seven taxa (44%) were found in one river only.

Seasonal differences in diatom diversity were evident in the RIVTYPE sites, which were sampled in the winter and spring seasons of 2002-2003: 149 taxa were identified from winter sampling and 119 from spring sampling. The average number of taxa per site was greater in spring (21.4) than in winter (18.7). Differences were evident in the types of taxa that were more common in sites from the two seasons. For example, *A. minutissimum*, *A. oblongella*, *C. placentula*, *C. minuta*, *C. silesiaca*, *F. capucina*, *F. capucina* var. *vaucherieae*, *G. olivaceum*, *G. parvulum*, *N. gregaria*, *N. lanceolata*, *N. dissipata*, *N. palea* and *T. flocculosa* were the most commonly-occurring taxa in winter samples, whereas in spring, *A. minutissimum*, *Amphora pediculus*, *C. placentula*, *C. minuta*, *G. minutum*, *G. olivaceum*, *Navicula cryptotenella*, *N. lanceolata*, *N. tripunctata*, *Rhoicosphenia abbreviata* and *S. ulna* were the most common.

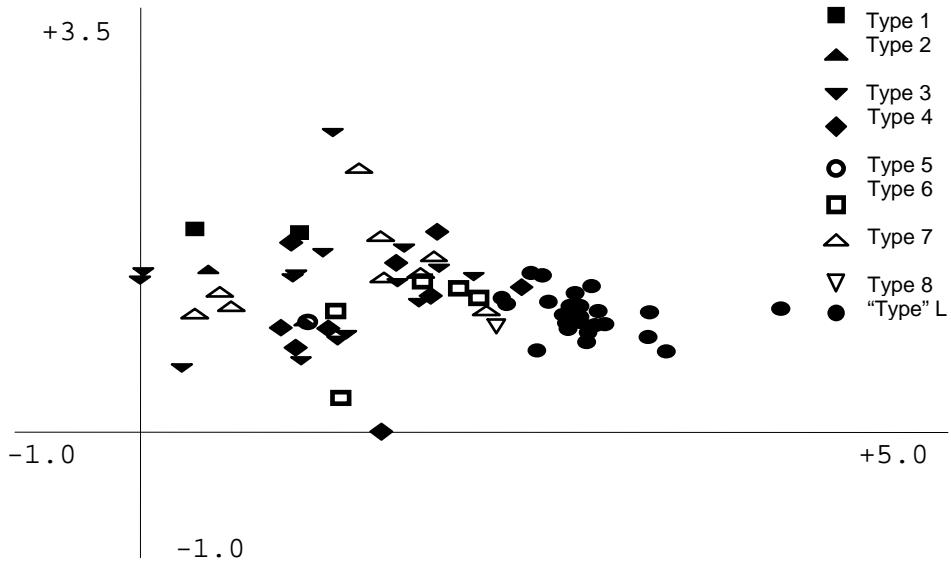


Fig. 2. DCA ordination of 45 RIVTYPE sites denoted by typology (Type 1-7) and 24 lowland sites (Type L) based on benthic diatom distribution.

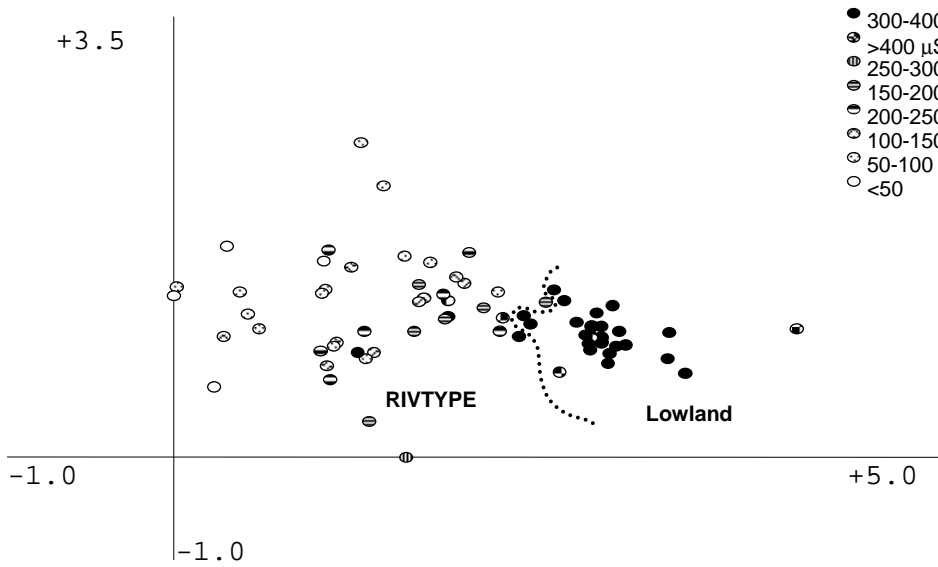


Fig. 3. DCA ordination of 45 RIVTYPE sites and 24 lowland sites based on benthic diatom distribution. Sites are denoted by the average water conductivity (mS cm^{-1}).

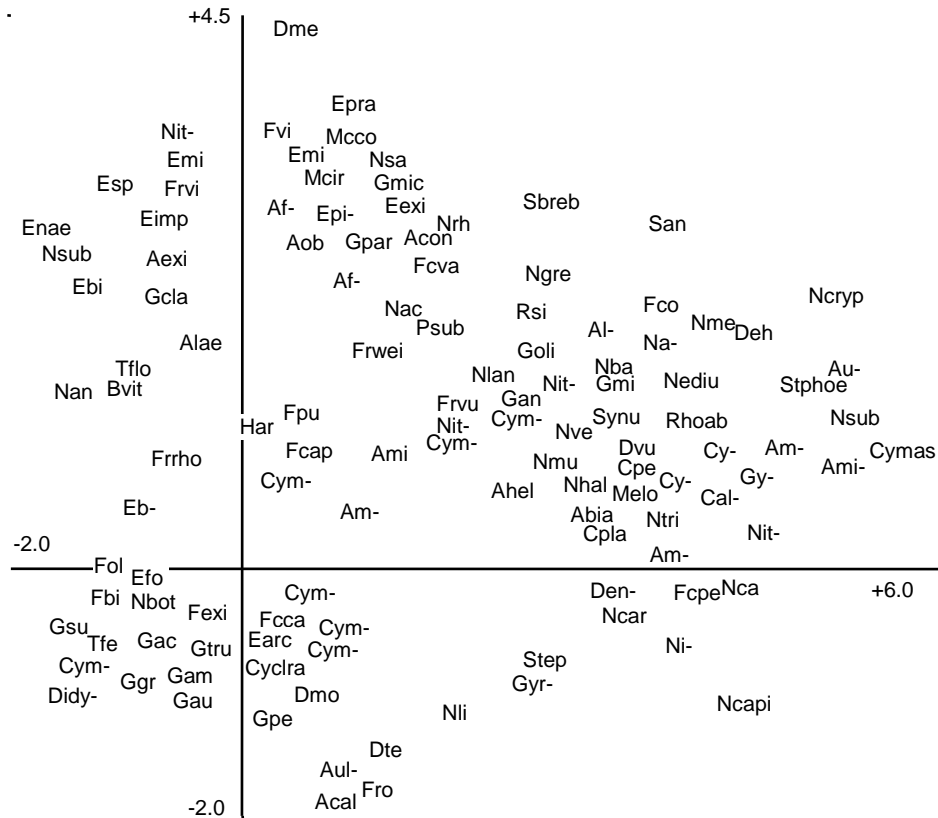


Fig. 4. DCA ordination of 45 RIVTYPE sites and 24 lowland sites based on benthic diatom distribution. Species ordination.

Achnanthes biasoletiana-Abias; *A. calcar*-Acal; *A. conspicua*? -Acons; *A. exigua*-Aexig; *A. flexella*-Aflex; *A. helvetica*-Ahelv; *A. laevis*-Alae; *A. lanceolata*-Alanc; *A. minutissima*-Amin; *A. oblongella*-Aobl; *Amphipleura lindheimeri*-Amphipl; *Amphora inariensis*-Aminar; *A. montana*-Ammon; *A. pediculus*-Amped; *Aulacoseira ambigua*-Aulamb; *A. granulata*-Aulgran; *Brachysira vitrea*-Bvit; *Caloneis bacillum*-Calbac; *C. pediculus*-Cped; *C. placentula*-Cplac; *Cyclotella meneghiana*-Cycmen; *C. radiosa*-Cyclrad; *Cymatopleura solea*-Cymat; *Cymbella aspera*-Cymasp; *C. cistula*-Cymcis; *C. gracilis*-Cymgra; *C. microcephala*-Cymmic; *C. minuta*-Cymmin; *C. prostrata*-Cympro; *C. silesiaca*-Cymsil; *Denticula tenuis*-Denten; *Diatoma ehrenbergii*-Dehr; *D. mesodon*-Dmes; *D. moniliformis*-Dmon; *D. tenuis*-Dten; *D. vulgare*-Dvul; *Didymosphenia geminata*-Didymos; *Epithemia adnata*-Epitadn; *Eunotia arcus*-Earc; *E. bilunaris*-Ebil; *E. bilunaris* var. *bilunaris*? -Ebv; *E. exigua*-Eexig; *E. formica*-Efor; *E. implicata*-Eimpl; *E. minor*-Emin; *E. naegeli*-Enaeg; *E. praerupta*-Eprae; *Eunotia* sp. 4-Esp4; *Fragilaria bicapitata*-Fbic; *F. capucina*-Fcapu; *F. capucina* var. *capucina*-Fccap; *F. capucina* var. *perminuta*-Fcper; *F. capucina* var. *vaucherie*-Fcvau; *F. construens*-Fcon; *F. exigua*-Fexig; *F. oldenburgiana*?-Fold; *F. pulchella*-Fpul; *F. robusta*-Frob; *F. virescens*-Fvir; *Frustulia rhomboides*-Frrhom; *F. rhomboides* var. *viridula*-Frvir; *F. vulgaris*-Frvul; *F. weinholdii*-Frwei; *Gomphonema acuminatum*-Gacu; *G. amoenum*-Gamo; *G. angustum*-Gang; *G. augur*-Gaug; *G. clavatum*-Gclav; *G. gracile*-Ggra; *G. micropus*-Gmicr; *G. minutum*-Gmin; *G. olivaceum*-Goll; *G. parvulum*-Gparv; *G. parvulum* var. *exilissimum*-Gpex; *G. subclavatum*-Gsub; *G. truncatum*-Gtrun; *Gyrosigma acuminatum*-Gyracu; *G. nodiferum*-Gyrnod; *Hannanea arcus*-Harc; *Melosira varians*-Melos; *Meridion circulare*-Mcirc; *M. circulare* var. *constrictum*-Mcon; *Navicula accomoda*-Nacc; *N. angusta*-Nang; *N. atomus*-Natom; *N. bacillum*-Nbac; *N. bottnica*-Nbot; *N. capitata*-Ncap; *N. capitatoradiata*-Ncapit; *N. cari*-Ncari; *N. cryptotenella*-Ncrypt; *N. gregaria*-Ngreg; *N. halophila*-Nhalo; *N. lanceolata*-Nlanc; *N. menisculus*-Nmene; *N. mutica*-Nmut; *N. rhychocephala*-Nrhy; *N. saprophila*-Nsap; *N. subhamulata*-Nsubh; *N. subtilissima*?-Nsubt; *N. tripunctata*-Ntrip; *N. veneta*-Nven; *Nedium dubium*-Nedium; *Nitzschia dissipata*-Nitdis; *N. fonticola*-Nifon; *N. inconspicua*-Nitiinc; *N. linearis*-Nlin; *N. palea*-Nitpal; *N. recta*-Nitrec; *P. subcapitata*-Psubc; *Reimeria sinuata*-Rsin; *Rhoicosphenia abbreviata*-Rhoabb; *Stauroneis phoenicenteron*?-Stphoen; *Stephanodiscus hantzschii*-Steph; *Surirella angusta*-Sang; *S. brevissonii*-Sbrevi; *Synedra ulna*-Synul; *Tabellaria fenestrata*-Tfen; *T. flocculosa*-Tflo

Seventy-one epilithic diatom taxa were identified from the twenty-four lowland Irish rivers sampled in summer 2002. Three species were common to all twenty-four rivers; *A. minutissimum*, *A. pediculus* and *C. placentula* (Table 3). Twenty-four taxa (34%) were found in one river only. In RIVTYPE sites, the total number of taxa per site recorded ranged between 6 and 38 (mean = 20), while in lowland rivers the range was 17 to 32 (mean = 23.5). The highest number of taxa was recorded was 32 and the lowest was 17.

Epilithic diatom distribution

DCA was employed to determine if differences in typology (Table 1) were expressed to any extent in the distribution of diatom taxa between the 45 sites. The twenty-four lowland sites were also included in these analyses. DCA (Figure 2) showed that this group of rivers were quite different in terms of diatom diversity to the RIVTYPE sites. The species ordination of the diatom assemblage is illustrated in Figure 4. Ubiquitous species such as *A. minutissimum*, *C. minuta* and *C. placentula* are found towards the centre of the species cluster and were found in most of the RIVTYPE and lowland sites. *Achnanthes lanceolata*, *A. pediculus*, *Caloneis bacillum*, *Cocconeis pediculus*, *Diatoma vulgare*, *G. minutum* and *N. cryptotenella* were much more frequent in the lowland sites than at the RIVTYPE sites. In contrast, *A. oblongella*, *B. vitrea*, *Cymbella gracilis*, *C. silesiaca*, *Diatoma moniliformis* and *T. flocculosa* among others were much more frequent in the RIVTYPE sites than in the lowland sites (Tables 2 and 3).

There was some differentiation between the RIVTYPE sites in respect of diatom diversity. *B. vitrea*, *Frustulia rhomboides*, *Gomphonema acuminatum*, *T. flocculosa*, *T. fenestrata* and a number of *Eunotia* spp. were more frequent at sites with low conductivities. *Gomphonema angustum*, *G. olivaceum*, *N. palea*, *Frustulia vulgaris* and *Navicula halophila*, among others, were more abundant at the sites with higher conductivities.

The typological classification of the RIVTYPE sites was not reflected to any extent in the site ordination based on diatoms (Figure 2). However, the DCA analysis did show that there was a greater variation in diatom diversity between the RIVTYPE sites than the lowland sites. It also suggests some differentiation of the RIVTYPE sites, because some of the more alkaline sites were more similar in diatom composition to the lowland sites than to the less alkaline RIVTYPE sites. This is seen more clearly in Figure 3 where the average water conductivity values of the sites are shown in an ordination diagram. Less alkaline sites of low conductivity are located towards the left of the ordination and sites with higher alkalinity and higher conductivity are clustered to the right. Axis 1 scores for sites are significantly correlated with conductivity ($r = 0.62$,

$P < 0.05$). Conductivity was strongly correlated with alkalinity but the correlation of axis 1 scores with alkalinity was somewhat weaker ($r = 0.56$, $P < 0.05$).

DISCUSSION

The diatom samples from both the RIVTYPE and lowland river surveys generally had high species richness and were characterized by a small number of dominant taxa and a large number of taxa that occurred at single sites only. This pattern of abundance has also been documented in a number of other diatom river studies (Van Dam 1982, Round 1993, Kelly and Whitton 1995, Allott and Flower 1997, Goldsmith 2000, Ní Chatháin 2002). A larger number of taxa was identified from the RIVTYPE sites in comparison with the lowland sites and this may be due to a number of factors, including the larger number of rivers with more varied hydrology, two-season sampling and also the higher water quality of the rivers in the RIVTYPE dataset. In addition, the lowland sites were quite different in character from most of the RIVTYPE sites as most were medium sized eutrophic rivers with calcareous substrata with Q-ratings of 3, 3-4 and 4 (moderately polluted, slightly polluted and unpolluted sites). There was also greater variation in diatom species richness between the RIVTYPE sites than the lowland sites, as a consequence probably of greater variation in hydrology of the rivers in the RIVTYPE study.

DCA revealed distinct differences in diatom distribution between the RIVTYPE and lowland rivers. Ubiquitous species such as *A. minutissimum*, *C. minuta* and *C. placentula* were found in most of the RIVTYPE and lowland sites. This was not surprising, as these species appear to occupy a broad ecological range. For example, *A. minutissimum* in particular is present and often abundant in the majority of epilithic diatom river studies in Ireland, Britain, Europe, Canada and the US (Ní Chatháin 2002, Sabater *et al.* 1988, Tomas and Sabater 1985, Cox 1990, Dixit and Smol 1994, Pan *et al.* 1996, Rott *et al.* 1998, Àces *et al.* 2003), and has a widespread distribution in circumneutral rivers (Kelly 2000). *C. placentula* is also a widely-occurring species and a well-known epiphyte of the green alga *Cladophora glomerata* in rivers, but is also found growing abundantly on rocks and is an abundant species across a wide range of chemical conditions (Kelly 2000). Species more common in the lowland sites such as *A. pediculus*, *C. pediculus*, *D. vulgare* and *G. minutum* among others, are common species in rivers with slight to moderate pollution. *A. pediculus*, although found commonly in RIVTYPE sites, was found at lower abundances than at the lowland sites. *C. pediculus*, *D. vulgare* and *G. minutum* were also found at lower abundances in the RIVTYPE sites in comparison with lowland sites. These species were also common and abundant throughout a two-year

study of the eutrophic River Deel in Ireland (Ní Chatháin 2002). *A. pediculus*, *D. vulgare*, *N. tripunctata* and *N. dissipata* tend to be found in eutrophic rivers free from significant organic pollution (Kelly 2000). *D. vulgare* is also abundant in nutrient-enriched rivers (Patrick and Reimer 1966, Áces and Kiss 1991).

In contrast, species that were rarely found in the lowland rivers were found in more abundance and more frequently at the RIVTYPE sites *e.g.* *A. oblongella*, *B. vitrea*, *C. silesiaca* and *T. flocculosa*. *A. oblongella* and *B. vitrea* are assigned an ecological indicator value of 1 (*i.e.* oligotrophic species) by Van Dam (1994), and seem to prefer nutrient-poor waters. Kelly (2000) also found that *B. vitrea* and *T. flocculosa* were found more frequently in nutrient poor waters with low conductivities ($<100 \mu\text{S cm}^{-1}$) and this was also the case for the RIVTYPE dataset.

The original typological classification of eight typologies was not reflected to any extent in the DCA ordination of the sites RIVTYPE based on diatoms. This was hardly surprising, as the classification is based on hydrological criteria such as catchment size (*e.g.* Types 3 and 7) rather than biotic or ecological criteria. However, DCA indicated that there was significant differentiation between the RIVTYPE sites in respect of community composition of benthic diatoms. RIVTYPE sites at the alkaline end of the spectrum were more similar to the lowland sites than to the less alkaline RIVTYPE sites and a number of taxa were confined to, and clearly distinguish the latter sites (*e.g.* *Tabellaria* spp., *B. vitrea*). This suggests that a number of diatom taxa could be used to characterize rivers of low base and low nutrient status in Ireland, however this would require further investigation. It would be interesting to evaluate the impact of eutrophication on the diatom flora of this type of river.

Rivers of high water conductivity and alkalinity typical of lowland Irish rivers are poorly represented among the RIVTYPE sites. Nonetheless, some diatom species (*A. pediculus*, *C. pediculus*, *G. angustum*, *N. tripunctata*) were typical of these sites. Many of the other diatom species were distributed along the gradient between these two extremes. Water conductivity and alkalinity are unlikely to be the only factors influencing diatom distribution, but at present there is insufficient information on the hydrology, water chemistry and ecology of these sites to assess the contribution of other factors. However, this situation is likely to be remedied in the near future. From these preliminary results, therefore, it appears that benthic diatoms could contribute to an ecological classification of the RIVTYPE reference sites along with information on macrophytes, macronivertebrates and other algal groups.

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