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A comparison of the efficacy of pond-net and box sampling methods in turloughs – Irish ephemeral aquatic systems

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Abstract

Two methods were used to sample aquatic macroinvertebrates in three turloughs. Turloughs are systems that flood periodically from groundwater, in response to local rainfall patterns and contain rare aquatic species and assemblages. The first method used a standard pond net that was swept through the water column, while the second involved fixing a rectangular, open-bottomed box to the substrata and removing all organisms with a net. Similar overall sampling effort was applied to each method and individual box samples were found to take longer to gather than pond net samples. The box method, however, gave the maximum yield for a given sampling effort. Significantly more beetle species and individuals were recorded per unit area of bottom at all three turloughs using the box method. Multivariate analysis segregated samples, firstly according to site and secondly, with respect to method. The box method is a viable alternative to sampling with a pond net. It is more quantitative, objective, specific and reliable. This is particularly important in habitats distinguished by rare species and assemblages, and for which monitoring is driven by legislative needs.

Introduction

Lotic macroinvertebrate sampling methods have received a disproportionate amount of attention in the literature (e.g. Macan, 1958; Cummins, 1962; Crossman & Cairns, 1974; Furse et al., 1981; Mackey et al., 1984; Humphries et al., 1998; Metzeling & Miller, 2001) compared with lentic methods (Macan, 1977; Cheal et al., 1992; Muzaffar & Colbo, 2002). This is largely a result of the widespread use of aquatic invertebrates in the biological monitoring of stream/river quality, e.g. RIVPACS in the UK (Wright et al., 1984; Cox et al., 1995); Q-value system in Ireland (Flanagan & Toner, 1972) and AusRivAs in Australia (Marchant et al., 1997; Turak & Waddell, 2002). Although a variety of samplers have been described (e.g. Macan, 1958; Cummins, 1962; Hilsenhoff, 1969; Maitland, 1969; Crossman & Cairns, 1974; Croset et al., 1976; Elliott & Tullet, 1978), the pond net remains the most popular device employed in freshwaters. While procedures have been described for pond net sampling of lotic systems (SAC, 1978; I.S.O., 1985) no standardised technique has been designed for lentic systems. Pond nets may be swept or 'shuffled' (in a modification of the lotic kick-sampling method) and the size of the sample can be determined by time, distance, area or number of sweeps. Sweeping is best adapted to macrophyte beds and soft substrata, while 'shuffling' is used in stony or gravelly substrata. Although the pond net is a flexible, easily employed and cost-efficient method, it does present a number of problems. Pond netting is not quantitative, it does not capture organisms which are hidden in dense vegetation or buried in the substrata and it cannot be used to sample specific, homogenous micro-habitats. In order to overcome these difficulties, freshwater biologists have repeatedly come up with the same solution: a device to physically isolate an area of bottom and volume of water from which all trapped macroinvertebrates are netted or sieved (Bates, 1941; Goodwin & Eyles, 1942; Macan, 1958; James & Nicholls, 1961; de Eyto, 1999; O Connor, 2000). The shape of these devices has varied from cylinders (Macan, 1958; O Connor, 2000), to squares and rectangles (Bates, 1941; Goodwin & Eyles, 1942; Macan, 1958; James & Nicholls, 1961; de Eyto, 1999), with the area of bottom enclosed also varying widely. In this study, the sampling efficiency of a rectangular frame or 'box' was compared with a pond net method, for collection of invertebrates in the lentic environment of turloughs.

Turloughs are ephemeral systems found over karstified Carboniferous limestone, which fill and empty in response to fluctuations in the local water table (Reynolds, 1982, 1996; Coxon, 1987a; Reynolds et al., 1998). This generally means they are flooded in the northern hemisphere winter and are dry in summer. They are a priority for conservation, as their distribution is more or less restricted to Ireland and because of the importance of their geological, hydrological and biological characteristics, and are listed as priority habitats in Annex I of the European Union's Habitats Directive (92/43/EEC). Turloughs are distinguished from other temporary water bodies in that they fill from, and empty to, the groundwater through conduits known as 'swallow-holes' or 'slugaire' (Coxon, 1987b, 1994; Reynolds, 1996). Rare species and assemblages of flora and fauna have been recorded in turloughs (Praeger, 1932; MacGowran, 1979; O'Connell et al., 1984; Coxon, 1987b; Lott & Bilton, 1991; Goodwillie et al., 1997; Owen, 1997; Good & Butler, 2001; Ní Bhríaín et al., 2002; Ryder et al., 2003; Ryder et al., in press), including diverse and characteristic communities of crustaceans (Reynolds, 1985, 1996, 2000; Ali et al., 1987; Duigan & Frey, 1987a, b; Duigan, 1988; Grainger & Holmes, 1989; Reynolds & Marnell, 1999) and aquatic beetles (Bilton, 1988; Bilton & Lott, 1991; Foster et al., 1992; Bradish et al., 2002). Turlough littoral zones can include terrestrial grassland plants, limestone pavement, stone wall field boundaries and even woodland ground flora. Within Ireland (Fig. 1), the majority of turloughs are found to the west and north of the River Shannon (Coxon, 1987a).

Description of sites studied

Three turloughs in south-east county Galway were chosen for their similarity in area (Goodwillie et al., 1997) (Fig. 1). Caranavoodaun (051° 14′ 17″ N, 010° 50′ 36″ W, Irish grid

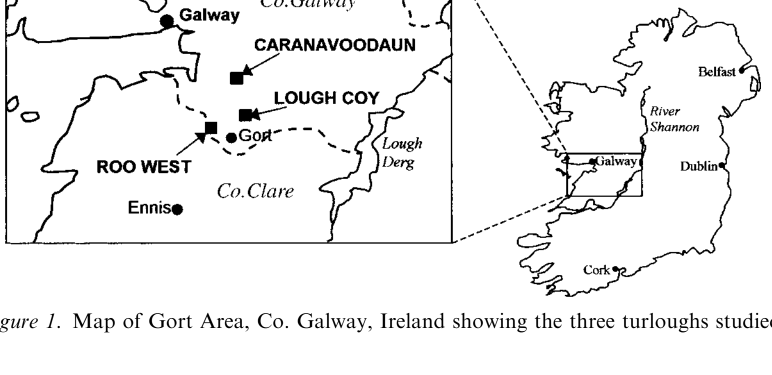

Figure 1. Map of Gort Area, Co. Galway, Ireland showing the three turloughs studied.

reference M455155, area 31 ha) is surrounded by hazel woodland and limestone heath and has a shallow basin containing areas of exposed limestone and extensive stone walls. It retains a small pool of water throughout most years. Lough Coy (051° 14′ 17″ N, 010° 50′ 35″ W, M490075, 36 ha) is separated from intensively managed agricultural land by a boundary of scrub. It occupies a deep, steep-sided depression, has some rock outcrops and retains a small permanent lake. Roo West (051° 14′ 14″ N, 010° 5′ 40″ W, M385022, 28 ha) is surrounded by an extensive area of exposed karstified limestone, is situated in a shallow basin and has a small permanent marl pond. All three turloughs are grazed: Caranavoodaun by horses and cattle, Lough Coy by cattle and Roo West by cattle and sheep.

Materials and methods

Roo West was sampled over three days, the 5th, 8th and 12th November 2001, while Caranavoodaun was sampled on 19th November and Lough Coy on 22nd November 2001.

Pond net sampling

Thirty sampling stations were selected randomly at Caranavoodaun and Lough Coy, while 40 were chosen at Roo West. Random distribution of sampling stations was achieved by pacing the shoreline of the turlough and then generating 30–40 random numbers between zero and the total number of paces. One sample was taken at each sampling station. A standard FBA pond net (0.9 mm mesh, 24.5 × 28.0 cm D-shaped frame) collected samples at a water depth of 25–30 cm. The net was swept sideways along a 1 m path and turned back along the same path to collect dislodged organisms, allowing the frame to graze the surface of the substrata with each pass.

Box sampling

Sixteen samples were taken from Caranavoodaun and Lough Coy and 18 from Roo West. The objective of the box sampling was to examine the spatial distribution of macroinvertebrates in turlough microhabitats and an approximately equal number of samples was taken from both open sward and along stonewalls. The box frame was created by cutting the bottom out of a sturdy, plastic storage box (39.0 cm long × 31.5 wide × 23.0 high). At each sampling station, the box was rapidly and vertically lowered through the water column and held firmly against the substrata (submerged terrestrial vegetation). Organisms trapped within the box were then removed using an aquarium-style fish net (0.8 mm mesh, 12.5 × 10.0 cm frame). This procedure was repeated three times at each sampling station along contiguous areas of bottom and the contents pooled to give one sample.

An individual operator employed each method in the field (pond net – S.B., box – T.R.). Both operators spent a roughly equivalent length of time sampling each turlough, resulting in the pond net method collecting almost double the number of samples for the same unit of time. All samples were sieved and preserved in 75% industrial alcohol in the field. Samples were sorted in the laboratory, invertebrates were identified using the keys of Macan (1977), Elliott & Mann (1979), Elliott et al. (1988), Friday (1988), Savage (1989), Wallace et al. (1990) and Gledhill et al. (1993). Oligochaetes, bivalves, water mites and dipteran larvae were not identified further than family, but were included in the analysis as operational taxonomic units.

Summary statistics and graphs were generated using Microsoft Excel. The data were ordinated by Nonmetric Multidimensional Scaling (NMS) using PC-ORD 4.17 for Windows. Mann–Whitney *U* tests were computed on medians using GraphPad InStat 3.05.

Results

The pond net method yielded lower overall species richness than the box method at all three turloughs, with 32 species captured using the pond net method compared with 50 with the box method at Caranavoodaun, 27 compared with 37 at Lough Coy and 27 compared with 38 at Roo West (Table 1). At Caranavoodaun, six species captured with the pond net were not taken with the box, while 24 species were captured with the box but not the pond net. Results were similar at

Table 1. Full species list and number of individuals sampled for the three turloughs. P-N – pond net, B – box, *n* – total number of samples

	Caranavoodaun		Lough Coy		Roo West	
	P-N	B	P-N	B	P-N	B
n	30	16	30	16	40	18
PLANARIIDAE						
Polycelis nigra	214	8			32	5
HYGROBIIDAE						
Potamopyrgus jenkinsi			4		1	
Bithynia tentaculata	65	16		3	24	23
LYMNAEIDAE						
Lymnaea palustris	56	6			27	
Lymnaea peregra	17	3	7		8	
PHYSIDAE						
Physa fontinalis		1				1
Aplexa hypnorum	9				1	
PLANORBIDAE						
Planorbis albus	10		13	3	55	
Planorbis carinatus				1		
Planorbis leucostoma	26	2			37	1
Planorbis planorbis						8
SUCCINEIDAE						
Succinea sp.		7		1		
ZONITIDAE						
Zonitoides sp.	1		15			
Bivalves		1				1
Oligochaetes		5				30
GLOSSIPHONIIDAE						
Glossiphonia complanata		1		5		
Theromyzon tessulatum		2	13	18		1
HIRUDINIDAE						
Haemopis sanguisuga		1				
Hydracarina	19	25		1	10	6
ASELLIDAE						
Asellus aquaticus		1	2	64		
Asellus meridianus					3	1
GAMMARIDAE						
Gammarus duebeni						1
Gammarus lacustris	2	5	7	13	19	10
BAETIDAE						
Cloeon dipterum	155	20			1	
Dragonfly larvae	21	6				
NEPIDAE						
Nepa cinerea				1		
NOTONECTIDAE						
Notonecta glauca	4	1		2	1	2

Continued on p. 137

Table 1. (Continued)

	Caranavoodaun		Lough Coy		Roo West	
	P-N	B	P-N	B	P-N	B
n	30	16	30	16	40	18
CORIXIDAE						
Arctocorisa germari			19	1		
Callicorixa praeusta			1	1		
Corixa panzeri			10	3		
Sigara concinna			252	23	1	
Sigara dorsalis				1		
Sigara falleni			370	26		
Sigara lateralis	3	1	16	2		
Sigara scotti			1			
Sigara other			2			
Corixid larva	1			1		
LIMNEPHILIDAE						
Grammotaulius nigropunctatus	147	75	15	1	28	2
Limnephilus affinis/incisus			3	6	47	26
Limnephilus auricula	5		15			
Limnephilus binotatus	10	22	3	3		19
Limnephilus decipiens		2				
Limnephilus flavicornis		21				
Limnephilus rhombicus	140	169	19		23	30
Limnephilus vittatus	94	23			271	13
Dipteran larvae	39	12				61
Unidentified larvae		11		4		5
HALIPLIDAE						
Haliplus confinis		3		2		
Haliplus fulvus		1				
Haliplus lineatocollis					3	
Haliplus rufficollis-group		2				
Haliplus variegatus	2	4		1		
HYGROBIIDAE						
Hygrobia hermanni			1			
NOTERIDAE						
Noterus clavicornis		6		1		
DYTISCIDAE						
Hygrotus inaequalis		9		48	2	4
Hygrotus quinquelineatus	2	2	1		1	2
Hygrotus impressopunctatus		2				
Hydroporus erythrocephalus		1		2		3
Hydroporus memnonius						1
Hydroporus palustris	26	22	7	16	7	3
Hydroporus pubescens						1
Porhydrus lineatus	7	1		1		
Graptodytes bilineatus	1	2			50	22
Suphrodytes dorsalis						1

Continued on p. 138

Table 1. (Continued)

	Caranavoodaun		Lough Coy		Roo West	
	P-N	B	P-N	B	P-N	B
n	30	16	30	16	40	18
DYTISCIDAE						
Agabus bipustulatus		4		1		6
Agabus labiatus	4	4			2	5
Agabus melanocornis		1				1
Agabus nebulosus		5	1	10	9	19
Ilybius fuliginosus				1		
Colymbetes fuscus		7		3		19
Laccophilus minuntus	1	11	1	81	2	5
Dystiscus circumcinctus			1			
Dytiscus semisulcatus	1					
HYDRAENIDAE						
Ochthebius minimus		1				
HELOPHORIDAE						
Helophorus brevipalpis	1	7			1	4
Helophorus grandis						4
HYDROPHILIDAE						
Berosus signaticollis	38	42				
DRYOPIDAE						
Dryops species		7		3		20
Beetle larvae	47	45	20	16		7
Total number of individuals	116	636	819	370	666	373
Total number of species	32	50	27	37	27	38

Lough Coy and Roo West, with 11 and nine species, respectively, taken with the pond net and not found in box samples and 21 and 20 species, respectively, recorded with the box only.

Summary statistics were calculated for the number of species and the number of individuals per pond net and box sample (Tables 2 and 3). In order to compare the sampling methods more quantitatively, means and medians per 1000 cm^2 of bottom were calculated. Each pond net sample was estimated to cover an area of 2450 cm^2 (24.5×100 cm), while each box sample was known to cover an area of 3686 cm^2. No significant differences in median number of species were found between sampling methods. The median number of individuals per sample was not significantly different between methods at Caranavoodaun and Roo West (Table 3), but the pond

Table 2. The mean (± standard deviation), median and range of number of species per 1000 cm^2 of bottom (*n* is the total number of samples)

	Caranavoodaun				Lough Coy				Roo West			
	n	Mean	Median	Range	*n*	Mean	Median	Range	*n*	Mean	Median	Range
Pond-net	30	3 ± 1	3	1–6	30	2 ± 1	2	0–4	40	2 ± 1	2	0–4
Box	16	3 ± 1	3	1–5	16	2 ± 1	2	1–4	18	2 ± 1	2	0–4

Tabla 3. The mean (± standard deviation), median and range of number of individuals per 1000 cm^2 of bottom (n is the total number of samples)

	Caranavoodaun				Lough Coy				Roo West			
	n	Mean	Median	Range	n	Mean	Median	Range	n	Mean	Median	Range
Pond-net	30	20 ± 15	15	4–57	30	11 ± 9	9*	0–35	40	7 ± 6	4	0–24
Box	16	12 ± 6	13	1–24	16	6 ± 5	6*	1–22	18	6 ± 6	5	1–9

Values with asterisks indicate significant differences between the medians.

net yielded significantly greater numbers per 1000 cm^2 at Lough Coy ($U = 138$, $p = 0.0193$, Mann–Whitney U test). The pond net method sampled lower beetle species richness than the box at each turlough, respectively 10 compared with 22 at Caranavoodaun, six compared with 13 at Lough Coy and nine compared with 17 at Roo West (Table 1). Median species richness for beetles was also significantly less using the pond net method when adjusted for area of bottom (Caranavoodaun $U = 94.5$, $p = 0.0008$; Lough Coy $U = 123$, $p = 0.007$; Roo West $U = 175$, $p = 0.0019$; Mann–Whitney U test). The pond net method also produced a lower total catch of beetles at all turloughs (83 compared with 144 at Caranavoodaun, 12 compared with 170 at Lough Coy and 77 compared with 120 at Roo West) (Table 1). The median number of beetles captured per 1000 cm^2 using the pond net method, was significantly lower at Caranavoodaun ($U = 125.5$, $p = 0.0086$; Mann–Whitney U test), Lough Coy ($U = 104$, $p = 0.0017$; Mann–whitney U test) and Roo West ($U = 181$, $p = 0.0027$; Mann–Whitney U test). Corixids were abundant at Lough Coy and significantly greater median numbers per 1000 cm^2 were captured with the pond net ($U = 61.5$, $p < 0.0001$; Mann–Whitney U test).

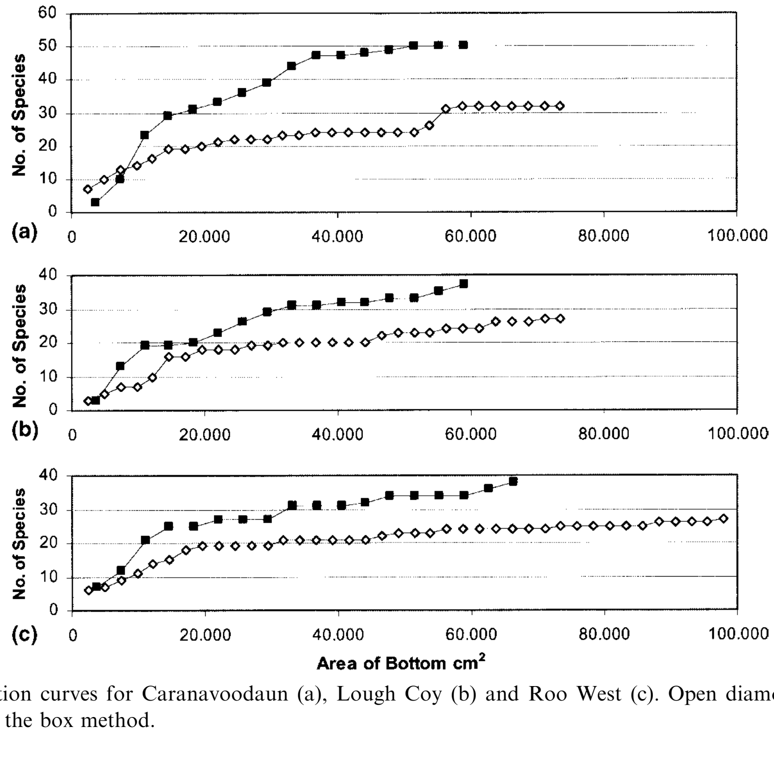

Figure 2. Family accretion curves for Caranavoodaun (a), Lough Coy (b) and Roo West (c). Open diamonds show the pond-net method, closed squares the box method.

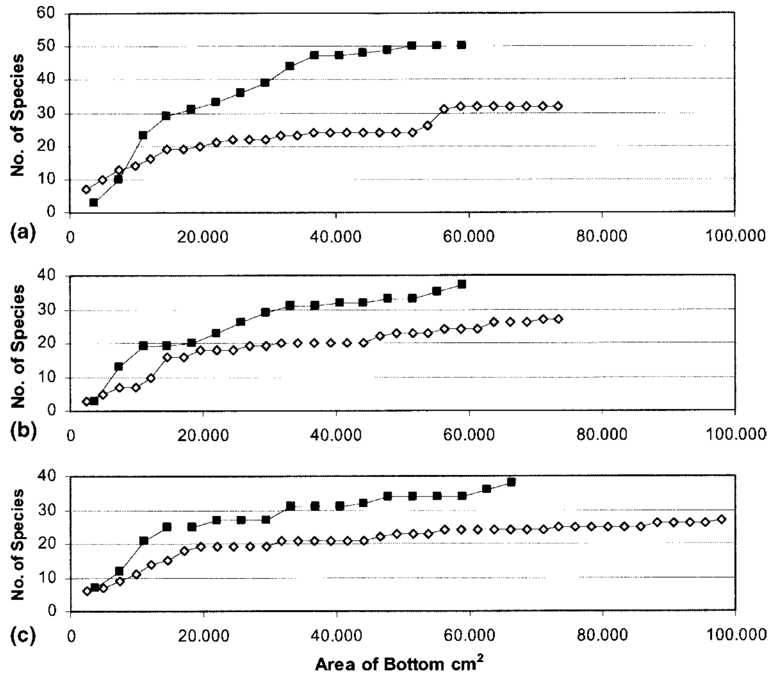

Figure 3. Species accretion curves for Caranavoodaun (a), Lough Coy (b) and Roo West (c). Open diamonds show the pond-net method, closed squares the box method.

Taxon accretion curves were drawn for families (Fig. 2) and species (Fig. 3). As the sampling stations were selected randomly, samples were arranged sequentially and the cumulative number of families/species counted. Fewer families were recorded using the pond net method than the box method at all three turloughs (eight fewer families at Caranavoodaun, five at Lough Coy and three at Roo West) (Fig. 2). The initial increase in families with area sampled was more rapid with the box method. After 11 057 cm^2, or three samples, 63% of all families recorded using the box method were captured at Caranavoodaun, 71% at Lough Coy and 67% at Roo West. After 12 250 cm^2, or five pond net samples, respectively 53, 58 and 53% were captured at Caranavoodaun, Lough and Roo West. The number of families collected using the box method appeared to level off after sampling of 33 000 cm^2 of bottom at both Lough Coy and Roo West, while the Caranavoodaun curve continued to rise. The number of families taken with the pond net continued to rise in a slow step-wise fashion as the area sampled increased.

The species accretion curves show more continuous and steeper increases for both methods and again illustrate the disparity in species richness between methods (Fig. 3). The box species accretion curves continued to increase until the final sample in all three turloughs, indicating both methods failed to record the full species richness for the sites. This is to be expected as taxon accretion curves are unlikely to ever reach an asymptote (Mackey et al., 1984). After 29 484 cm^2 or eight samples, however, 89% of all species were recorded at both Caranavoodaun and Roo West and 94% at Lough Coy, using the box method.

NMS was used to ordinate samples using the proportional abundance of species. Before ordination, species occurring in 5% or less of sample units were removed and the data set was transformed using an arcsine square root transformation. The Sorensen distance measure was used on random starting configurations. Three dimensions captured most of the variance in macroinvertebrate communities, with axes one, two and three containing 41.3, 20.2 and 16.9%, respectively, of the information (cumulative = 78.5%). Higher dimensions improved the model very little. Axis one and two (cumulative = 61.5%) are shown here

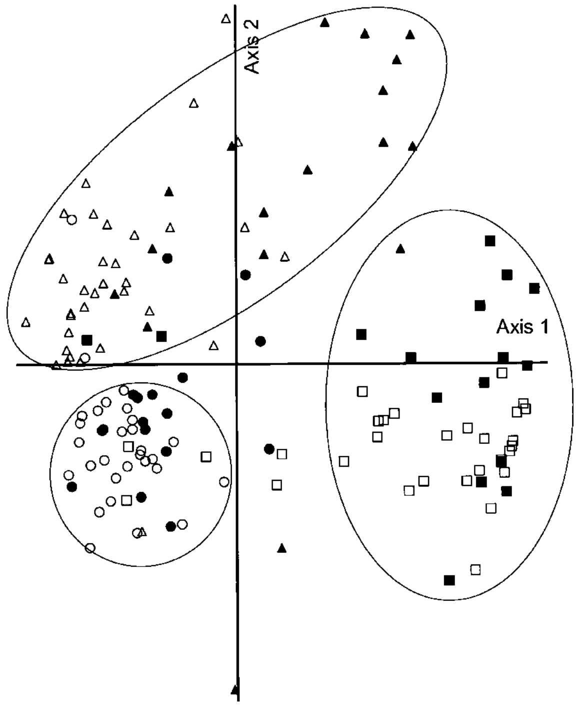

Figure 4. Nonmetric Multidimensional Scaling plot of samples using the proportional abundance of species. Circles represent Caranavoodaun; squares, Lough Coy and triangles, Roo West. Open symbols are pond net samples and closed symbols are box samples.

(Fig. 4). The final three-dimensional solution took 80 iterations, had a final stress of 17.54 and a final instability of 0.00042. A Monte Carlo test performed with 20 randomized runs gave a probability of 0.048 that a similar final stress could have been obtained by chance. The plot shows a broad separation of the samples according to turlough. Samples from Lough Coy separated from those of Caranavoodaun and Roo West along axis one, while Caranavoodaun and Roo West samples were split along axis two. It is also evident that within the turlough clusters there is a separation of samples according to sampling method, particularly at Roo West.

Discussion

Biological monitoring of the invertebrate fauna of water bodies requires that samples be representative of the community present. In priority habitats, such as turloughs, which are often distinguished by rare species and assemblages, it is crucial to retrieve as close to the full species compliment as possible (Cao et al., 1998). Clearly, for the same investment of operator energy and time, the box method was far more successful than the pond net method in sampling the macroinvertebrate species richness of turloughs. Other quantitative devices have been described as less successful than pond nets in recording taxonomic richness (Macan, 1977; Mackey et al., 1984; Humphries et al., 1998). Hilsenhoff (1969) found, however, that his quantitative artificial substrate sampler collected more lotic taxa than a pond net.

Although the overall species richness was considerably greater using the box at all three turloughs, there was no clear difference in the effectiveness of the methods at the 1000 cm^2 level. Yet, the box method did prove to be significantly more effective than the pond net in sampling beetle diversity and density at the 1000 cm^2 level, allowing vigorous investigation of the dense vegetation, into which beetles dive when disturbed.

At Lough Coy, the significantly greater catch of macroinvertebrates per 1000 cm^2 taken with the pond net can be explained by the dominance of corixids in those samples. Humphries et al. (1998) also found Hemiptera dominated pond net, sweep samples from lowland rivers. The abundance of corixids in pond net samples, along with the fact that *Dytiscus* spp. were captured at Caranavoodaun and Lough Coy using the pond net, but not with the box, suggests that the box method underestimated and may be inappropriate for sampling larger and faster swimming invertebrates.

Although neither method recorded all families/species, each pond net taxon accretion curve appeared to approach an asymptote. This suggests the maximum potential of the method was being reached and that pond nets are not capable of capturing some of the more elusive turlough species, including those firmly anchored in the substrata. While further box sampling may have increased the diversity recorded, it would have been prohibitively time consuming.

The differences between methods were not sufficiently large to misclassify many samples using NMS. Both methods were successful in separating the turloughs, indicating each is sufficient to identify broad patterns among sites. That pond net samples were more tightly clustered and more clearly separated than box samples, reflects the greater specificity of box samples, demonstrated by the high overall diversity, but low diversity per 1000 cm^2. Box samples appeared to portray more accurately the natural aggregation of species.

It may be possible to use a combination of data gathered by both methods to segregate turloughs using NMS. The separation of samples according to sampling method indicates the methods are not directly comparable, however, and a combination of the methods could not be used to examine within-turlough patterns.

The high final stress value for the NMS probably reflects the large number of sampling units involved. While this reduces the robustness of the plot, it is possible to make a general interpretation. Separation of turloughs along axis one appeared to be determined by water permanence, the scarce summer water at Caranavoodaun and Roo West contrasting with the small permanent lake at Lough Coy. Differences between Caranavoodaun and Roo West identified along axis two, may reflect higher productivity and greater microhabitat diversity at Caranavoodaun.

Conclusions

Sampling with the box was more efficient and accurate than with the pond net. Pond netting, however, was protracted by the need to bottle sweeps individually and pooling sweeps to form a single sample per turlough would increase its efficiency. Despite this, an increase in the number of sweeps would be unlikely to reveal the full diversity of macroinvertebrates in a turlough. The box method was more thorough, if slightly more time-consuming overall.

Part of the success of the box method was owing to its ability to capture organisms hidden in the more structurally complex parts of the substrata, such as amongst mosses or stones. By contrast the pond net sampled the water column more efficiently. The box method detected species aggregations and was suitable for investigating invertebrate microhabitats. Unlike the pond net, the box method was highly quantitative, enabling an expression of numbers per area of bottom or volume of water, and provided more reliable sampling. To test its objectivity, however, the box method should be tested for inter-operator differences. Pond nets have demonstrated significant inter-operator differences when used in streams (Furse et al., 1981; Mackey et al., 1984).

Turloughs are unique and require a sampling strategy that reflects their high biodiversity importance. Sampling is complicated by the availability of water, which can vary among years and even among turloughs, and the variability of the substrata, which comprises semi-aquatic vegetation communities and physical structures such as limestone pavement and stone walls. The timing and frequency of turlough sampling needs consideration and testing. As a rule of thumb, we recommend turloughs are sampled a minimum of twice per year, once before the water recedes in spring/summer and once following 3 or 4 weeks flooding in autumn.

Adequate sampling of a turlough, particularly by those who lack experience of these systems, should incorporate both box and pond net methods. Based on our species accretion curves, we suggest ten box samples (or 36 860 cm^2) be taken per turlough and supplemented by a small number of sweeps with the pond net. This should maximise the quality of the data, while minimising the time and effort expended. The box samples should be taken from the variety of microhabitats present, preferably using stratified random sampling. Such a standardised sampling regime, although requiring further statistical validation, would be particularly appropriate to monitoring the persistence of the rare, relict and threatened turlough species.

The box sampling method could be applied to other freshwater habitats. Turloughs are recognised for the scarcity of their aquatic invertebrate populations (Reynolds, 1996, 2000), so the success of the box method could be expected to improve when applied to permanent, lentic littoral zones. The box is suitable for use in all shallow, lentic and lotic waters, from macrophyte beds to fine and coarse substrata, although modifications of the frame may be required to seal the bottom in cobbles and larger particle sizes (James & Nicholls, 1961). While many quantitative aquatic samplers are large and/or heavy, our small frame was light and was not an encumbrance to the operator.

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