north western international river basin district
North South Shared Aquatic Resource (NS SHARE)

neagh bann international river basin district
North South Shared Aquatic Resource (NS SHARE)

north eastern river basin district
North South Shared Aquatic Resource (NS SHARE)

North South Shared Aquatic Resource (NS Share)

Assessment of the Effectiveness and Suitability of Available Techniques for Sampling Invertebrates in Deep Rivers

Final Report: November 2006

T1(A5.8) - 1.1

Environment & Heritage Service
www.ehsni.gov.uk

An Agency within the Department of the Environment
www.doeni.gov.uk

AN ROINN COMHSHAOIL, OIDHREACHTA AGUS RIALTAIS ÁITIÚIL
DEPARTMENT OF THE ENVIRONMENT, HERITAGE AND LOCAL GOVERNMENT

INTERREG IIIA

Project part financed by the European Union

North **S**outh **Sh**ared **A**quatic **Re**source (NS Share)

Water Framework Directive

A Directive establishing a new framework for Community action in the field of water policy (2000/60/EC) came into force in December 2000. This Water Framework Directive (WFD) rationalises and updates existing legislation and provides for water management on the basis of River Basin Districts (RBDs). The WFD was transposed into national law in Northern Ireland by the Water Environment (Water Framework Directive) Regulations (Northern Ireland) 2003 and in the Republic of Ireland by the European Communities (Water Policy) Regulations 2003. The primary objective of the WFD is to maintain the "high status" of waters where it exists, prevent deterioration in existing status of waters and to achieve at least ***"good status"*** in relation to all waters by 2015.

NS Share Study Area

NS Share is a cross border project and incorporates three River Basin Districts as set out in the joint North/South Consultation paper *Managing our Shared Waters*:

1. North Western International River Basin District (NWIRBD);
2. Neagh Bann International river Basin District (NBIRBD);
3. North Eastern River Basin District (NERBD).

The NW and NB are International River Basin Districts as they share their waters between Northern Ireland (NI) and Republic of Ireland (ROI). The NERBD is contained wholly within NI.

NS Share Project

The overall objective of the project is to strengthen inter-regional capacity for environmental monitoring and management at the river basin district level, to improve public awareness and participation in water management issues, and to protect and enhance the aquatic environment and dependent ecosystems.

The NS Share project aims to facilitate delivery of the objectives of the WFD within the project area between August 2004 and March 2008.

The NS Share project is funded by the EU INTERREG IIIA Programme for Ireland / Northern Ireland. The Department of the Environment (NI) and the Department of the Environment, Heritage and Local Government (ROI) are implementing agents for the project. Donegal County Council is the project promoter. Technical support is proivded by the Environment and Heritage Service an agency within the Department of the Environment (NI), and the Environmental Protection Agency (ROI). RPS Consulting Engineers in association with Jennings O'Donovan are the principal consultants.

Assistance was also provided by the Marine Institute, Central Fisheries Board, Geological survey Ireland, Geological survey Northern Ireland, Loughs Agency, North West Regional Fisheries Board, and Cavan, Leitrim, Longford, Louth, Meath, Monaghan, and Sligo County Councils.

Project publications are available at www.nsshare.com/publications

PREFACE

The work presented in this paper was carried out as part of the NS SHARE project, which is funded by the European Union INTERREG IIIA programme for Ireland/Northern Ireland. The implementing agents for the NS SHARE project are the Department of Environment (DOE), Northern Ireland, and the Department of Environment Heritage and Local Government (DEHLG), Republic of Ireland. Donegal County Council (DCC) is the project promoter.

All data, drawings, reports, documents, databases, software and coding, website and digital media and publicity material produced as part of this project shall be the property of the DOE/DEHLG who will use, reproduce and distribute same as they see fit.

The views expressed in this document are not necessarily those of DOE, DEHLG or DCC. Their officers, services or agents accept no liability whatsoever for any loss or damage arising from the interpretation or use of the information, or reliance on views contained herein. This document does not purport to represent policy of any government.

ASSESSMENT OF THE EFFECTIVENESS AND SUITABILITY OF AVAILABLE TECHNIQUES FOR SAMPLING INVERTEBRATES IN DEEP RIVERS

By

M.W. NEALE, N.T. KNEEBONE, J.A.B. BASS, J.H. BLACKBURN, R.T. CLARKE, T.A. CORBIN, J. DAVY-BOWKER, R.J.M. GUNN, M.T. FURSE, J.I. JONES

Centre for Ecology & Hydrology
Winfrith Technology Centre
Dorchester
Dorset
DT2 8ZD
United Kingdom
Tel +44 01305 213 500 (Switchboard)
Tel +44 01305 213 560 (Direct line)
Fax +44 01305 213 600

CONTENTS

ACKNOWLEDGEMENTS

This project was supported by the EU INTERREG IIIA Programme for Ireland/Northern Ireland through the North South Share River Basin Management Project. We are grateful to all the organisations and individuals involved in this project, the European Commission, the Department of Environment, Heritage and Local Government, the Department of Environment, Northern Ireland, the Environment and Heritage Service, the Environmental Protection Agency, Kirk McClure Morton, and the University of Ulster. In particular we are grateful to Tony MacNally, Alan Barr, Brian Rippey, Peter Hale, John Lucey, John Murray-Bligh, Imelda O'Neill, Eamon Hagen and Mark Magee, for the provision of valued advice, data and project management. Thanks are also given to the University of Ulster for the loan of an outboard motor, and to the land owners, and the staff of the North Western Regional Fisheries Board (Bord Iascaigh Réigiúnach an Iarthuaiscirt) in particular, for assistance and access to the sites.

SUMMARY

Sampling macroinvertebrates in deep waters is inherently more difficult, hazardous and time-consuming than sampling shallow waters. However, such samples are required to assess the ecological status of water bodies that comprise deep river sites. The appropriate method(s) and protocols for sampling in deep waters need to be clearly defined.

Here we report the results of a field trial to examine the most appropriate technique(s), in terms of effectiveness and suitability, to be used when sampling benthic invertebrates from deep-water sites. Hence, we recommend a standard technique to be used when sampling benthic invertebrates from deep-water sites

Thirteen sites were selected to provide a range of representative deep-water sites known to support diverse macroinvertebrate communities. At each site three replicate samples were collected with each of the “deep water” techniques: light dredge, Mackey/Yorkshire pattern air-lift, long-handled pond net working from the bank and a marginal sweep with a standard pond net. At two sites there was sufficient wadeable area for a further three replicate “shallow water” standard RIVPACS kick samples to be collected within the study stretch, to enable comparison between deep and shallow water methods.

Analyses were undertaken to determine,

i) How efficient are the techniques in terms of the time and effort required to collect and process the samples? - The light dredge and long-handled pond net were the most efficient techniques in terms of the time and effort required to collect and process the samples, and the airlift the least efficient. All techniques were difficult to use in the field. However, the airlift always required a boat to collect a sample and a boat was required to collect a sample from the margin of narrow, deep rivers. Samples collected with the airlift took longer to sort and process than the other three techniques.

ii) How effective are the techniques at providing an adequate sample of the macroinvertebrate fauna present under all environmental conditions encountered? - The airlift provided the most adequate sample of the river channel fauna. The light dredge and long-handled pond net performed poorly relative to the airlift, in terms of all the metrics used, particularly when the substrate comprised a high proportion of boulders. The airlift had a significantly higher ASPT than all the other techniques. Samples collected at the margin sampled different components of the fauna to those collected from the river channel. Furthermore, the faunal assemblage of the margins seemed to be responding to different pressures to the fauna of the river channel.

iii) How precise are the techniques in terms of the uncertainty associated with each sample? - The airlift was the most precise of the techniques tested. The light dredge had such high sampling variance and low repeatability that it is effectively useless for assessing and discriminating the ecological status of sites of this type. Sampling precision has implications for confidence of site quality class. The technique with the lowest within site sampling variation will have the greatest confidence of class status.

iv) Which technique has the most cost effective precision? – The airlift was the most cost effective, in terms of the processing time required to achieve adequate precision. The increased costs in processing each airlift sample are outweighed by increased precision.

v) How comparable are the techniques to existing methodologies for sampling shallow rivers? - In terms of the time to process the samples, faunal composition and key metrics the samples collected with the airlift were the most similar to the standard RIVPACS kick sample. The light dredge and long-handled pond net collected a similar fauna to the kick and airlift, but less effectively, missing parts of the fauna and producing smaller, and lower scoring samples. The samples from the margin sampled a different fauna to the kick and airlift.

Consideration was also given to how suitable the techniques were in terms of health and safety.

It is recommended that the airlift is used for the routine monitoring of benthic macroinvertebrates at sites with extensive deep water habitats.

To permit the effective assessment of river quality at deep water sites, sampling activity should target both deep water habitats and margin habitat.

It was also recommended that deep water habitats should be integrated into existing shallow water models. The use of the airlift to sample deep water sites may enable deep waters to be classified with shallow waters. This has implications in that it will not be necessary to develop new independent models to assess deep waters. It should be possible to assess deep water sites with the Q system with little modification. In RIVPACS, modifications will be necessary, but the integration of shallow and deep sites will reduce the number of deep water reference sites needed to achieve the same level of group discrimination and precision of O/E (Observed/Expected) in a RIVPACS-type module.

1. INTRODUCTION

1.1 Background

This report forms the final stage of the NS-Share Deep Rivers Sampling Project. This project was commissioned as the result of a shared requirement to identify the most appropriate method(s) for collecting macroinvertebrate samples from deep river sites in both the United Kingdom and the Republic of Ireland. Such samples are required to assess the ecological status of water bodies that comprise deep river sites.

The sampling methodology developed for use at shallow river sites (timed pond net collections) is comparatively simple with the result that a high degree of standardisation is possible (McGarrigle *et al.* 1992; Murray-Bligh *et al*. 1997). In addition, much effort has been devoted in the UK to documenting and reducing sources of error from sampling variation, sorting and identification in order to improve the precision of the technique (Dines and Murray-Bligh, 2000; Clarke *et al.* 2002). In contrast, sampling deep waters is inherently more difficult, hazardous and time-consuming. The biologist has much less control of the sampling device and, as a consequence, it is difficult to sample all invertebrate habitats in proportion to their occurrence.

For the Environment Agency 1995 GQA survey (England & Wales), long-handled pond net sampling from the river bank was recommended for deep water sites on practical and safety grounds. However, the effectiveness of this technique has not been fully assessed. The long-handled pond net does not allow all habitats (marginal and benthic) to be sampled in proportion to their occurrence. This is necessary if a representative sample of the species present is to be achieved; an inaccurate assessment of quality will be given if species are missed at a site simply because they are out of range of the technique used. Much of the main channel of larger rivers is out of the range of the long-handled pond net and, as a consequence, mid-channel taxa are under-represented. A variety of other methods are in regular use for the assessment of deep-water sites across Britain and Ireland, with the methodology adopted determined at a regional level or by the individual collecting the sample.

However, more established methods for sampling benthos, such as dredges and airlifts, are more time consuming than the standard pond net technique and usually require several people, resulting in increased costs. A protocol on standard sampling effort has yet to be defined for deep river devices. Furthermore, the representation of the benthic community using such devices relative to that achieved using the standard shallow river pond net technique, has not been assessed so that the influence of the choice of technique (deep versus shallow technique) on the assessment of a site remains unknown.

The appropriate method(s) and protocols for sampling in deep waters need to be clearly defined. There is also a need to adopt standard approaches across ecoregions to ensure that in future, bio-assessments for deep rivers are as reliable as those currently available for shallow sites and comparable with them. In the context of the current work, deep water sites found on large rivers, impounded rivers and re-engineered channels are included but techniques used in canals, lakes and ponds are also pertinent. Nevertheless, biological monitoring strategies for some of these other water bodies are the subject of specific investigations within the NS-Share programme.

1.2 Objectives

An earlier report (Jones, Bass & Davy-Bowker 2005) recommended a field investigation designed to deliver clear guidance on the sampling method(s) to be used when collecting benthic invertebrate samples at deep-water sites.

The overall objective of this report is to present the results of field trials providing a comparison of sampling device performance using the techniques approved for testing by the project board at deep-water sites.

The macroinvertebrate monitoring methods chosen for use at deep-water locations need to be both scientifically defensible and practical. This requires a suitable balance between the adequacy of information obtained and the availability and cost of manpower, equipment, and time constraints. In addition, Health and Safety issues must be paramount (Rayson 2000). The (UK) National Biology Technical Group provided recommendations to Environment Agency staff on the use of invertebrate sampling equipment in deep waters (National Biology Technical Group 2000), but this may need to be reviewed.

The specific objectives of this review are:

1. To report the results of a field trial to examine the most appropriate technique(s), in terms of effectiveness and suitability, to be used when sampling benthic invertebrates from deep-water sites.

2. To recommend a standard technique (or combination of techniques) to be used when sampling benthic invertebrates from deep-water sites.

2 METHODS

2.1 Selection of Techniques

The techniques used for sampling benthic invertebrates from the natural substrata of deep rivers and streams were reviewed in Jones, Bass and Davy-Bowker (2005) building on previous reviews by Elliott *et al*. (1993), Wright *et al*. (1999) and Bass *et al.* (2000). Jones *et al*. (2005) recommended that the following methods should be included in any field trial:

- Light dredge
- Air-lift
- Long-handled pond net from the bank
- Marginal pond netting

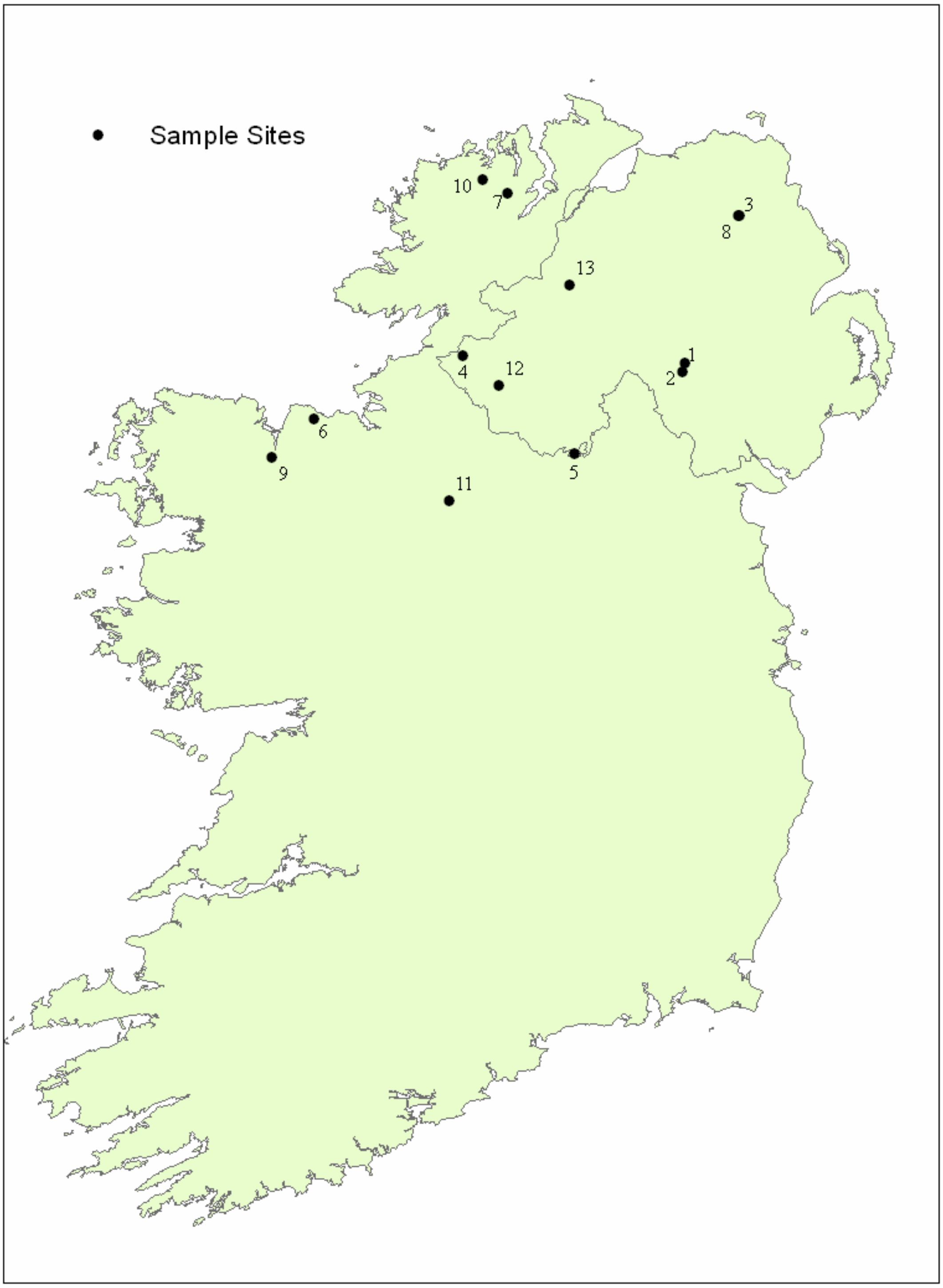
It was recommended that these methods should be tested at a number of sites of varying characteristics to ensure the general applicability of the recommended method. At each site replicate samples should be collected using each of the techniques under investigation. It was also recommended that the relationship between samples collected from deep water and the corresponding margin samples be fully investigated, to determine any inconsistencies between the two techniques.

Furthermore, it was recommended that where possible samples collected using deep water techniques were compared to those collected using standard shallow water techniques, to determine if the classification of a site as “deep” has any impact upon the assessment of the quality of the site.

2.2 Selection of Sites

Thirteen high quality, deep water sites were selected in conjunction with EHS and EPA staff, which covered the range of site characteristics likely to be encountered in the United Kingdom and the Republic of Ireland (Table 1). Sites with known poor water quality were excluded because the aim of this study was to compare sampler performance under a variety of physical conditions rather than site quality. All sites usually had a sufficient large area of deep water, defined here as greater than 1 m depth, to enable the collection of sufficient replicates of each of the selected techniques to be tested. Background environmental and biological data were compiled from EHS and EPA records (Table 3).

Figure 1. Geographical position of sites visited. For list of site names see Table 1.



	River	Site	IGR
1	Blackwater	Blackwatertown	H841524
2	Blackwater	Moy	H852559
3	Clogh	Glarryford	D062131
4	Erne	Rosscor Bridge	G987586
5	Finn	Wattle Bridge	H425203
6	Garavogue	Lough Gill	G413340
7	Leannan	Lough Fern	C164219
8	Main	Dundermot	D057130
9	Moy	Arran Bridge	G248190
10	Owencarrow	New Bridge	C066268
11	Shannon	Hartley Bridge	G938020
12	Sillees	Carr Bridge	H130471
13	Strule	Abercorn Bridge	H404861

Table 1. Site location. Column 1 refers to site labels on Figure 1. Details of the samples that were collected from the sites are given in Table 2, and chemical and biological characteristics in Table 3.

2.3 Field Procedures

2.3.1 Deep water sampling protocols

The sites were selected to provide a range of representative deep-water sites known to support diverse macroinvertebrate communities. This ensured there was a broad scope for comparisons between sampling methods. The sites were sampled in summer, between 10th and 19th August 2005. At each site three replicate samples of each of the “deep water” techniques were collected, namely light dredge, Mackey/Yorkshire pattern air-lift, long-handled pond net working from the bank and a marginal sweep with a standard pond net. Three operators (all CEH permanent staff) experienced in the use of these techniques collected the samples. At each site two different operators used each technique, one collecting one replicate and the other two replicates, thus enabling any variation in samples due to the influence of operators to be determined. The three operators varied which techniques they used so as to not bias sample collection.

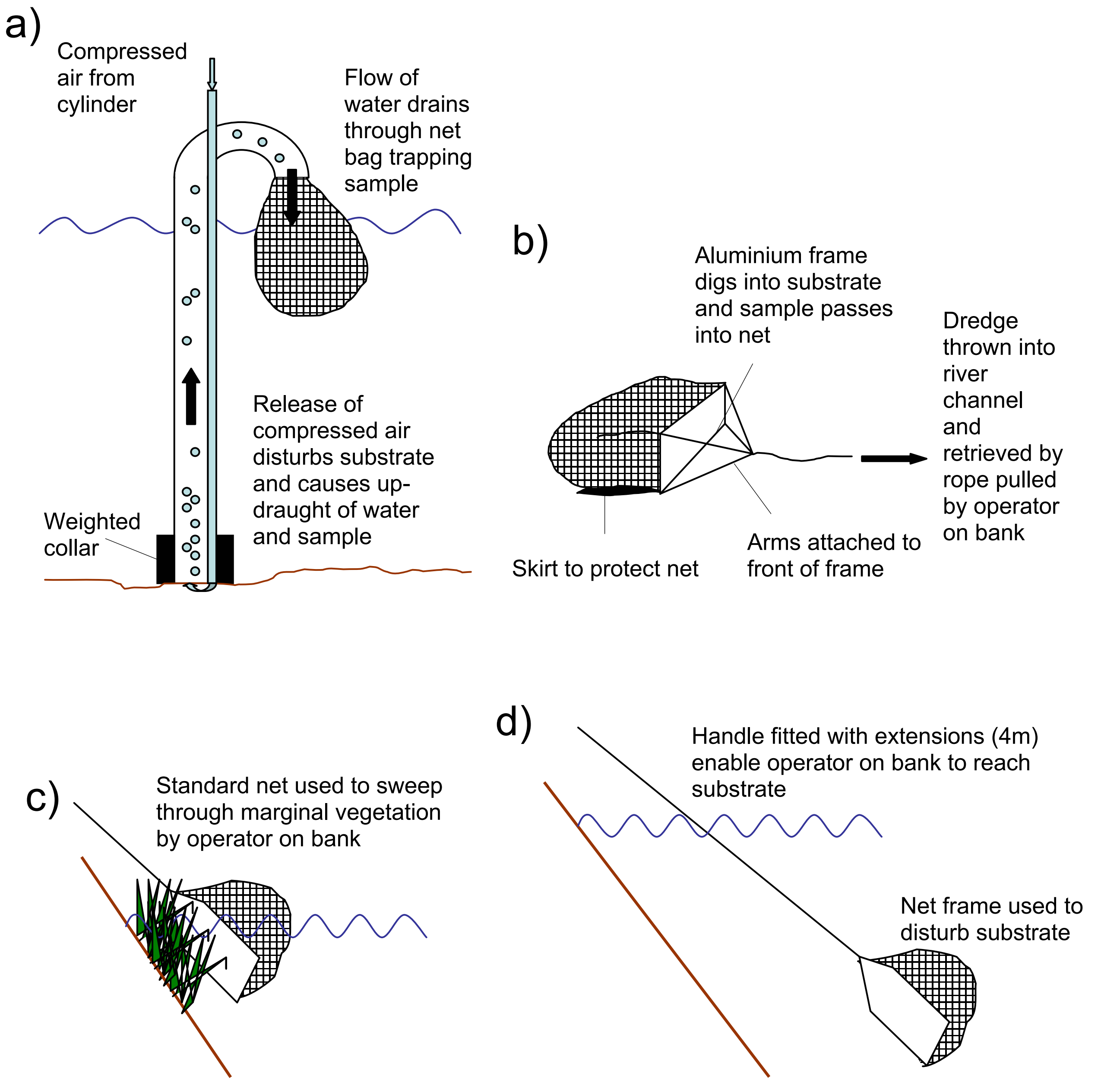
In order to compare the selected methods in a systematic way the sampling effort and range of habitat types sampled needed to be consistent between each replicate sample. Following the recommendations of Wright *et al.* (1999) and Bass *et al.* (2000), the sampler operators were asked to restrict their sampling effort for each deep-water replicate sample to an area of about 1.5 m^2 to ensure comparable areas of riverbed were covered by each method. This area corresponds approximately to the area sampled by a standard 5 m trawl of the dredge. The series of replicate samples for all techniques was taken within the same stretch of main channel. Samples were taken in an upstream sequence to avoid sampling the same area more than once, in a blocked design. This did not exclude downstream drifting as individual samples are taken, if this was the most practical method. Operators made every effort to maintain consistency among techniques.

The airlift comprises a vertical riser pipe, 10 cm diameter, with a u-bend at the upper end, onto which is attached a collecting bag. The lower, weighted end of the riser pipe is placed on the substrate and compressed air introduced into the lower end, which disturbs the sediment and causes the water, air and substrate mixture to rise up to the collecting bag (mesh size 1 mm). The airlift was used from a boat, and repeatedly raised and lowered (bounced) until the required area was sampled. Sufficient compressed air was required to take 3 replicate samples at each site and up to 3 sites per day.

The light dredge is an oblong aluminium frame with bag attached (mesh size 1 mm), which is thrown into the water and dragged across the substrate by means of a sinking rope, marked at metre intervals, attached to two arms that protrude from the sides of the frame in a triangle. A skirt protects the underside of the bag from damage, but extra net bags were taken as a precaution. The light dredge was used from the bank, thrown into the channel and dragged across the substrate until the required area was sampled, at which point it was retrieved rapidly through the water column.

Marginal sweep samples were taken with a standard pond net (mesh size 1 mm) from marginal vegetation, working from the bank where possible. Sampling effort was divided in proportion to the available habitats to cover the required area.

Figure 2 Schematic diagrams to illustrate the mode of operation of the four techniques used, a) airlift, b) light dredge, c) marginal sweep and d) long-handled pond net



The long-handled pond net is a standard pond net (mesh size 1 mm) with wooden extension poles to the handle attached to give a working length of 4 m. The long-handled pond net was used from the bank, extending as far as possible into the channel. Alternate short thrusts and retrieves with downward pressure applied to the net were used to disturb the substrate until the required area was sampled. The samples were then washed at the surface to remove excess fine sediment.

Despite the use of bankside techniques, it was necessary, or useful, on occasion to use a boat to collect samples or retrieve equipment.

It was anticipated that some of the sites selected may be unsuitable for certain techniques. Operators were mandated to decide on the day whether to proceed with sampling a site or not. The following guidance notes were provided.

Is there:

Suitable access for the boat and equipment?
Safe river conditions?
Suitable water depth/velocity to use the deep water equipment?
Suitable water depth/velocity to collect a standard kick sample?

If there was not a sufficiently large area of deep water (>1 m depth) to collect all three replicate samples for each deep water technique, without re-sampling previously disturbed areas, the site was not sampled. It was not necessary to abandon any sites due to difficulties of access or safety. At one site (River Strule at Abercorn Bridge) marginal sweep samples were not collected as the river level had dropped and the marginal vegetation was some distance above the water level on the day of sampling.

At two sites there was sufficient wadeable area for a further three replicate “shallow water” Standard RIVPACS kick samples to be collected within the study stretch, following the same protocol as that used for the deep water methods (Table 2). These standard RIVPACS kick samples followed the UK agencies standard protocol, comprising a three minute kick together with a one minute search where the water surface is sampled and rocks, plants, logs or other submerged objects are examined and all attached invertebrates removed and incorporated into the sample.

Where a replicate sample was excessively large (with quantities of organic/inorganic debris), no more than 2.5 litres was retained (including sufficient preservative volume). In this case a sub-sample was taken after elutriating the whole sample thoroughly to reduce the bulk. Large pebbles/cobbles were removed after checking and retaining any attached fauna. Any noticeable large taxa (Unionidae, Astacidae), or noticeable taxa of high conservation value, were removed, recorded and returned to the site live. Where sub-samples were retained, the proportion of the sample volume that was preserved and retained was recorded (ignoring any stones that had fauna removed by hand and been discarded).

All replicate samples were fixed in the field with approximately 4% formaldehyde in individual, labelled pots. Formaldehyde was used to ensure preservation of macroinvertebrates during a long period of storage, as the sorted samples were to be archived for potential further identification in the future. More stringent health and safety precautions are required when using this preservative rather than IMS, and were adhered to both in the field and the laboratory.

	River	Site	Airlift	Dredge	Margin	LHPN	Kick
1	Blackwater	Blackwatertown	*	*	*	*	
2	Blackwater	Moy	*	*	*	*	
3	Clogh	Glarryford	*	*	*	*	
4	Erne	Rosscor Bridge	*	*	*	*	
5	Finn	Wattle Bridge	*	*	*	*	
6	Garavogue	Lough Gill	*	*	*	*	
7	Leannan	Lough Fern	*	*	*	*	*
8	Main	Dundermot	*	*	*	*	
9	Moy	Arran Bridge	*	*	*	*	
10	Owencarrow	New Bridge	*	*	*	*	
11	Shannon	Hartley Bridge	*	*	*	*	
12	Sillees	Carr Bridge	*	*	*	*	
13	Strule	Abercorn Bridge	*	*		*	*

Table 2. Sites from which samples were collected using the different techniques tested. At two sites there was sufficient shallow water to collect kick samples in the same reach. At one site it was not possible to collect a margin sample as the water level had dropped below the level of the marginal vegetation, leaving it dry.

At the time of sampling a field sheet was completed, where the following variables were recorded, together with a site map showing the location of the individual replicate samples:

River name
Site name
Grid reference
Date
Average width
Centre channel depth
Macrophyte % cover
Velocity category (<10 cms^{-1}, 10-25 cms^{-1}, 25-50 cms^{-1}, 50-100 cms^{-1}, >100 cms^{-1})
Substrate % composition (boulders & cobbles, pebbles & gravel, sand, silt & clay)
Water clarity (turbid/clear)
Any other notes of relevance (tidal, boats, etc.)

2.3.2 Worker questionnaire

On return from the field, each worker scored each site for:

1. How easy it was to access the site with each set of equipment (scored 1-5, increasing difficulty),

2. How easy each technique was to use under the site conditions (scored 1-5, increasing difficulty),

3. Whether a boat was required to take an effective sample from the site with each device (scored 1-3 = no requirement for a boat, a boat was helpful to collect an adequate sample, a boat was necessary to collect an adequate sample).

2.4 Sample Processing

On return to the laboratory the following procedures were adopted for sample processing and data recording:

1. The replicate sample was washed free of preservative using a fine sieve (0.5 mm mesh). This process was undertaken in a fume cupboard.

2. The washed material to be sorted was dispersed in shallow water in a white tray. The whole tray was scanned and representatives of all macroinvertebrate taxa detected were removed and re-preserved in 70% IMS. The time involved in this process and the operator's name were recorded. Where particularly large numbers of certain taxa were present, all specimens from a defined fraction of the tray area were removed and counted. Where a known proportion of a particularly abundant taxon was counted, the total number present was calculated by extrapolation.

3. The macroinvertebrates were identified to BMWP family level and the Log abundance of each BMWP family in the replicate sample estimated. The identifier's name and the total time taken to process the sample were recorded.

Complete independent checks were made on all samples, in terms of:

1. The accuracy and number of taxa recorded,

2. The derived BMWP scores

3. The accuracy of data transferred to an Access database (primary storage medium).

2.5 Historic Data

Further biological and physicochemical data were kindly provided by EHS and EPA from their data holdings collected during routine monitoring.

River	Site	Alk mgL^{-1}	Cond μScm^{-1}	pH	BOD mgL^{-1}	DO %	NO_3 mgL^{-1}	NH_3 μgL^{-1}	P(sol) μgL^{-1}	SS mgL^{-1}	ASPT	BMWP	No Taxa (mean)
Blackwater	1	172	447	7.9	2.44	93	1.43	2.5	73	14.9	5.34	98.3	18.4
Blackwater	2	165	438	7.9	2.65	92	1.48	3.2	103	16.3	4.60	74.1	16.1
Clogh	3	92	268	7.7	1.82	93	1.92	1.8	84	5.6	5.67	120.7	21.3
Erne	4	75	241	7.9	1.39	93	0.80	2.2	59	3.0	5.16	132.6	25.7
Finn	5	170	428	7.8	1.84	83	1.17	1.8	68	5.5	5.0	137.4	27.5
Garavogue	6		230	8.0									
Leannan	7												
Main	8	94	272	7.7	1.88	92	2.07	2.0	86	6.1	5.21	117.3	22.5
Moy	9		375	8.1									
Owencarrow	10												
Shannon	11		96	7.1									
Sillees	12	99	259	7.7	1.68	88	0.35	1.5	53	7.6	5.72	172.7	30.2
Strule	13	54	194	7.4	2.08	79	1.40	1.7	84	8.4	5.79	148.1	25.6

Table 3. Site chemical and biological characteristics where available. Column 2 refers to site labels on Figure 1.

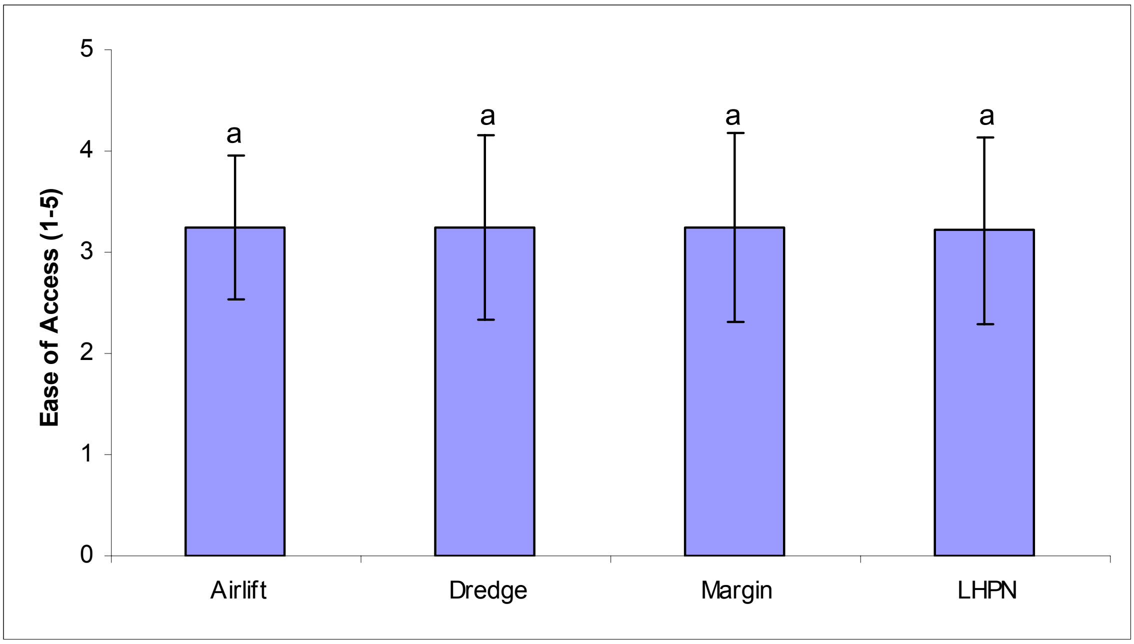
3 RESULTS

3.1 Worker Questionnaire

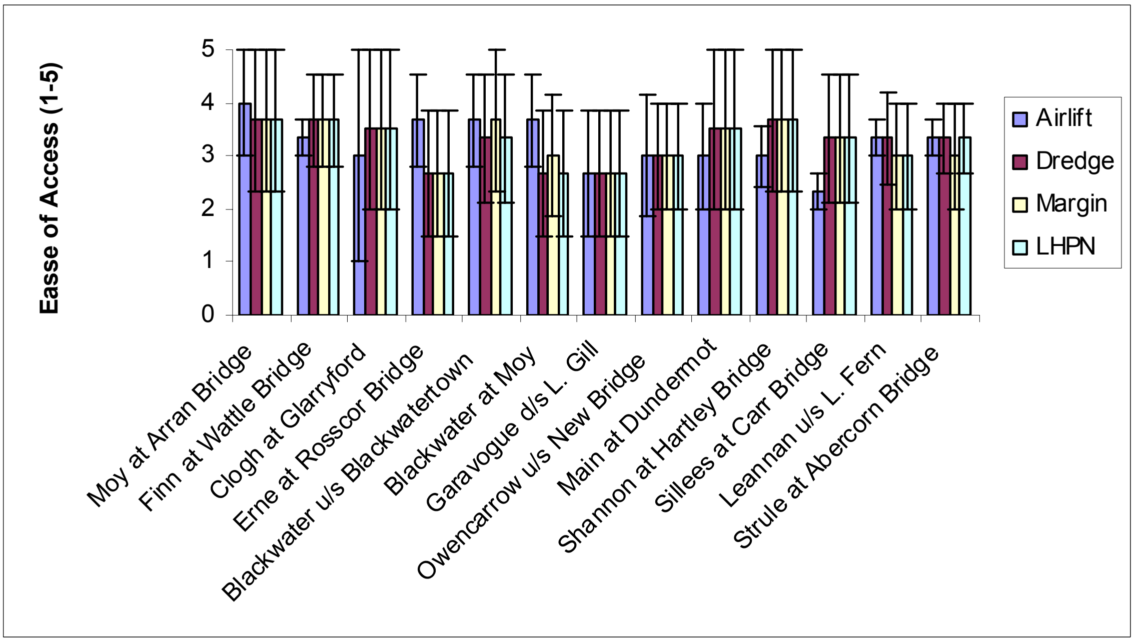
In the opinion of the three field workers who collected the primary samples (all permanent CEH staff) there was no significant difference among the techniques in how easy it was to access the river with the equipment (Figure 3a). There were differences among the sites, with some sites being more difficult to access than others, but there was no consistent pattern within sites as to which technique was the most difficult to access the river (Figure 3b).

Figure 3. Worker opinion of ease of access to river to obtain a sample. a) mean score ±SE (5 = difficult, 1 = easy) for the four deep water techniques tested, b) mean score for the four deep water techniques tested by site. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference.

a)

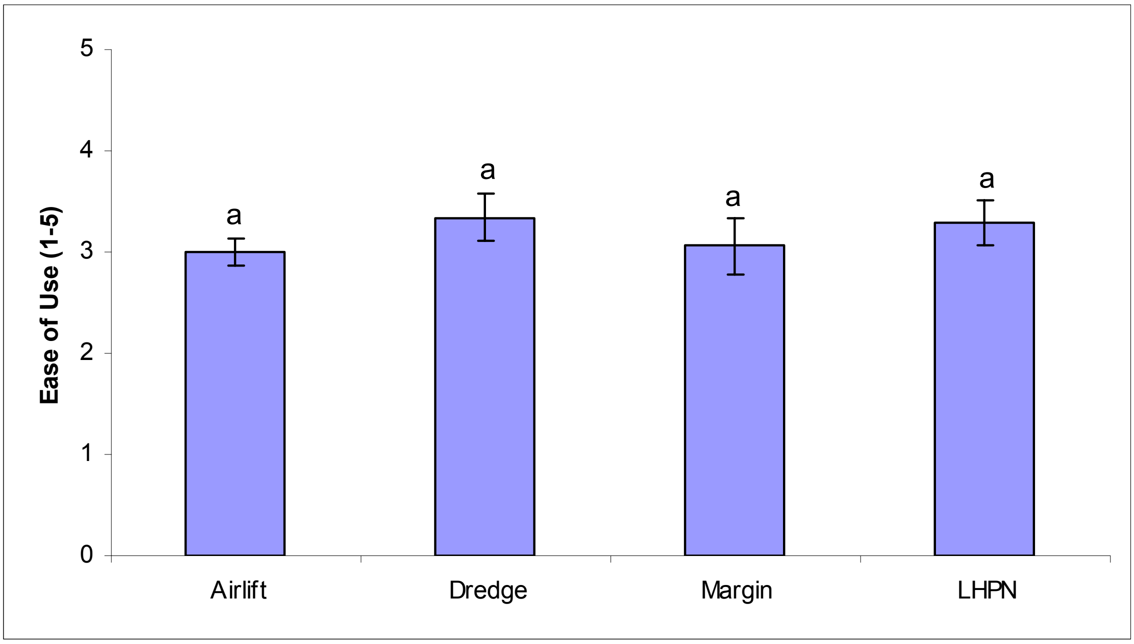


b)



In the opinion of the three field workers there was no significant difference in how easy the four deep water techniques tested were to use (Figure 4). On average across the range of sites, all techniques were considered to be of moderate difficulty to use. Whilst it should be noted that the workers have experience of using all the techniques tested, these techniques are not regularly used and the workers were advised to score the techniques relative to a standard kick sample.

Figure 4. Worker opinion of ease of use of the four deep water techniques tested to obtain a sample, mean score ±SE (5 = difficult, 1 = easy). Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference.



There were significant differences among the techniques as to whether the field workers were of the opinion that a boat was necessary, or useful, to collect an adequate sample (Figure 5). The airlift always needed a boat to collect a sample. At certain sites a boat was necessary or useful to collect a sample using the "bankside" techniques (Figure 5). The requirement for the use of a boat was inversely related to the width of the river: In some narrow deep rivers it was the field workers' opinion that a boat was useful when collecting light dredge and long handled pond net samples, and necessary when collecting margin samples (Figure 6). This result is more likely to be a consequence of the profile of the river bank rather than a feature of the river channel; narrow deep rivers are often the result of engineering and have steep bank profiles, which make access to the river channel more difficult. The light dredge frequently became snagged in the sites with narrower channels, and the workers felt that a boat was useful to retrieve the equipment.

Figure 5. Worker opinion of the requirement of a boat to obtain an adequate sample using the four deep water techniques tested by site, mean score ±SE (1 = no requirement for a boat, 2 = a boat useful, 3 = a boat necessary).

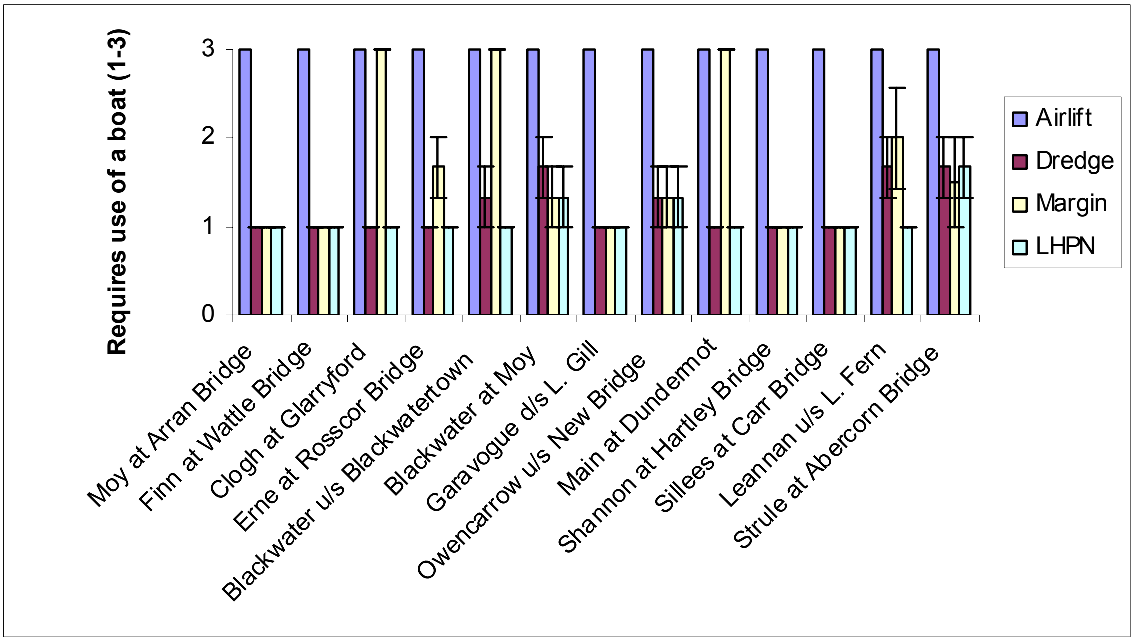
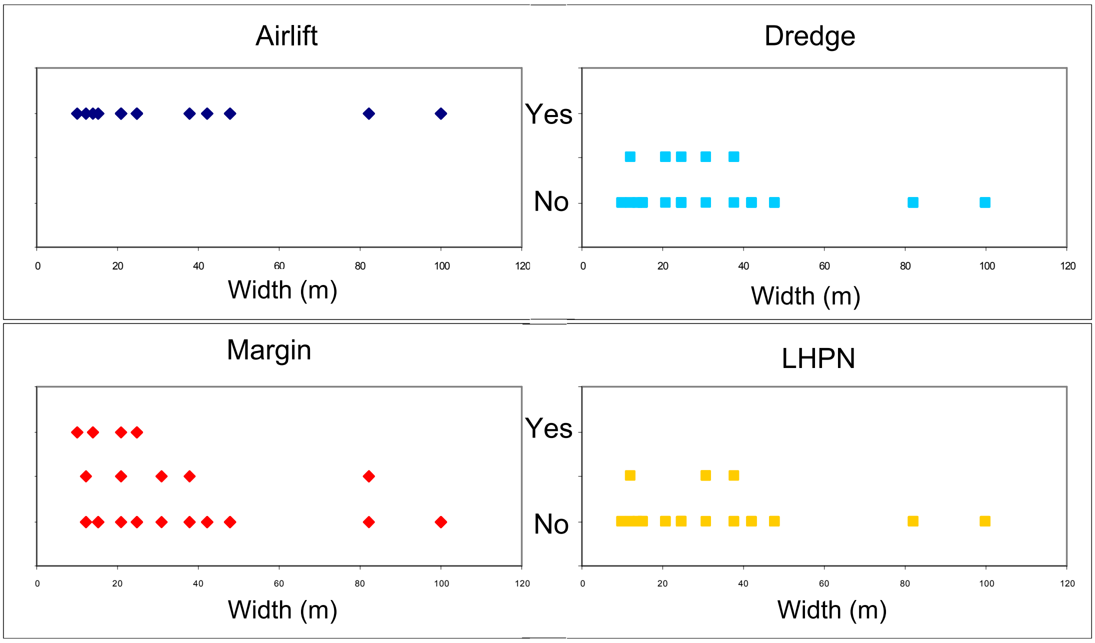


Figure 6. Relationship between width of river and worker opinion of the requirement of a boat to obtain an adequate sample using the four deep water techniques tested (No = no requirement for a boat, mid-point = a boat useful, Yes = yes a boat necessary). Technique * Width p ≤ 0.0001 from Ancova.



The four techniques were ranked according to the mean score in this survey of workers' opinion, thus enabling an average rank position in terms of ease of use to be established. Ranks were shared where there was no significant difference in the mean score among the techniques. Ranks do not reflect the score given by the workers, merely the techniques' position relative to one another.

	Technique			
	Airlift	Dredge	Margin	LHPN
Ease of Access	1	1	1	1
Ease of use	1	1	1	1
Boat	4	1	3	1
Average rank	2	1	1.66	1

Table 4. Ranking of the field sampling techniques (airlift, dredge, margin and LHPN) for relative ease of sampling in terms of ease of access, ease of use, requirement for a boat, and overall. Rank of 1 is the easiest to use.

3.2 Sample Processing

For a potential technique to be suitable for sampling invertebrates in deep rivers, as well as being easy to use, safe and practical in the field, it should be efficient in terms of the time it takes to process the samples produced. This will influence the time and cost involved in the assessment of the site. In order to compare this aspect of the deep water sampling techniques, a comparison was made of the time it took to sort the samples (influenced by the volume and composition of the sample matrix and the number of individuals) and the total time to process the samples (further influenced by the number of taxa and ease of identification).

There were some significant differences among the sites in the time it took to sort the samples, with the samples from the Shannon at Hartley Bridge taking the longest time to sort (Figure 7b). There were also significant differences among the deep water techniques tested in the time it took to sort the samples: The mean time it took to sort airlift samples was over 200 mins, more than twice as long as the samples collected with the other techniques (Figure 7a). These differences in the time it took to sort the samples are a reflection of differences in the total sample volume, matrix and the number of individual macroinvertebrates in the sample.

As a consequence of the differences in the time it took to sort the samples, there were significant differences in the total time it took to process the samples, with the mean time it took to process the airlift samples significantly longer than the other three techniques (Figure 8a). Again, there were differences in the mean time it took to process the samples from the different sites reflecting the varied nature of the sites selected (Figure 8b). Although the mean time to process the airlift samples was significantly longer than the time to process the samples collected with the light dredge, the long-handled pond net or from the margin, the actual mean time taken to process the samples collected with latter three techniques was as long as that for the airlift samples at some sites (Figure 8c); this interaction (site * technique) was not statistically significant, however.

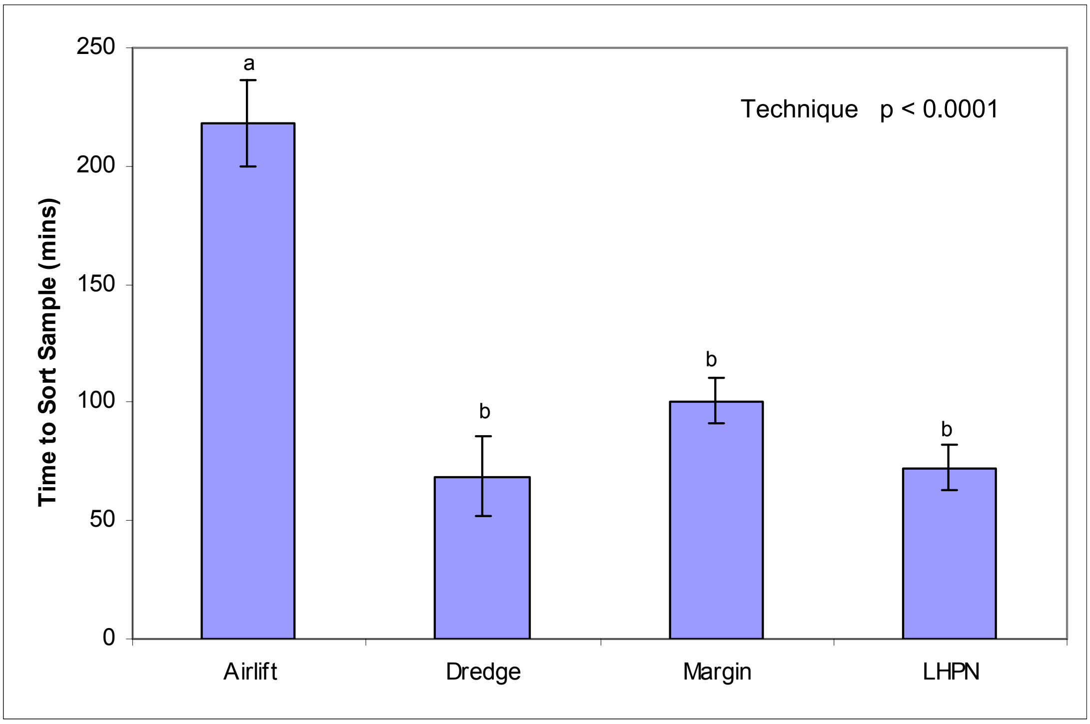
The four techniques were ranked according to the mean time it took to process the samples, thus enabling an average rank position in terms of ease of processing to be established. Ranks were shared where there was no significant difference in the mean time among the techniques. Ranks do not reflect the time taken, merely the techniques' position relative to one another.

	Technique			
	Airlift	Dredge	Margin	LHPN
Sort	4	1	1	1
Process	4	1	1	1
Average rank	4	1	1	1

Table 5. Ranking of the field sampling techniques (airlift, dredge, margin and LHPN) for the time taken to process the samples, and overall. Rank of 1 is the quickest to process.

Figure 7. Influence of technique and site on the time taken to sort the samples collected with the four deep water techniques tested, a) influence of technique, b) influence of site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference, multiple letters indicate overlapping groupings of means.

a)



b)

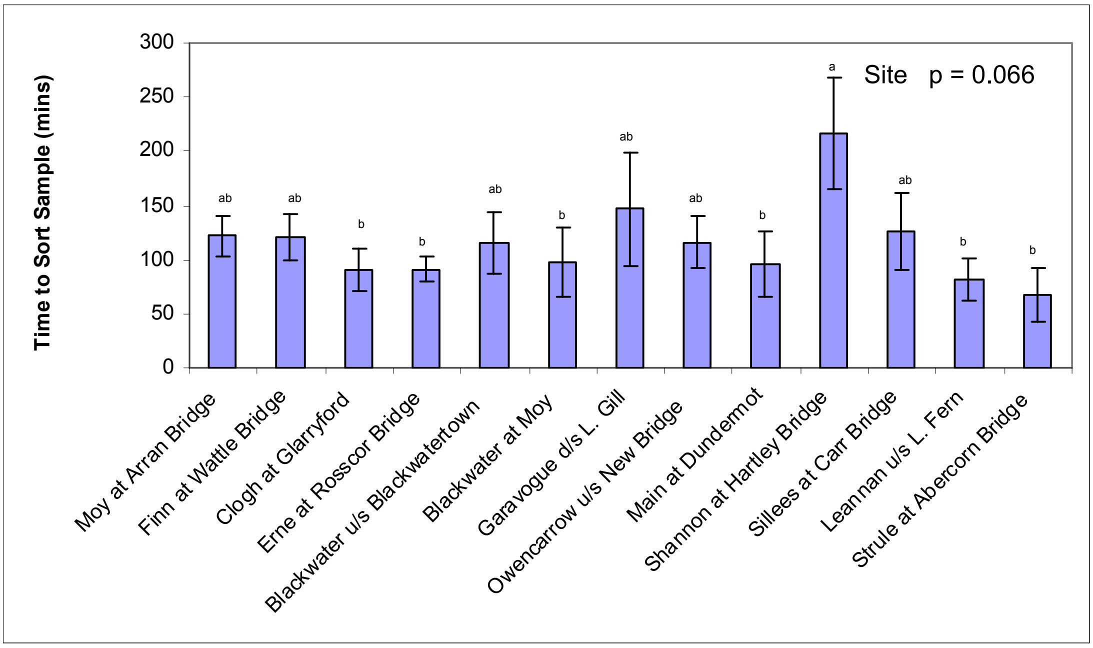
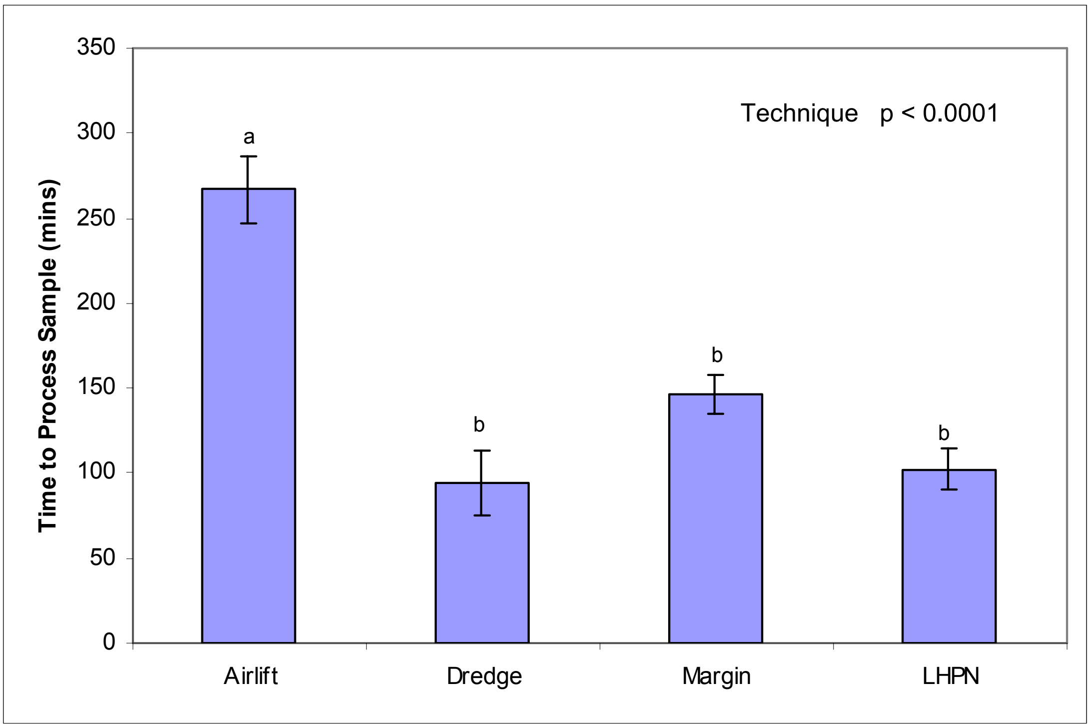
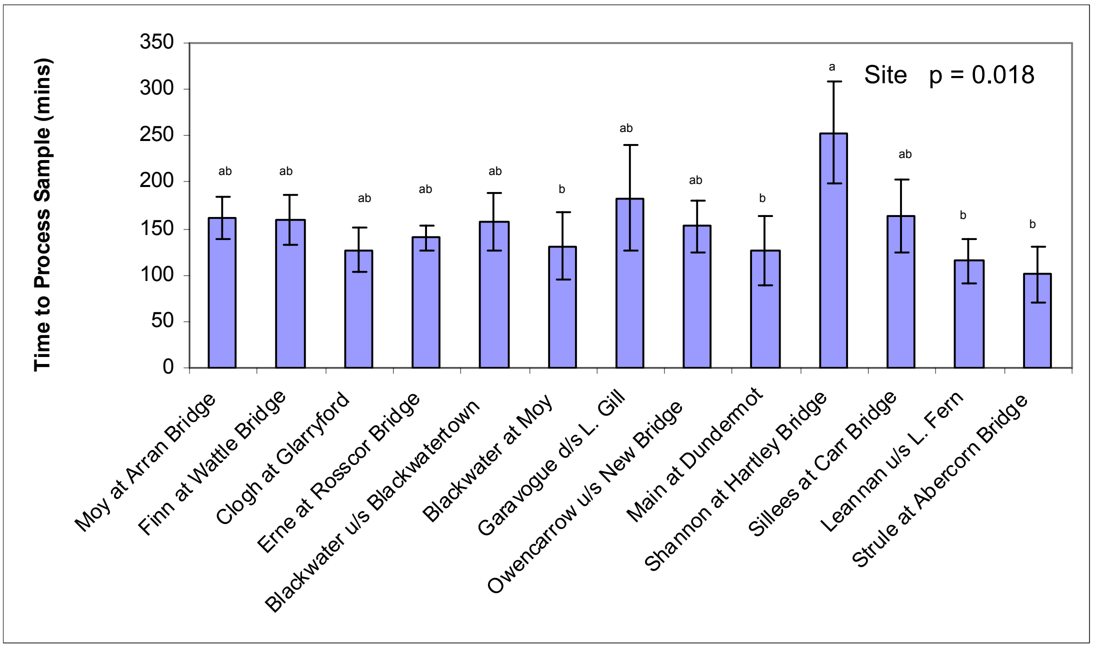


Figure 8. Influence of technique and site on the total time taken to process the samples collected with the four deep water techniques tested, a) influence of technique, b) influence of site, c) influence of technique by site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference, multiple letters indicate overlapping groupings of means.

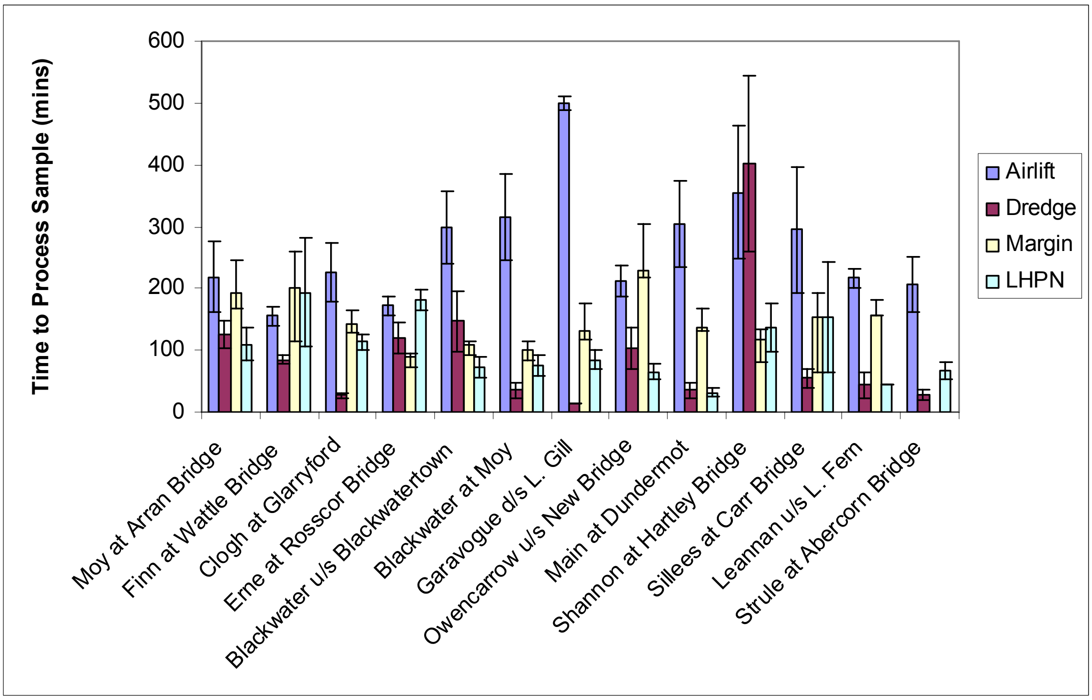
a)



b)


Time to Process Sample (mins)
350
300
250
200
150
100
50
0
Site p = 0.018
ab
ab
ab
ab
ab
b
ab
ab
b
a
ab
b
b
Moy at Arran Bridge
Finn at Wattle Bridge
Clogh at Glarryford
Erne at Rosscor Bridge
Blackwater u/s Blackwatertown
Blackwater at Moy
Garavogue d/s L. Gill
Owencarrow u/s New Bridge
Main at Dundermot
Shannon at Hartley Bridge
Sillees at Carr Bridge
Leannan u/s L. Fern
Strule at Abercorn Bridge

c)


Time to Process Sample (mins)
600
500
400
300
200
100
0
Airlift
Dredge
Margin
LHPN
Moy at Arran Bridge
Finn at Wattle Bridge
Clogh at Glarryford
Erne at Rosscor Bridge
Blackwater u/s Blackwatertown
Blackwater at Moy
Garavogue d/s L. Gill
Owencarrow u/s New Bridge
Main at Dundermot
Shannon at Hartley Bridge
Sillees at Carr Bridge
Leannan u/s L. Fern
Strule at Abercorn Bridge

3.3 Biotic Indices

For a sampling technique to be effective, the technique needs to provide a representative sample of the fauna, perform well for key biotic indices relative to other techniques, and perform equally well across all sites.

3.3.1 Number of BMWP scoring taxa

Overall there were significant differences in the number of BMWP scoring taxa per sample among the deep water techniques tested. The samples collected from the margin had the highest number of scoring taxa, the samples collected with the light dredge and long-handled pond net the lowest, and the samples collected with the airlift intermediate (Figure 9a). The mean number of BMWP scoring taxa in the samples from the margin was approximately twice that of the samples collected with the light dredge or the long-handled pond net. The mean number of BMWP scoring taxa in the samples collected with the airlift was approximately 50% greater than in that of the samples collected with the light dredge or the long-handled pond net.

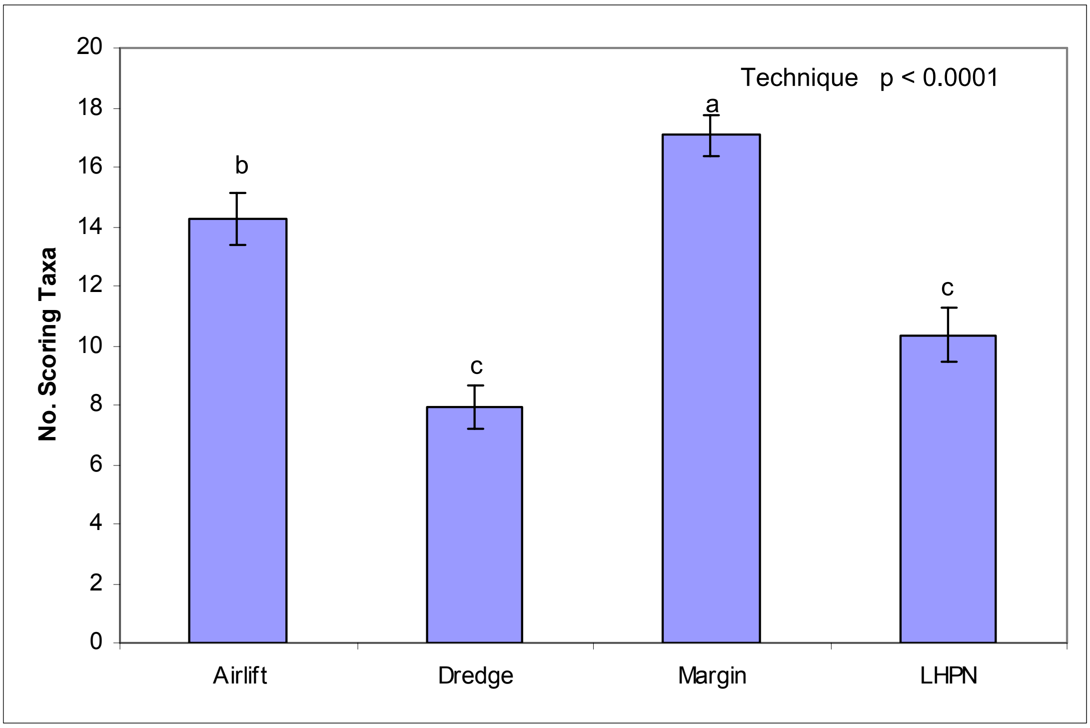
There were significant differences in the number of scoring taxa per sample among the sites; the Blackwater at Blackwatertown and Owencarrow at Newbridge had the lowest mean number of scoring taxa per sample and the Moy at Arran Bridge the highest (Figure 9b). These differences were expected as the sites had been chosen to reflect a range of conditions, to enable a test of the effectiveness and suitability of the deep water sampling techniques over the range of conditions that may be encountered. An interaction occurred between site and technique indicating that the techniques did not perform equally well in all sites (Figure 9c). This appears to be due largely to variations in the taxon richness of the samples collected from the margin relative to those from the river channel, but the relative performance of the river channel techniques did vary among sites also.

There was no effect of operator on the number of scoring taxa per sample, nor any interaction involving operator.

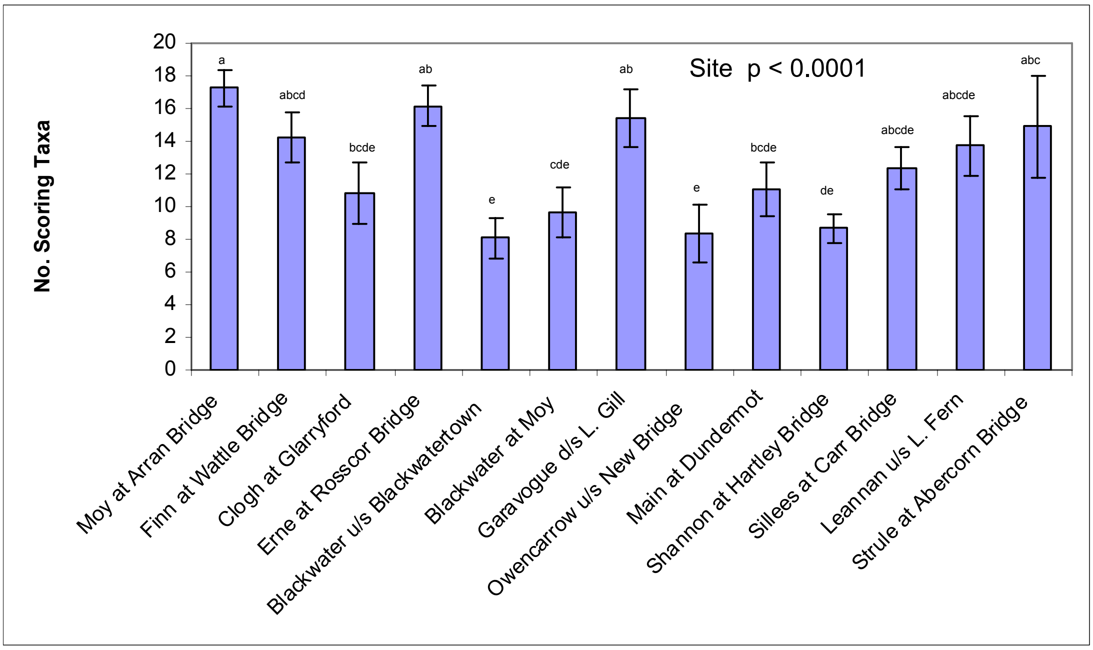
In a previous study it was shown that there were differences in taxon accretion rates among techniques for sampling deep waters (Bass *et al*. 2000). In general, the airlift accretion curves flattened out after fewer replicates at higher Ntaxa and at noticeably more sites than the accretion curves for dredge samples [NB a heavier dredge was used]. Some series of long-handled pond net samples also reached a taxon accretion plateau, but in these cases the Ntaxa were considerably lower than recovered by other sampling devices at the same sites.

Figure 9. Influence of technique and site on the number of BMWP scoring taxa in the samples collected with the four deep water techniques tested, a) influence of technique, b) influence of site, c) influence of technique by site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference, multiple letters indicate overlapping groupings of means.

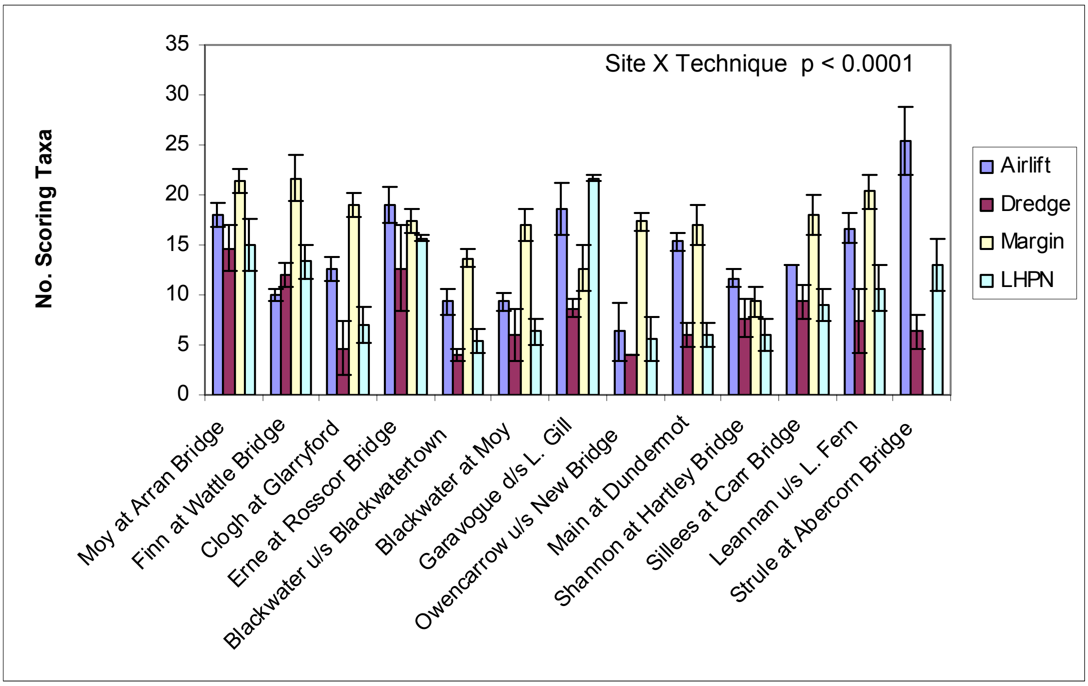
a)



b)



c)



3.3.2 BMWP

Overall there were significant differences in the total BMWP score per sample among the deep water techniques tested. The samples collected from the margin and with the airlift had a significantly higher BMWP score than the samples collected with the light dredge and long-handled pond net (Figure 10a). The total BMWP score of the samples collected in the margin and with the airlift was approximately twice that of the samples collected with the light dredge and the long-handled pond net.

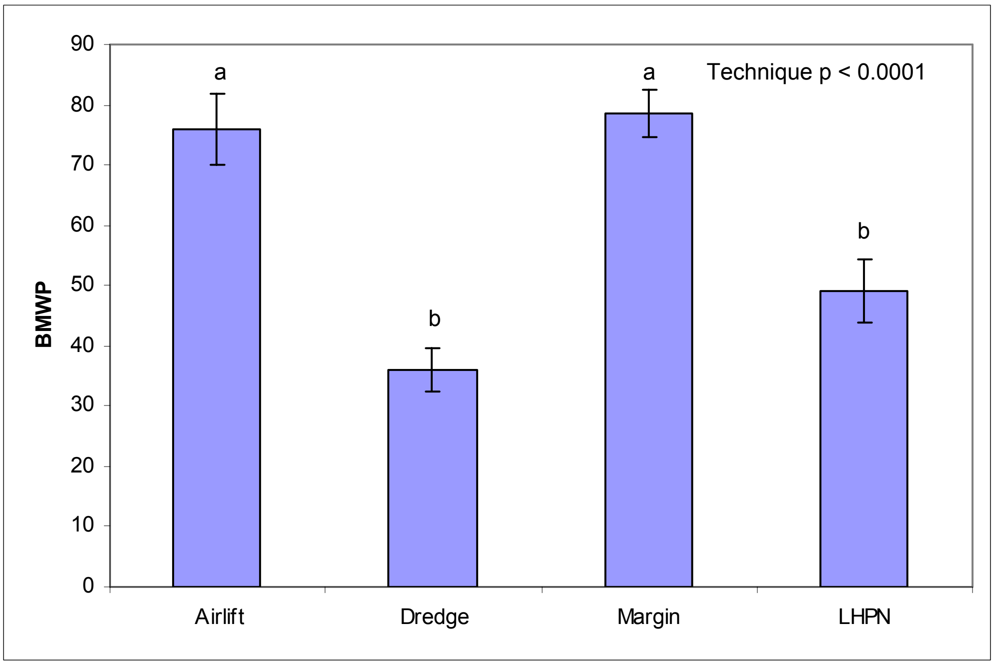
Again there were differences among the sites, in terms of total BMWP score (Figure 10b), but these differences were expected as the sites had been selected to provide a variety of conditions for testing the techniques.

The interaction between site and technique was significant (Figure 10c), indicating that at least one technique did not perform equally well across all sites. This may be the result of differences in response of the invertebrate community to pressures between the samples collected from the margin and those collected using the river channel techniques (see section 3.4), or could be due to a failure of some techniques under certain conditions (see section 3.6).

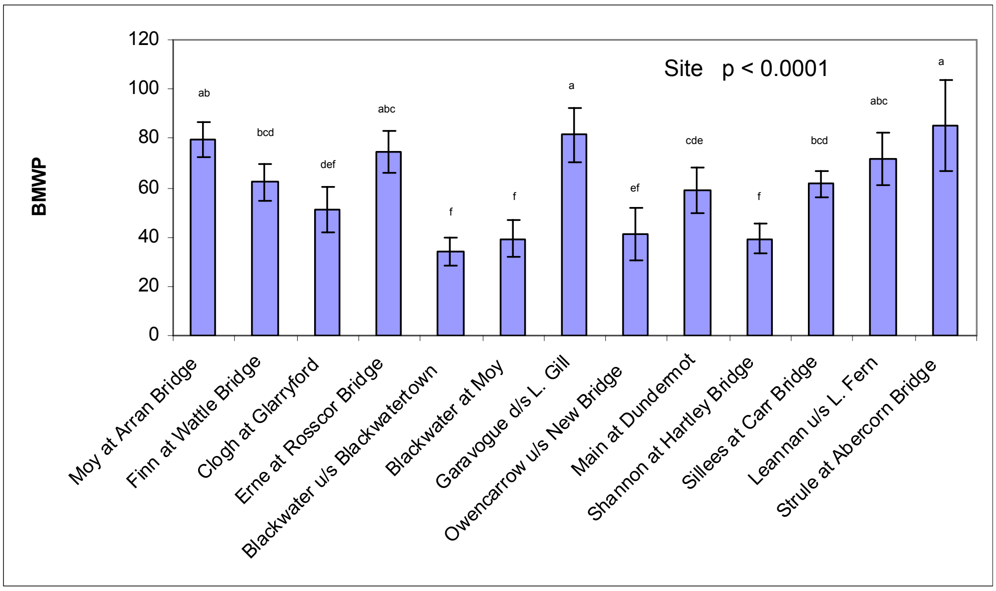
There was also an interaction between site, technique and operator, suggesting that, for at least one technique, the operators were not equally efficient at all sites.

Figure 10. Influence of technique and site on the total BMWP score of the samples collected with the four deep water techniques tested, a) influence of technique, b) influence of site, c) influence of technique by site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference, multiple letters indicate overlapping groupings of means.

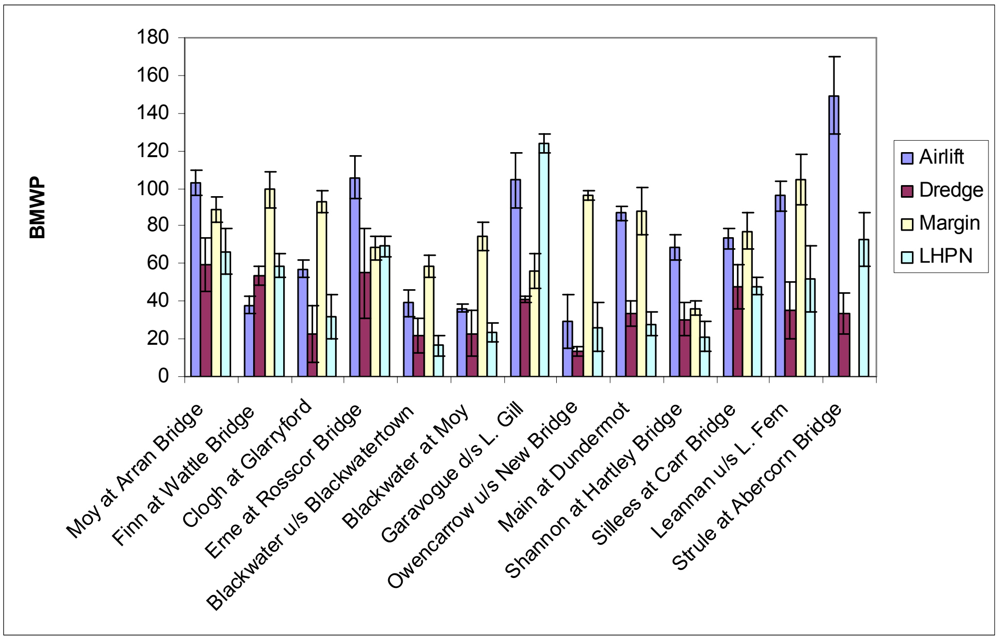
a)



b)



c)



3.3.3 ASPT

Overall there were significant differences in the ASPT per sample among the deep water techniques tested. The samples collected with the airlift had a significantly higher ASPT than the samples collected with the light dredge and long-handled pond net, or from the margin (Figure 11a). At the site level, the airlift had the highest or joint highest ASPT in 9 out of 13 sites (Figure 11c). This difference must reflect a difference in the fauna collected with the different techniques. Whilst a high ASPT does not necessarily indicate that a certain technique should be preferred, it does indicate that more sensitive taxa within the community present at a site are being collected by that technique. This has important implications for monitoring as high scoring sensitive taxa are more likely to disappear first as a site becomes more stressed. Thus, change in site quality is more likely to be detected using the airlift: in a moderately stressed site the O/E_{ASPT} for airlift is likely to be lower than for other techniques and have greater statistical power to detect stress.

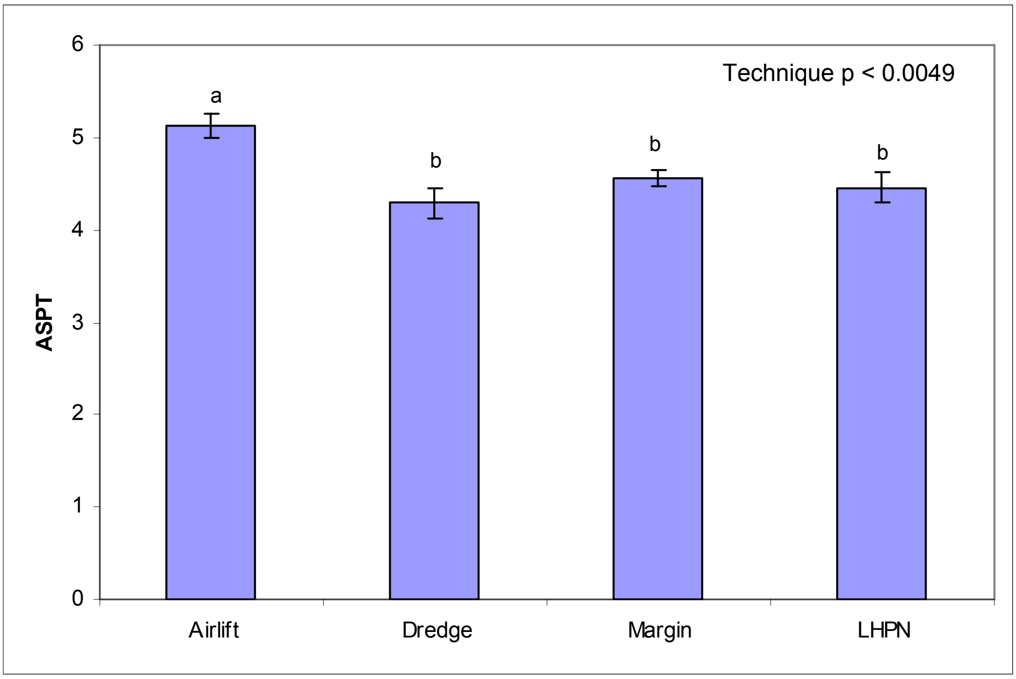
Again there were differences among the sites, in terms of ASPT, but these differences were expected as the sites had been selected to provide a variety of conditions for testing the techniques (Figure 11b).

The interaction between site and technique was not significant, i.e. the relative performance of the techniques was the same across all sites, with the airlift producing samples with a statistically higher ASPT (Figure 11c).

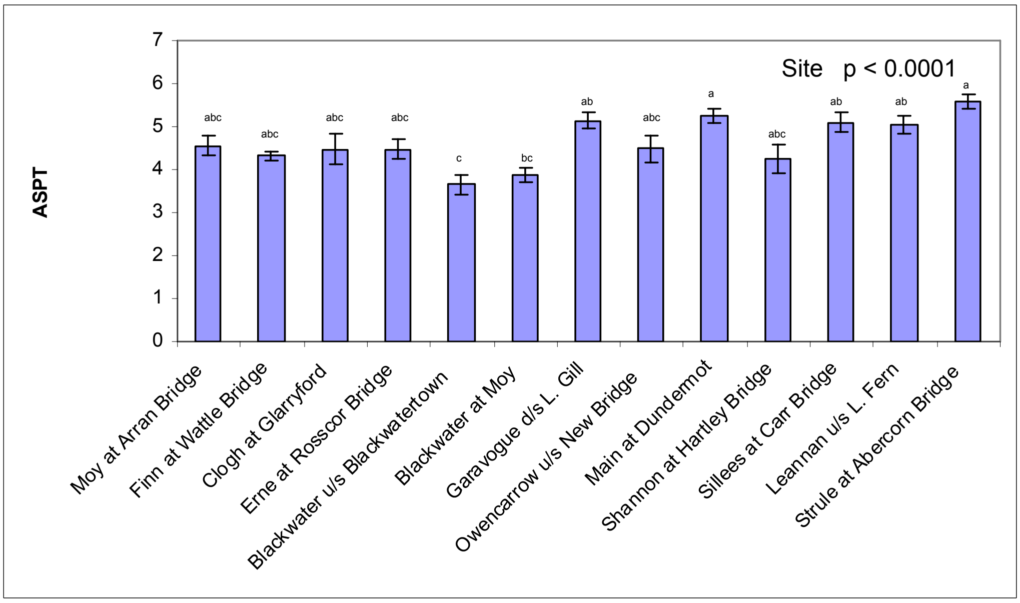
There was no effect of operator on ASPT, nor any interaction involving operator.

Figure 11. Influence of technique and site on the ASPT of the samples collected with the four deep water techniques tested, a) influence of technique, b) influence of site, c) influence of technique by site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference, multiple letters indicate overlapping groupings of means.

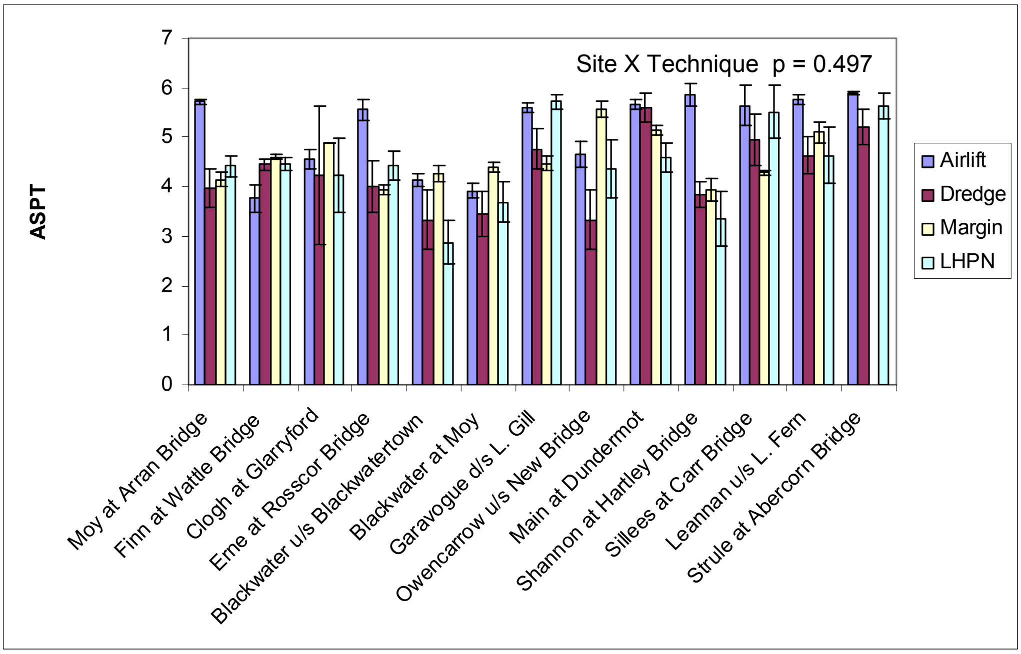
a)



b)



c)



The four techniques were ranked according to the mean score of the metrics used here to assess the effectiveness of sampling the macroinvertebrate fauna, thus enabling an average rank position to be established. Ranks were shared where there was no significant difference in the mean metric score among the techniques. Ranks do not reflect the score, merely the techniques' position relative to one another.

	Technique			
	Airlift	Dredge	Margin	LHPN
No Taxa	2	3	1	3
BMWP	1	3	1	3
ASPT	1	2	2	2
Average rank	1.33	2.33	1.33	2.33

Table 6. Ranking of the field sampling techniques (airlift dredge, margin and LHPN) for relative ease of number of BMWP scoring families, Total BMWP score, ASPT, and overall. Rank of 1 is the highest metric score.

3.4 Correlation Amongst Techniques

For a sampling technique to be effective, the technique needs to provide a representative sample of the river fauna. If all techniques provide a representative sample of the fauna, a standard technique can be selected on its performance and suitability relative to other techniques. However, if techniques do not show correspondence, consideration will have to be given to the sensitivity of the techniques to the pressures of interest and how well they represent the fauna before they can be considered. In such circumstances it may be necessary to adopt a combination of techniques.

To determine the extent to which the techniques were comparable, the relationship between the key metric values from pairs of replicates collected by the four different techniques was determined by correlation. The scatter in the points reflects both variations in the quality of the sites and variation between replicate samples within a site. The latter source of variation, i.e. uncertainty, is dealt with in section 3.5.

The total BMWP score of the samples collected with the three river channel techniques (airlift, light dredge, long-handled pond net) showed some correlation, which was strongest between the airlift and the long-handled pond net. However, there was little correlation between the total BMWP score of the samples collected from the margin and those collected with the river channel techniques (Figure 12).

The number of scoring taxa per sample showed strongest correlation (R = 0.48) between the long-handled pond net and airlift, and the long-handled pond net and dredge (Figure 13). Correlation was weak between the samples collected with the airlift and margin, and the long-handled pond net and margin, and intermediate between the samples collected with the airlift and dredge, and margin and dredge (Figure 13).

The ASPT of the samples collected with the three river channel techniques (airlift, light dredge, long-handled pond net) showed consistent moderate correlation with an R of 0.31 to 0.33 (Figure 14). However, there was only weak correlation between the ASPT of the samples collected from the margin and those collected with the river channel techniques (R = 0.07 to 0.1).

These results indicate that the samples collected from the margin represented a different community of taxa that were responding to different pressures or insensitive to the pressures that affected the main river channel.

Figure 12. Matrix showing correlation between BMWP scores of the four deep water techniques, using pairs of matched replicates from the same site reach. R is shown in the top right hand corner for each combination.

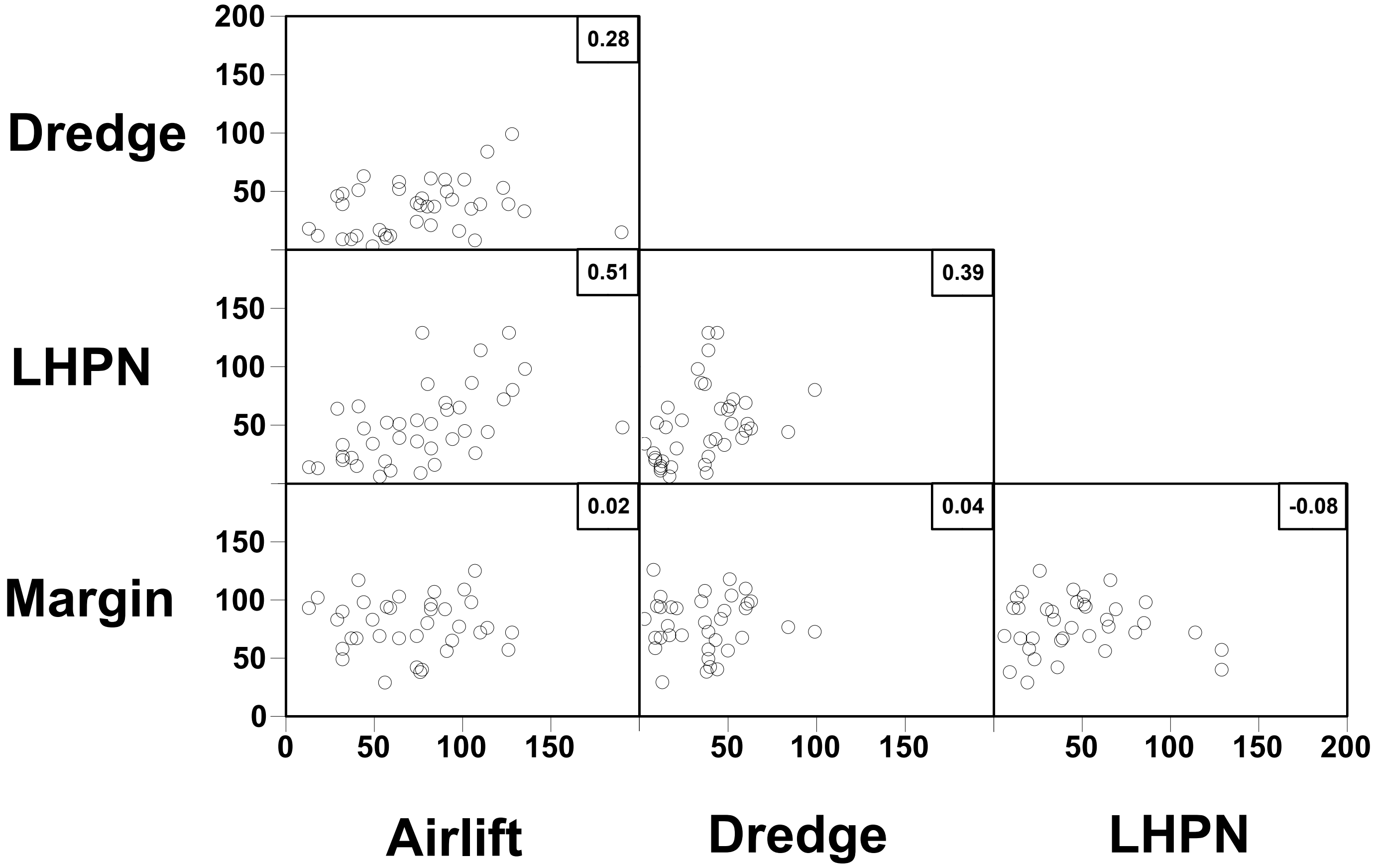


Figure 13. Matrix showing correlation between number of scoring taxa of the four deep water techniques, using pairs of matched replicates from the same site reach. R is shown in the top right hand corner for each combination.

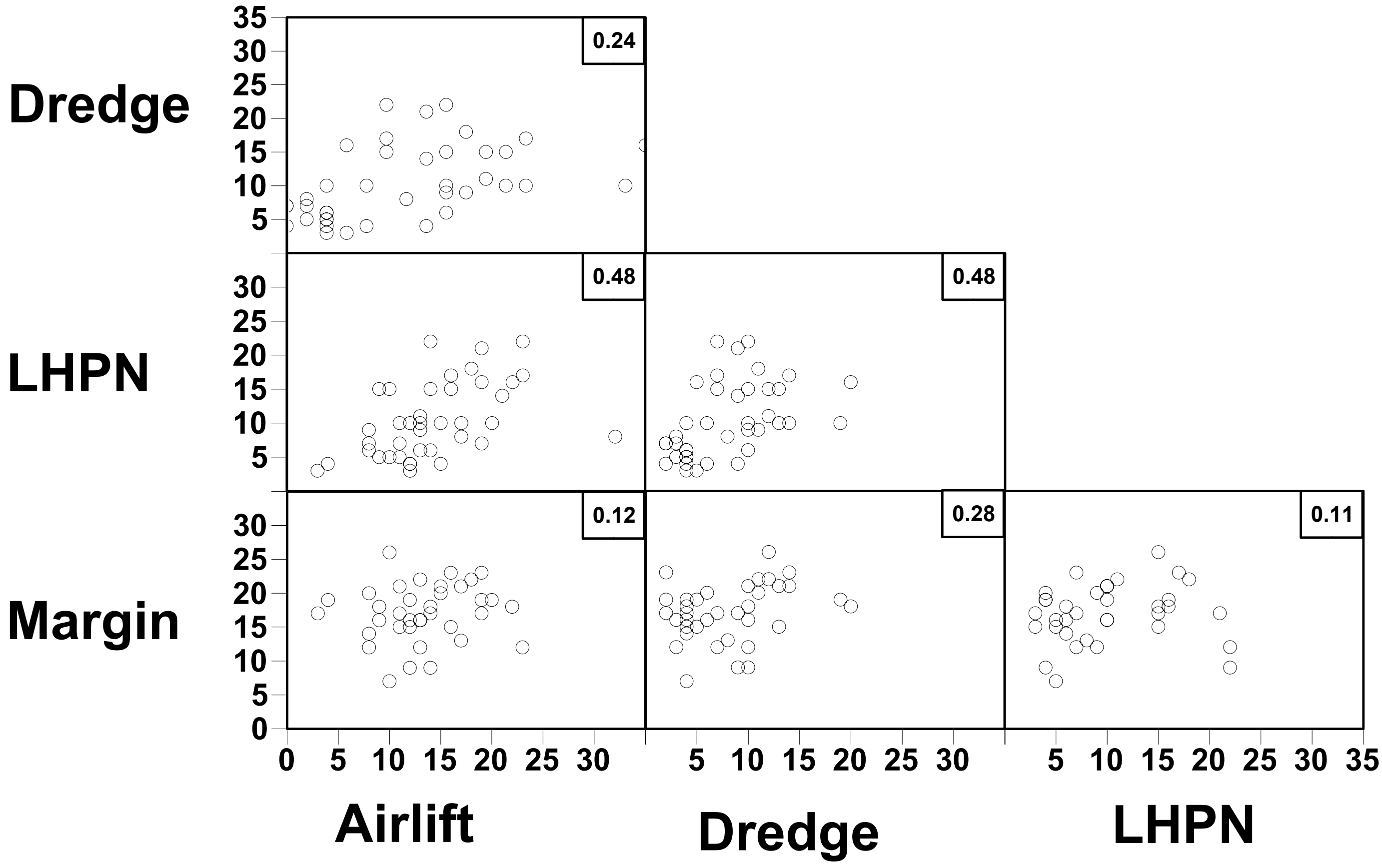
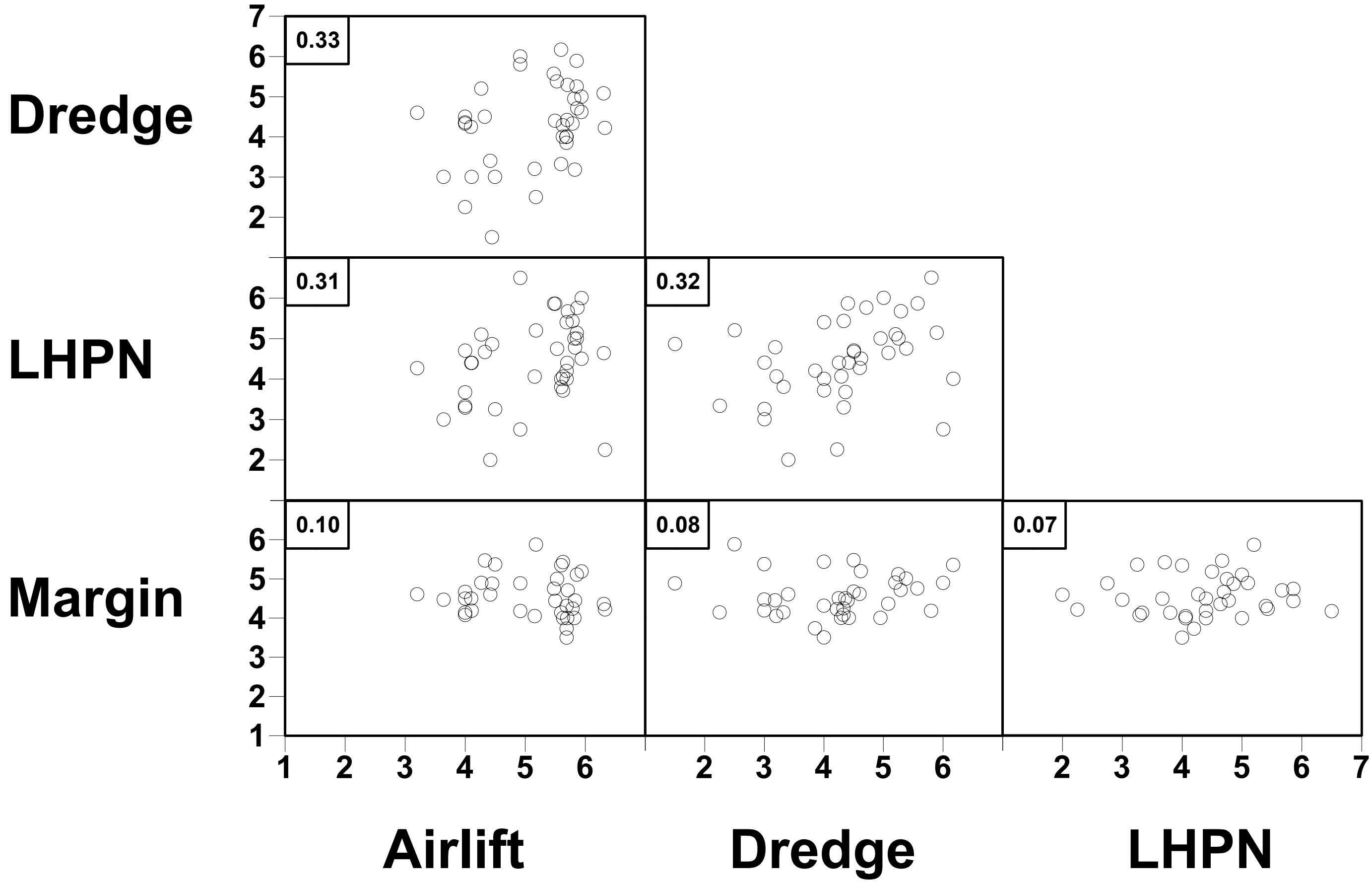


Figure 14. Matrix showing correlation between ASPT of the four deep water techniques, using pairs of matched replicates from the same site reach. R is shown in the top left hand corner for each combination.



3.5 Comparing Estimates of Sampling Uncertainty

For a sampling technique to be effective, sampling variability within a site and time period needs to be small relative to the real differences between sites in their biota and in their values for key biotic indices.

Statistical hierarchical analysis of variance (ANOVA) techniques were used to estimate the variance in each of the three biotic indices (BMWP Score, Number of BMWP taxa (TAXA) and Average Score Per Taxon (ASPT)) due to differences between sites, differences between operators (person taking the field sample) at the same site (Operator effects) and differences between replicate samples taken by the same operator at the same site (Table 7).

Specifically, if Y_{ijk} is the value of the metric for replicate sample k taken by operator j at site i, then Y_{ijk} can be expressed in terms of the sum of the components contributing towards the overall variation in its values, namely:

$$Y_{ijk} = \mu + a_i + b_{ij} + c_{ijk}$$

where μ = overall mean value of Y within the river type;

a_i = deviation of mean value for site i from the overall mean value μ

b_{ij} = deviation of mean value for operator j at site i from the mean for site i

c_{ijk} = deviation of replicate k by operator j at site i from the mean for sampling operator j at site i

and where

σ_I^2 = variance of the a_i = variance due to differences between sites in mean value

σ_J^2 = variance of the b_{ij} = variance due to differences between operators within a site

σ_K^2 = variance of the c_{ijk} = variance due to differences between replicate samples taken by the same operator at the same site

This approach correctly distinguishes and estimates that part of the overall variance of metric values at a site which is due to systematic differences between people in the way they take the sample (namely σ_J^2) from that part due to pure replicate sampling variability arising from small-scale spatial heterogeneity in fauna and sampling variability at the site (namely σ_K^2).

The total variance (σ_T^2) in metric values across all deep rivers is estimated by:

$$\sigma_T^2 = \sigma_I^2 + \sigma_J^2 + \sigma_K^2$$

The within-site variance (σ_W^2) in metric values, that which is due specifically to small-scale sampling variation within a site, is estimated by:

$$\sigma_W^2 = \sigma_J^2 + \sigma_K^2$$

The percentage ($P_{W/T}$) of the overall total variance (σ_T^2) in metric values across all deep rivers which is due specifically to small-scale sampling variation within a site is estimated by:

$$P_{W/T} = 100\sigma_W^2 / \sigma_T^2 .$$

If $P_{W/T}$ is large, then the sampling process and metric jointly give results which are imprecise and cannot reliably be used to detect differences between sites and, thus, different status classes of deep rivers. Conversely a small 'percentage within-site sampling variance' indicates high statistical precision and repeatability of results. It is a separate consideration whether the metric provides a meaningful and hence accurate (rather than merely precise) ecological indicator of river site condition.

The variance components are often quoted in their standard deviation (SD) form (e.g. $SD_W = \sqrt{\sigma_W^2}$ denotes the overall sampling SD within a station).

The estimates of the above variance components using each method are given in Table 7, together with estimates of what percentage they each contribute to the total variance in metric values across the 13 deep water sites. Due to the limited sampling (only two replicates by one operator and one by a second operator, within one season at each site), these estimates of variance components will themselves be subject to estimation error, but gross differences across the 13 sites should indicate major differences in sampling precision between sampling techniques.

Sampling variability within sites comprised only 19% of the total variance in BMWP values based on airlift samples across all 13 deep river study sites. Only 4% of the total variance in

BMWP score based on airlift samples was estimated to be due to operator differences, but these inter-operator differences were not statistically significant. Thus all, or nearly all of the within site variability is pure replicate sampling variability due to small-scale spatial heterogeneity in invertebrate composition.

In contrast, using the dredge sampling technique led to all of the total variance in BMWP Score being attributable to within-site sampling variability with, on average, an estimated 64% due to operator effects (Table 7). Operator differences in both BMWP score and ‘Number of taxa’ were statistically significant for dredge samples (but not for any other sampling technique). This suggests that different operators in practice did not use the dredge in exactly the same way and this had an effect on the fauna obtained and thus the BMWP score.

For both the ‘Margin’ and LHPN sampling techniques, about two-thirds (60% and 68% respectively) of the total variance in BMWP score values across the study sites was attributable to differences between sites.

The variation in BMWP score, number of BMWP taxa (TAXA) and average score per taxon (ASPT) among the replicate samples collected by the different workers at the different sites by the four different techniques is shown graphically in Figures 15, 16 and 17 respectively.

		Technique			
	Variance	Airlift	Dredge	Margin	LHPN
BMWP	Between-Site	1349	0	335	692
	Operator	60	351	58	138
	Replicate	248	201	168	191
	Total	1657	552	561	1021
	%Site	81 ***	0	60 **	68 **
	%Operator	4	64 *	10	13
	%Replicate	15	36	30	19
	% Within-site	19	100	40	32
TAXA	Between-Site	29.05	5.18	8.5	19.2
	Operator	0.06	9.42	5.02	5.11
	Replicate	8.88	7.85	4.79	5.54
	Total	37.99	22.45	18.31	29.85
	%Site	76 ***	23	46 *	64 **
	%Operator	0	42 *	28	17
	%Replicate	24	35	26	19
	% Within-site	24	77	54	36
ASPT	Between-Site	0.616	0.062	0.264	0.495
	Operator	0	0	0	0
	Replicate	0.182	1.093	0.061	0.752
	Total	0.798	1.155	0.325	1.247
	%Site	77 ***	5	81 ***	40 **
	%Operator	0	0	0	0
	%Replicate	23	95	19	60
	% Within-site	23	95	19	60

Table 7. Estimates of sources of variance in BMWP Score, number of taxa (TAXA) and ASPT for each of the field sampling techniques (airlift, dredge, margin and LHPN). *, ** and * denote site or operator variance component was statistically significant in ANOVA tests at the 0.05, 0.01 and 0.001 test probability level.**

The four techniques were ranked for each metric in terms of the percentage of the total variance in metric values across the 13 study sites, which was estimated to be within-site sampling variability. The lowest percentage indicates the highest sampling precision and is given a rank of 1 (Table 8). On this basis and based on these biotic indices, the airlift appears to have the highest sampling precision of all four techniques, followed by the 'Margin' and LHPN sampling techniques; the dredge method had the lowest sampling precision for all three indices (Table 8).

	Technique			
	Airlift	Dredge	Margin	LHPN
BMWP	1	4	3	2
TAXA	1	4	3	2
ASPT	2	4	1	3
Average rank	1.33	4	2.33	2.33

Table 8. Ranking of the field sampling techniques (airlift, dredge, margin and LHPN) for relative sampling precision (% Within-site variance) in terms of BMWP Score, number of taxa (TAXA) and ASPT, and overall.

Figure 15. Sampling variation in BMWP Score at each site for the field sampling techniques (a) Airlift and (b) Dredge, coded by field sampler (1) ●, (2) ■ and (3) ♦. See Table 1 for site name.

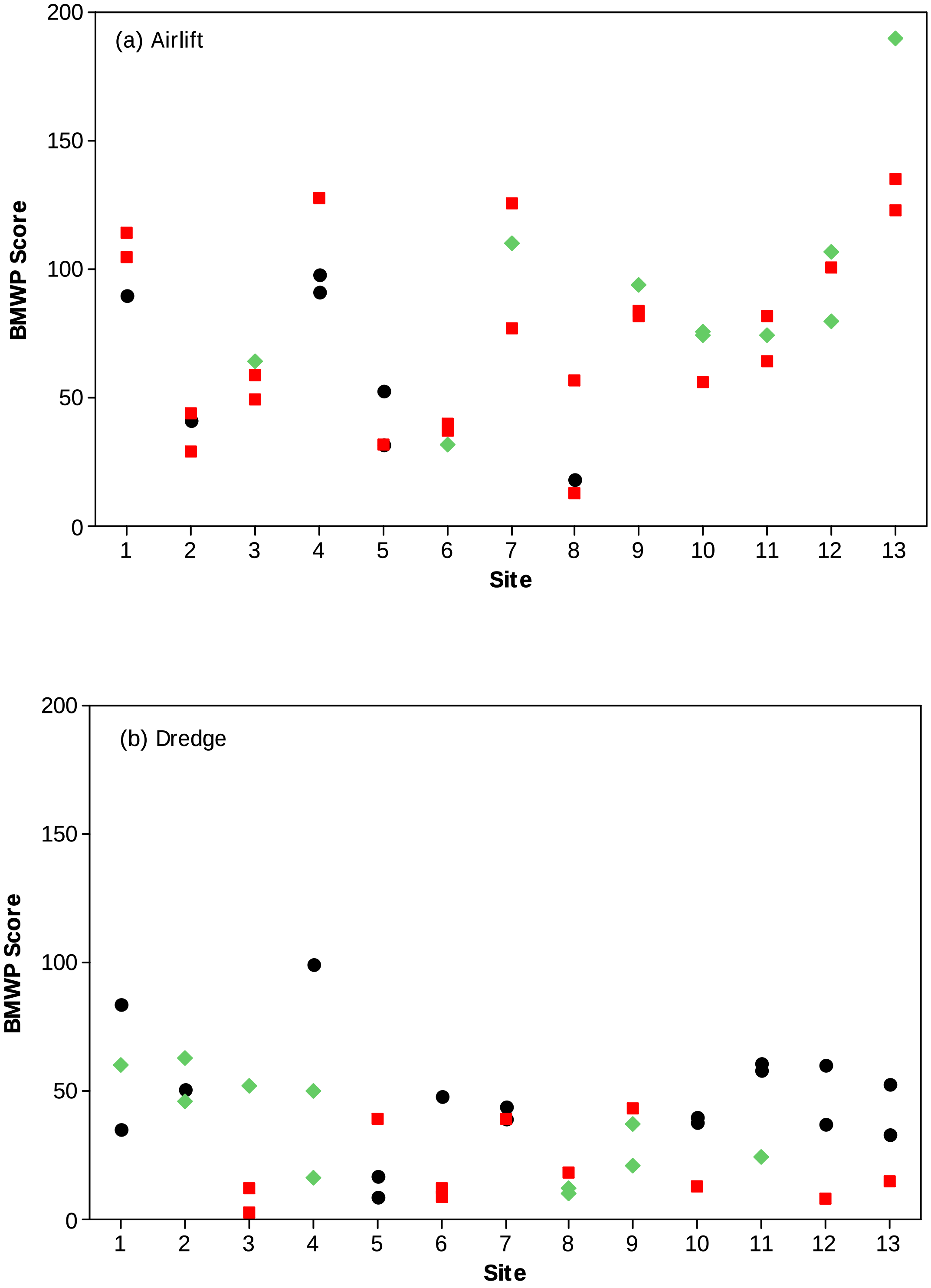
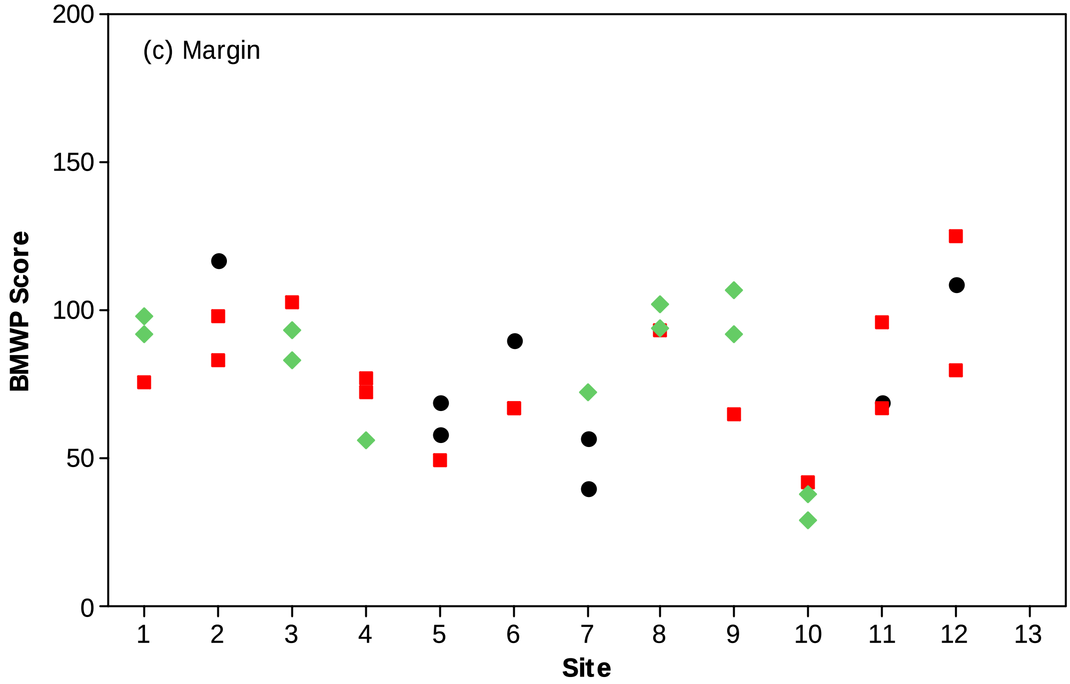


Figure 15. Sampling variation in BMWP Score at each site for the field sampling techniques (c) Margin and (d) LHPN , coded by field sampler (1) ●, (2) ■ and (3) ♦. See Table 1 for site name.



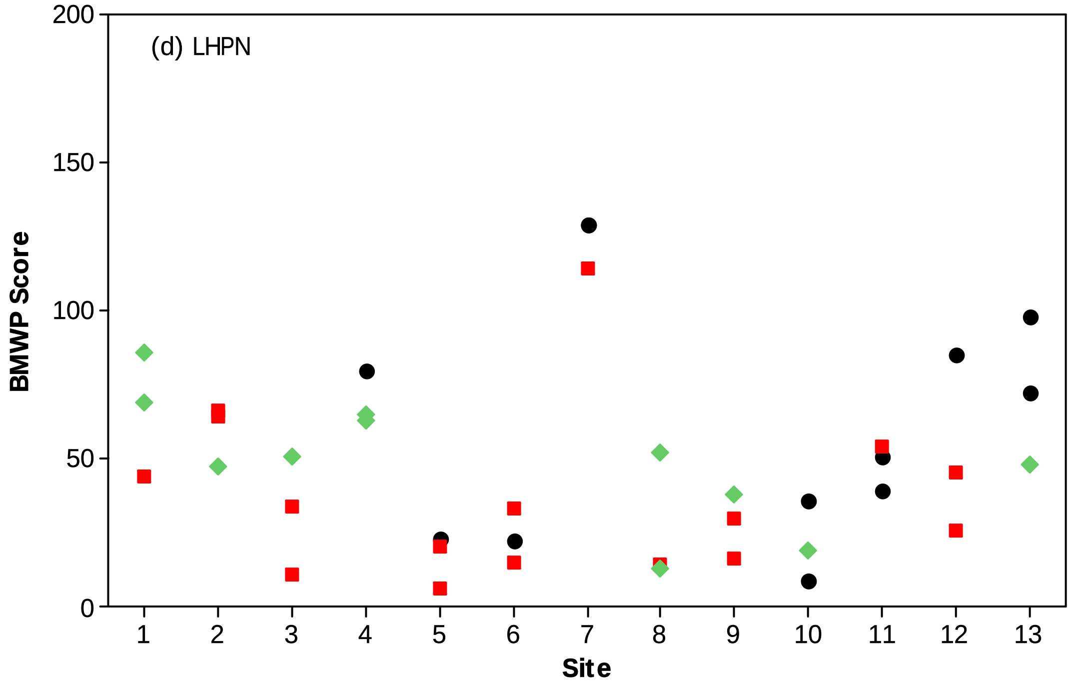
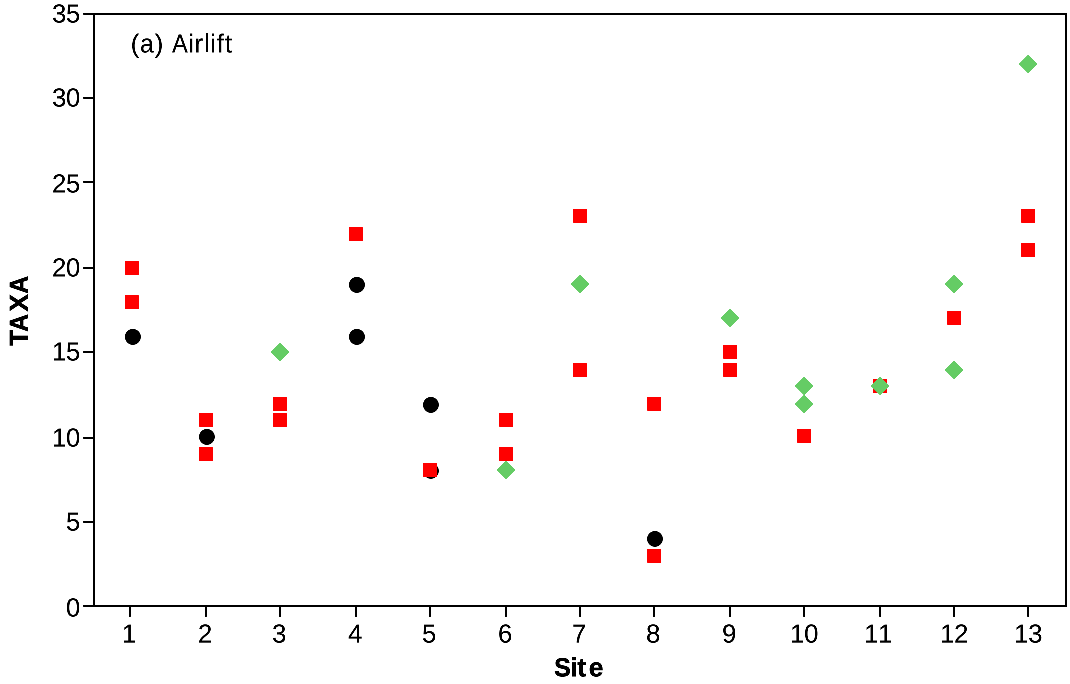


Figure 16. Sampling variation in 'Number of TAXA' at each site for the field sampling techniques (a) Airlift and (b) Dredge , coded by field sampler (1) ●, (2) ■ and (3) ♦. See Table 1 for site name.



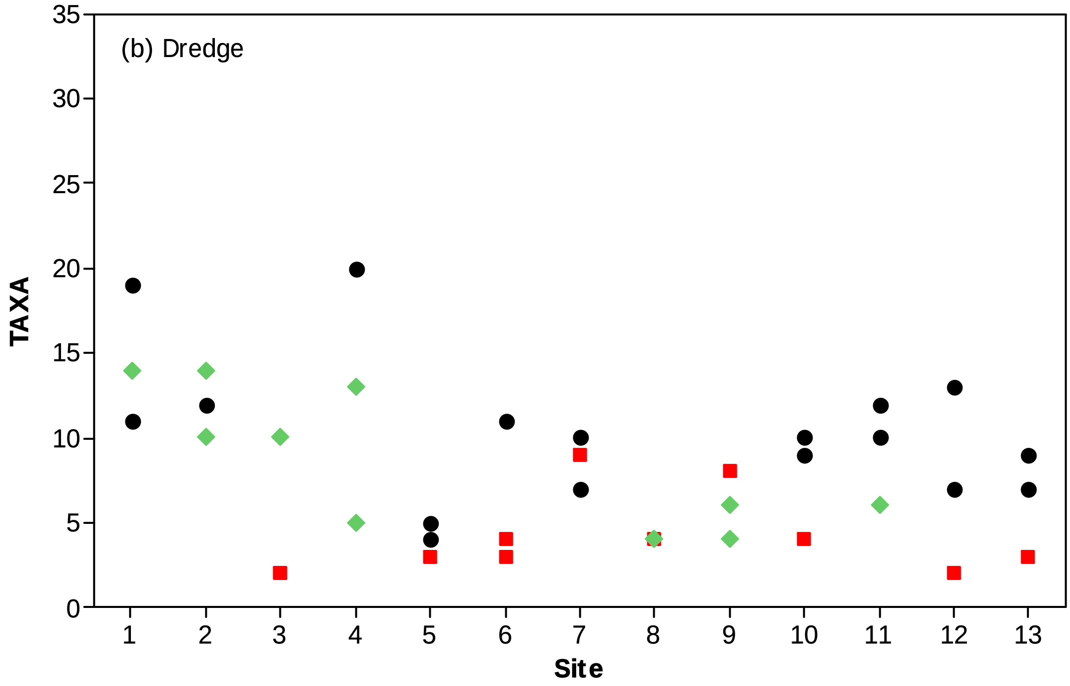


Figure 16. Sampling variation in 'Number of TAXA' at each site for the field sampling techniques (c) Margin and (d) LHPN, coded by field sampler (1) ●, (2) ■ and (3) ♦. See Table 1 for site name.

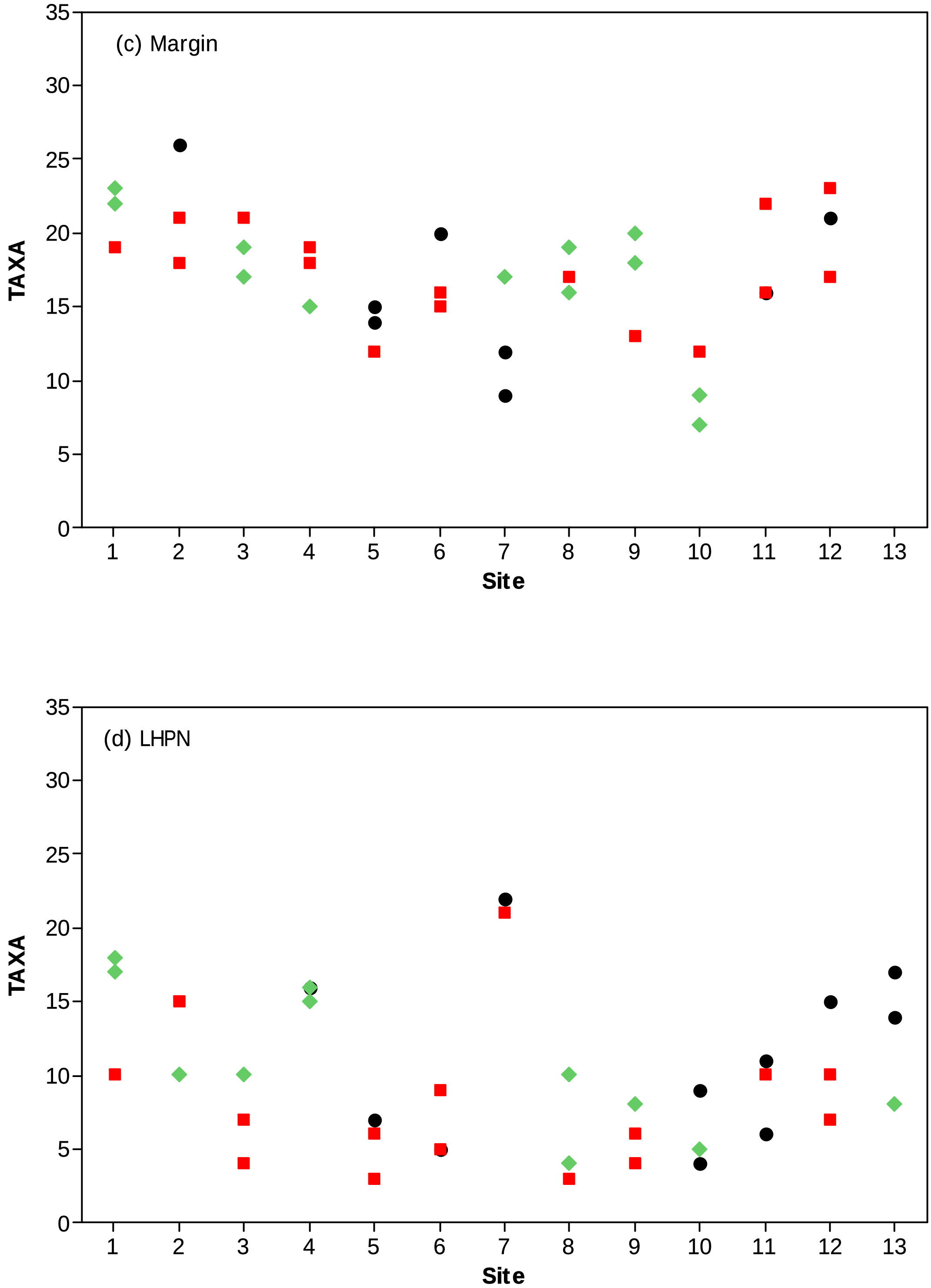
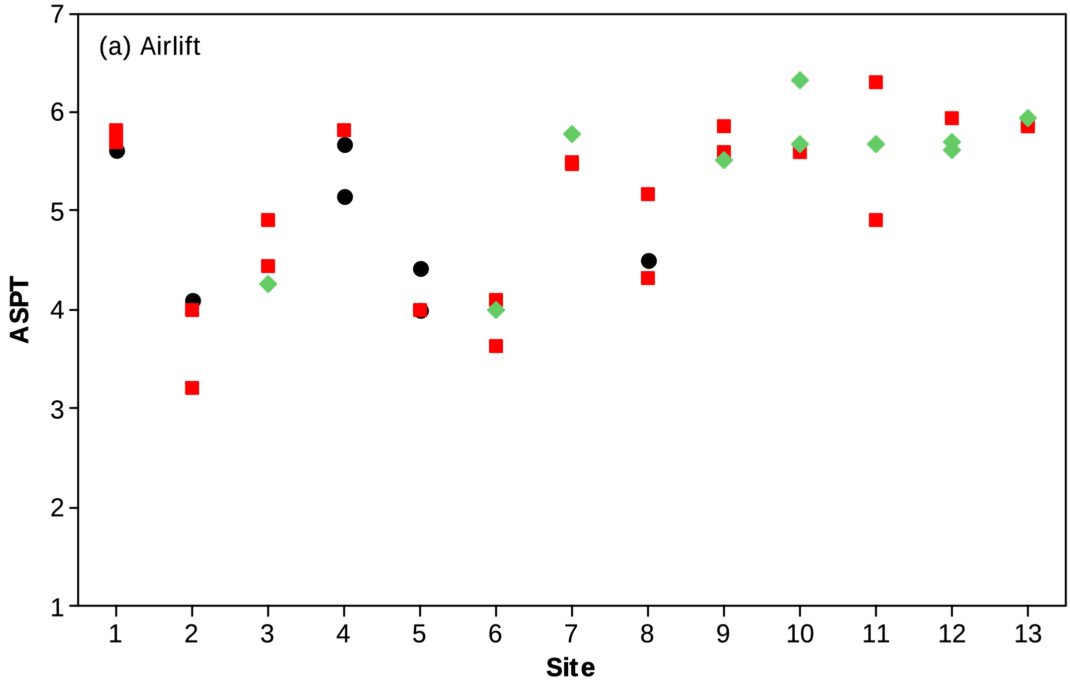


Figure 17. Sampling variation in ASPT at each site for the field sampling techniques (a) Airlift and (b) Dredge, coded by field sampler (1) ●, (2) ■ and (3) ♦. See Table 1 for site name.



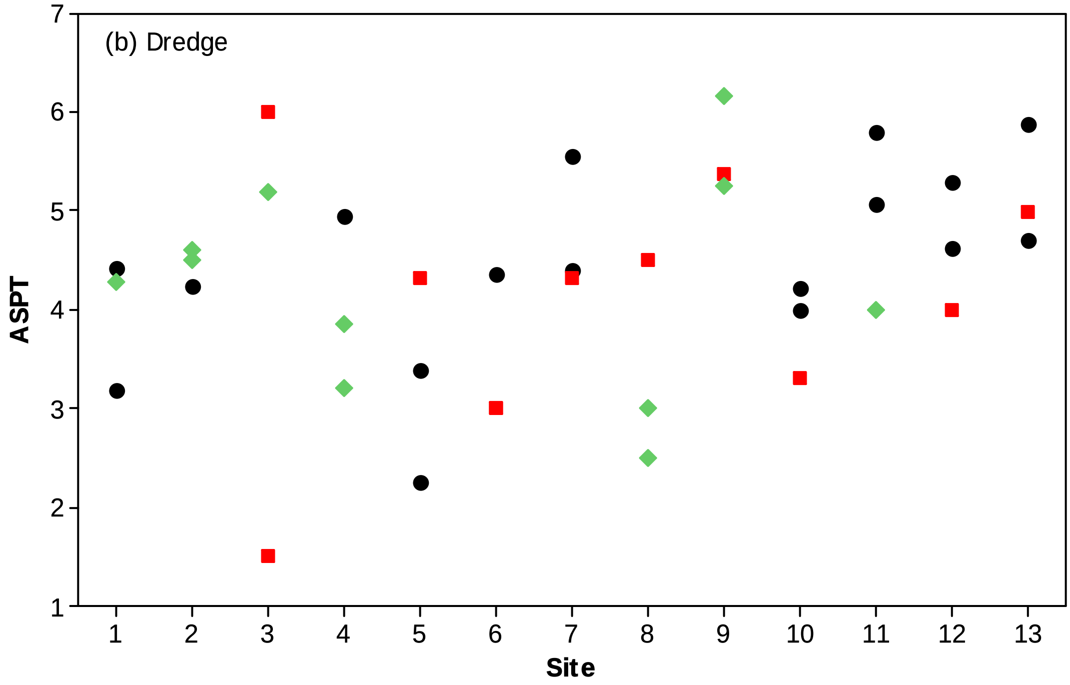
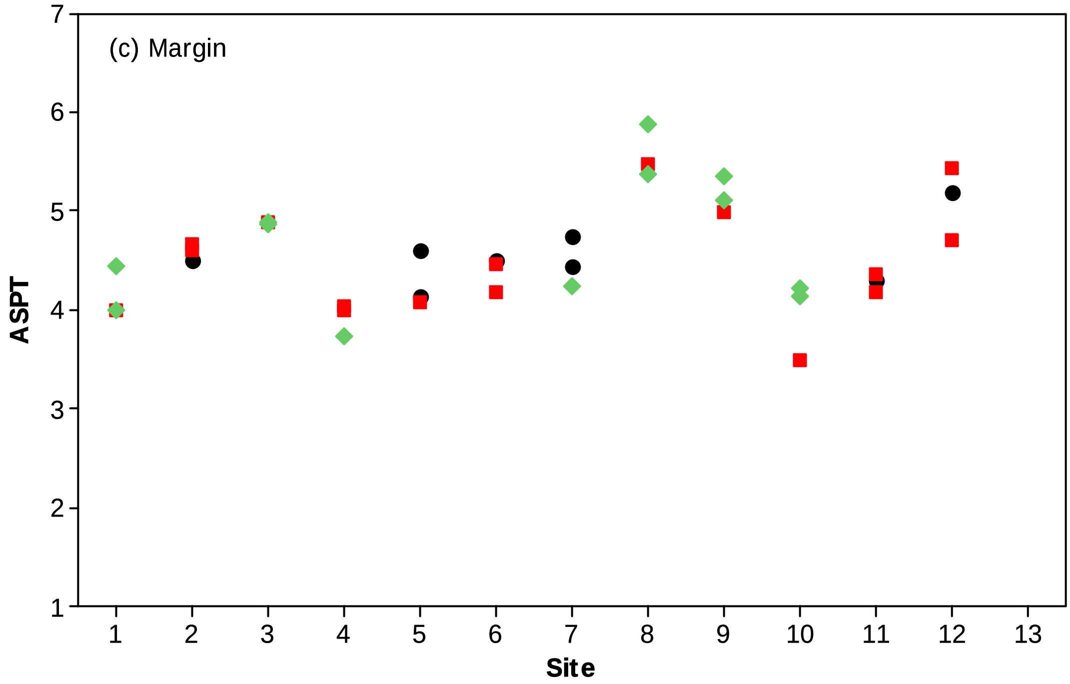
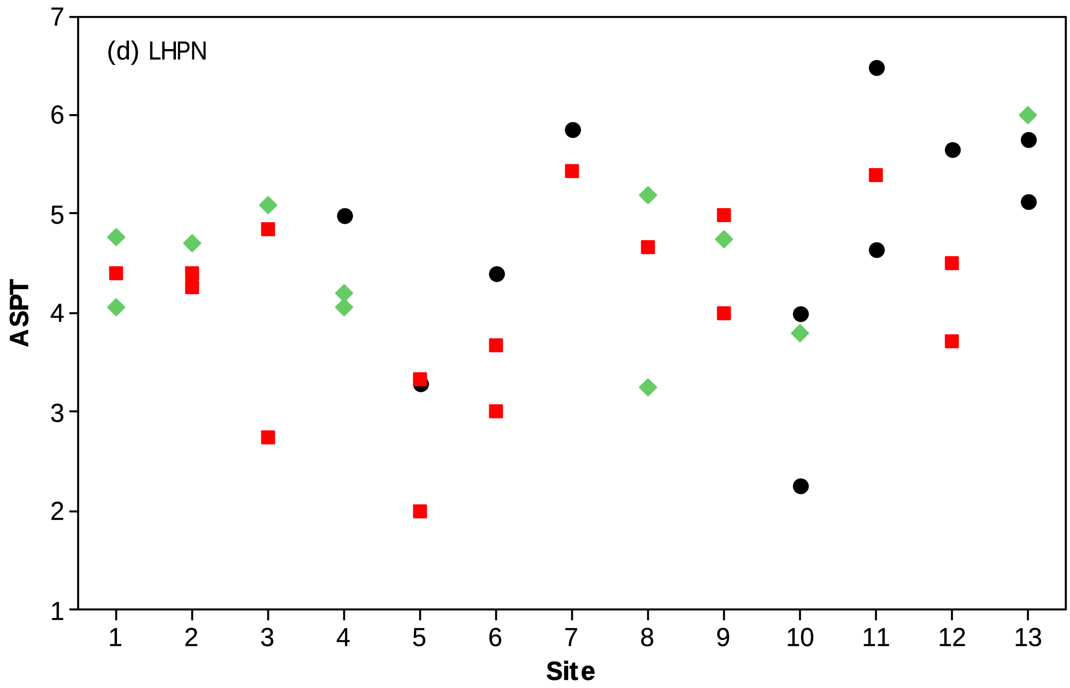


Figure 17. Sampling variation in ASPT at each site for the field sampling techniques (c) Margin and (d) LHPN, coded by field sampler (1) ●, (2) ■ and (3) ♦. See Table 1 for site name.





3.6 Comparing Cost Effective Precision

The airlift has been shown to have the lowest 'percentage within-site sampling variance' and thus the highest statistical precision and repeatability of results amongst the four techniques assessed (see section 3.5). The light dredge, on the other hand, has been shown to have such high sampling variance and low repeatability that it is effectively useless for assessing and discriminating the ecological status of sites and need not be considered further.

However, a single airlift sample has been shown to take a longer time to sort and process and therefore costs more per sample than either a margin or LHPN sample (see section 3.2).

It is possible to combine these two aspects, namely sampling precision and sample processing time costs, to estimate the relative cost effectiveness of each technique.

Specifically, if σ_I^2 and σ_W^2 denote the estimates of the variance between sites and overall within-site sampling variance respectively, then the sampling variance of the estimate of the index value based on the mean of *n* samples is σ_W^2 / n, and its sampling variance expressed as a percentage of the total variance in estimates across all sites is:

$$P_{W(n)} = 100(\sigma_W^2 / n)/(\sigma_I^2 + \sigma_W^2 / n)$$

To compare the sampling processing cost effectiveness of the four techniques, we calculate the number (*n*) of samples required for each technique to achieve sampling precision of the estimate of the mean index value for a site of less than 10% of the total variance across all sites (i.e. $P_{W(n)} < 10\%$).

For example, to estimate BMWP score requires only one airlift sample to achieve <20% sampling variance, but three airlift samples are required for the mean BMWP for a site to have <10% sampling variance (Table 9). Airlift samples took on average 267 minutes to processing, indicating total sample processing times of 267 and 801 minutes to achieve 20% and 10% sampling precision for index estimates. In contrast, the margin technique, requires an estimated three and seven samples to achieve 20% and 10% precision, equivalent to total sample processing times, on average, of 441 and 1029 minutes.

Based on these calculations for each technique and index, we can rank the techniques in terms of their cost effectiveness to achieve critical sampling precisions (Table 10).

Obviously, these comparisons still ignore any differences in costs associated with collecting the samples in the field. They also ignore any differences in the metric values achieved with the different techniques.

	Q%	Technique			
		Airlift	Dredge	Margin	LHPN
Per sample (mins)		267	94	147	102
(a) BMWP					
σ_I^2		1349	0	335	692
σ_W^2		308	551	226	329
	20%	267 (1)	(>100)	441 (3)	204 (2)
	10%	801 (3)	(>100)	1029 (7)	510 (5)
(b) TAXA					
σ_I^2		29.05	5.18	8.5	19.2
σ_W^2		8.94	17.27	9.81	10.65
	20%	534 (2)	1316 (14)	735 (5)	306 (3)
	10%	801 (3)	2914 (31)	1617 (11)	612 (6)
(c)ASPT					
σ_I^2		0.616	0.062	0.264	0.495
σ_W^2		0.182	1.093	0.061	0.752
	20%	534 (2)	6674 (71)	147 (1)	714 (7)
	10%	801 (3)	15040 (160)	441 (3)	1428 (14)
Time to achieve precision in all 3 metrics	20%	534 (2)	(>100)	735 (5)	714 (7)
	10%	801 (3)	(>100)	1617 (11)	1428 (14)

Table 9. Comparison of the field sampling techniques (airlift, dredge, margin and LHPN) for sampling processing cost (time in minutes; number of samples shown in brackets) to achieve a sampling variance of less than Q% (20% or 10%) of the total variance amongst all sites in terms of BMWP Score, number of taxa (TAXA), ASPT, and all 3 metrics. σ_I^2 and σ_W^2 denote between- and within- site variance estimates.

	Technique			
	Airlift	Dredge	Margin	LHPN
BMWP	2	4	3	1
TAXA	2	4	3	1
ASPT	2	4	1	3
Average rank	2	4	2.33	1.67
Maximum rank	2	4	3	3

Table 10. Ranking of the field sampling techniques (airlift, dredge, margin and LHPN) for cost (sample processing time) to achieve sampling variance less than 10% of total variance in terms of BMWP Score, number of taxa (TAXA) and ASPT, and overall (average and maximum). Rank of 1 equals least time to achieve sampling variance less than 10% of total variance.

3.7 Influence of the Environment on Performance of Techniques

In order to assess the relative effectiveness of the different deep water sampling techniques, the influence of environmental conditions on key metrics was determined. If all techniques performed equally well relative to one another across all conditions, a standard technique can be selected on other measures of performance and suitability. However, if there are site conditions which influenced the relative performance of the techniques, the influence of environmental conditions on the techniques performance will need to be taken into account when choosing a standard technique.

Analysis of covariance was used to assess the influence of environmental conditions on the relative performance of the techniques. Here an interaction between the environmental variable being tested and the techniques used indicates divergence in the relative performance of the techniques associated with changes in that environmental variable.

Variation in sediment composition appeared to influence the relative performance of the deep water sampling techniques tested. Changes in the proportion of silt and pebbles/gravel were not correlated with changes in the relative performance of the techniques tested (Figures 18 & 20). An increasing proportion of sand in the sediment was correlated with an increase in the ASPT of the samples collected from the margin, but a decline in ASPT in the samples collected with the three river channel techniques (Figure 19b). There was no influence of sand on the relative performance of the techniques in terms of number of scoring taxa (Figure 19a). This difference is likely to be correlative and reflect the differences in fauna between the margins and the river channel (see section 3.4).

There was an increase in the number of scoring taxa with an increasing proportion of boulders/cobbles for the samples collected with the airlift and from the margin (Figure 20a), whereas there was no relationship with the samples collected with the light dredge and long-handled pond net. There was no effect of the proportion of boulders/cobbles on the relative performance of the techniques in terms of ASPT (Figure 21b). This difference may be a consequence of the poor ability of the dredge and long-handled pond net to penetrate the sediment or to extract macroinvertebrates from the interstices when the sediment comprised cobbles and boulders when compared to the airlift.

Macrophytes had no influence on the relative performance of the techniques (Figure 22), despite concerns that certain techniques may miss plant associated taxa or become clogged with plants.

Surprisingly, river width had a significant effect on the relative performance of the techniques in terms of ASPT. The ASPT of samples collected with the airlift increased with river width whilst those collected from the margin decreased, resulting in increasing separation of ASPT for these two techniques with increasing river width (Figure 23a). The ASPT did not follow a consistent change with increasing river depth, indicating that the change in relative performance of the techniques was unlikely to be a consequence of increasing depth with width (Figure 23b): Whilst all the wider rivers were deep, some of the narrow rivers sampled were relatively deep (Figure 24). It is possible that in narrow rivers representatives of the mid-channel fauna are present in the margins and caught by both techniques, whereas in wider rivers there is more spatial segregation between the margins and channel and together with the occurrence of sensitive, deep river taxa in the channel.

To summarise,

1. There is little influence of the environment on the performance of the different techniques used to sample the river channel, with the exception of the poor performance of the long-handled pond net and light dredge when the substrate is very coarse. These techniques appear to be unable to penetrate the substrate under these conditions, whereas the airlift appears to flush the invertebrates from the interstices between the boulders and cobbles.

2. Wide rivers cannot be effectively sampled at the margin alone as the high scoring mid-channel fauna are overlooked.

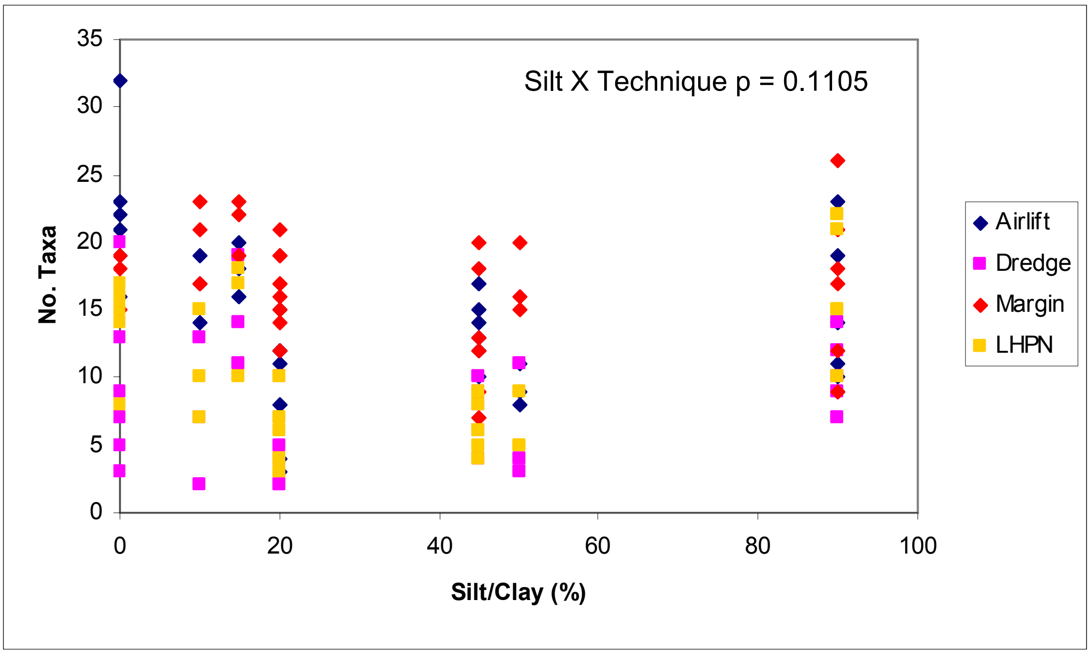
The four techniques were ranked according to the influence of environmental conditions on the metrics used here to assess the techniques' effectiveness at sampling the macroinvertebrate fauna, thus enabling an average rank position to be established (Table 11). Ranks were shared where there was no significant difference in the mean metric score among the techniques. Ranks do not reflect the extent of the influence of the environment, merely the techniques' position relative to one another where a rank of 1 indicates no influence.

	Technique			
	Airlift	Dredge	Margin	LHPN
Boulders/cobbles	1	3	1	3
Sand	1	1	4	1
Width	1	2	4	2
Average rank	1	2	3	2

Table 11. Ranking of the field sampling techniques (airlift, dredge, margin and LHPN) for influence of environment on performance in terms of physical conditions that had a significant influence on performance, proportion of bed substrate that comprised boulders or cobbles, proportion of bed substrate that comprised sand, width of river, and overall. Rank of 1 indicates no influence of changing conditions.

Figure 18. Influence of the proportion of silt and clay in the substrate on relative performance of the four deep water techniques tested, in terms of a) number of BMWP scoring taxa, and b) ASPT. Results of interaction between technique and proportion of silt and clay in the substrate from Ancova shown; this interaction indicates differences in relative performance if significant.

a)



b)

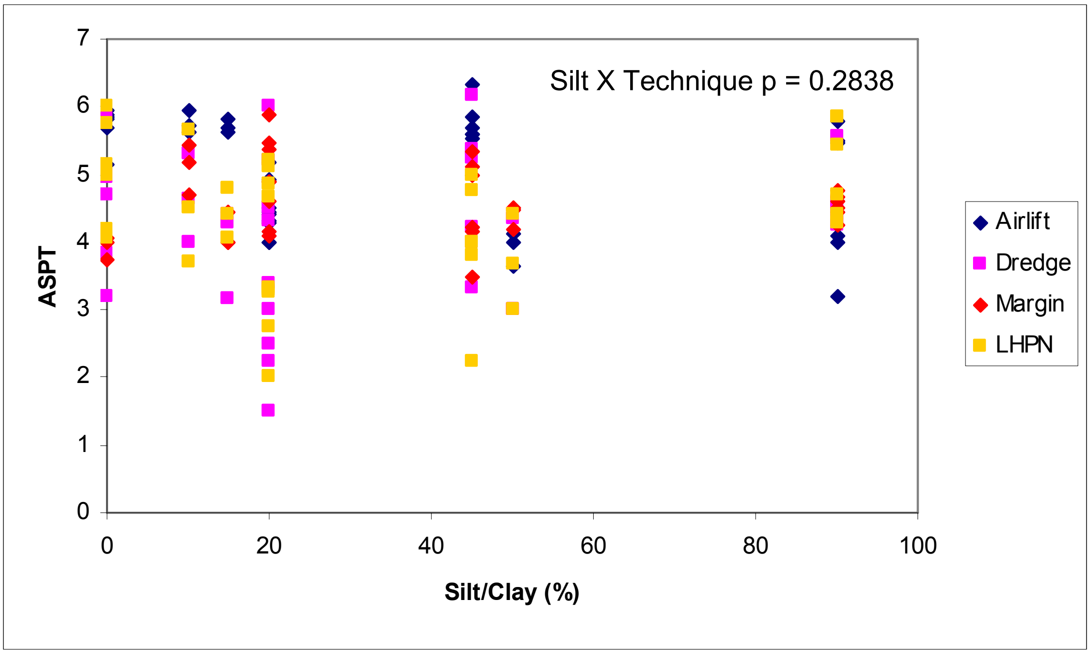
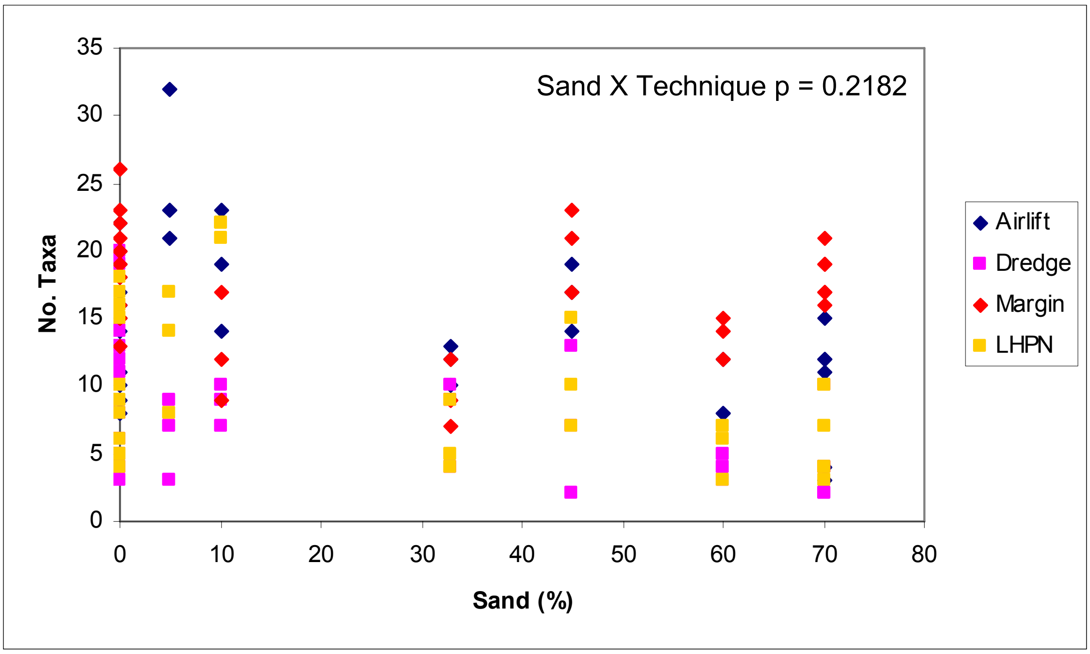


Figure 19. Influence of the proportion of sand in the substrate on relative performance of the four deep water techniques tested, in terms of a) number of BMWP scoring taxa, and b) ASPT. Results of interaction between technique and proportion of sand in the substrate from Ancova shown; this interaction indicates differences in relative performance if significant.

a)



b)

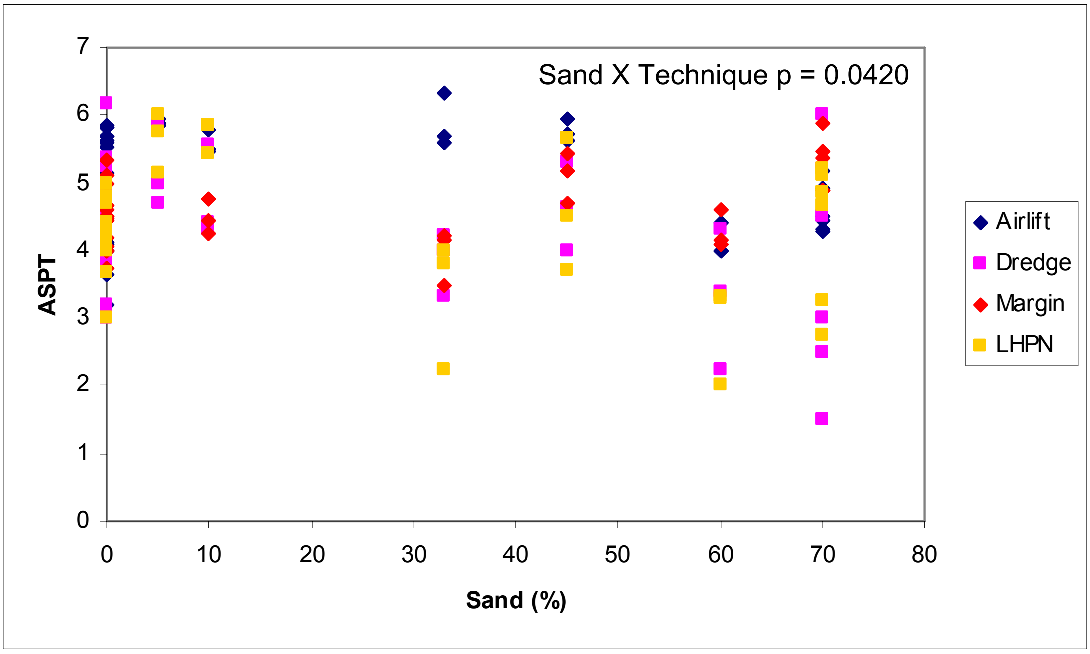
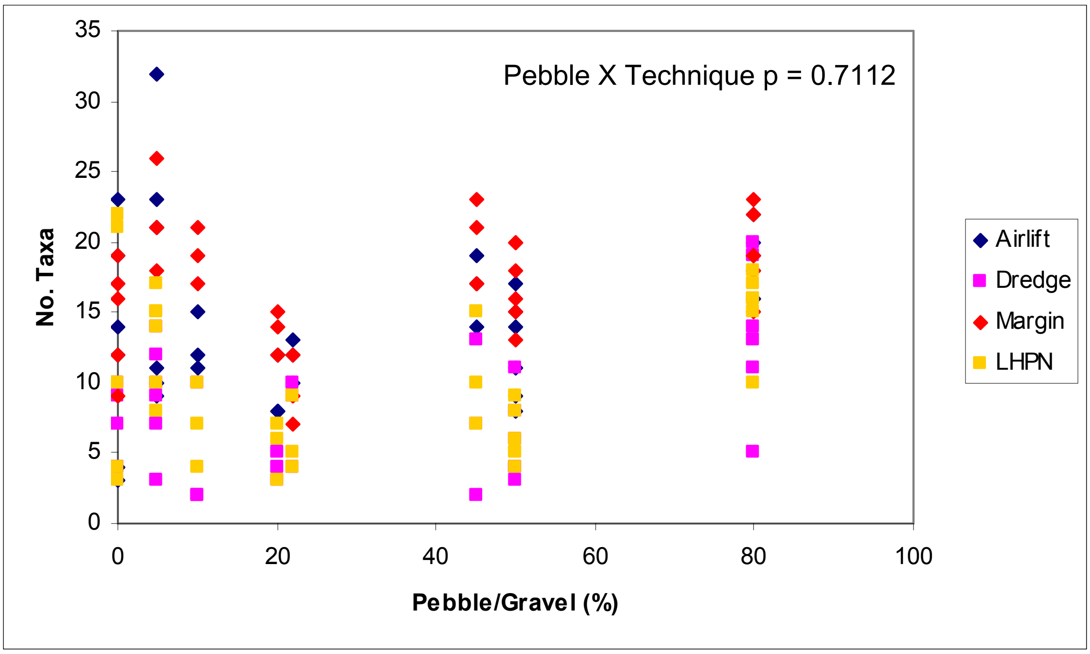


Figure 20. Influence of the proportion of pebbles and gravel in the substrate on relative performance of the four deep water techniques tested, in terms of a) number of BMWP scoring taxa, and b) ASPT. Results of interaction between technique and proportion of pebbles and gravel in the substrate from Ancova shown; this interaction indicates differences in relative performance if significant.

a)



b)

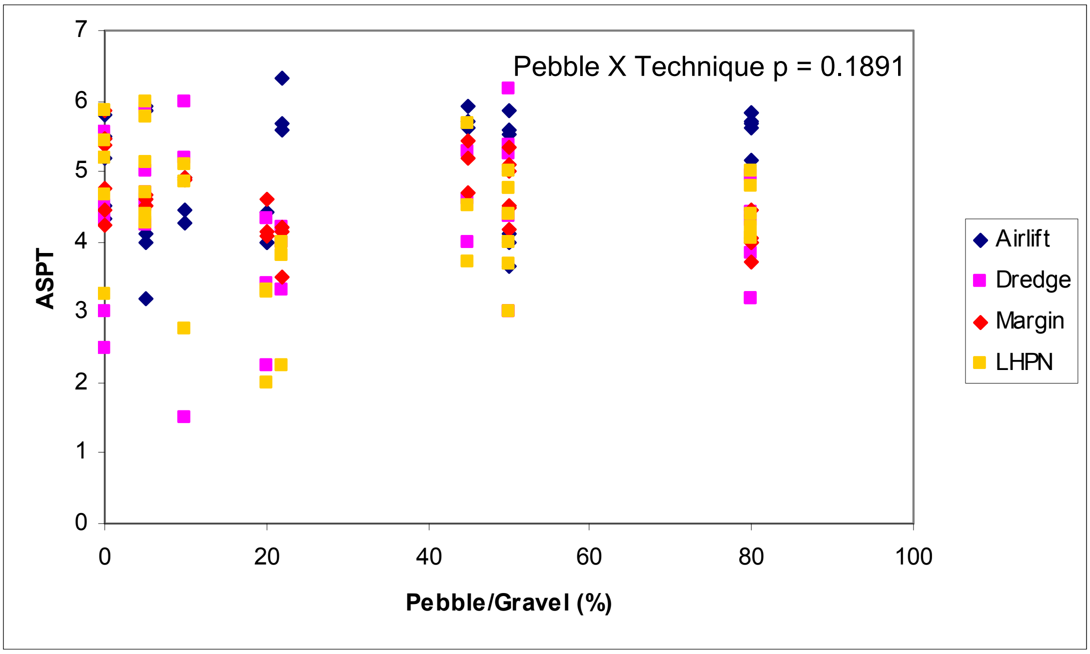
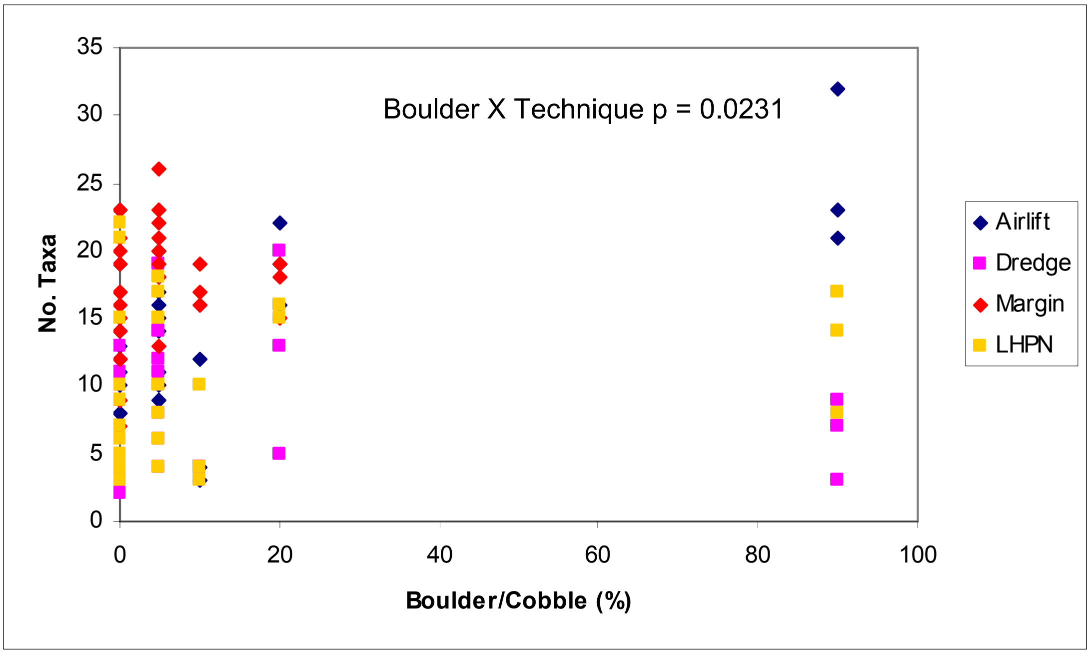


Figure 21. Influence of the proportion of boulders and cobbles in the substrate on relative performance of the four deep water techniques tested, in terms of a) number of BMWP scoring taxa, and b) ASPT. Results of interaction between technique and proportion of boulders and cobbles in the substrate from Ancova shown; this interaction indicates differences in relative performance if significant. (NB no sample was collected from the margin of the site with the highest proportion of boulders/cobbles)

a)



b)

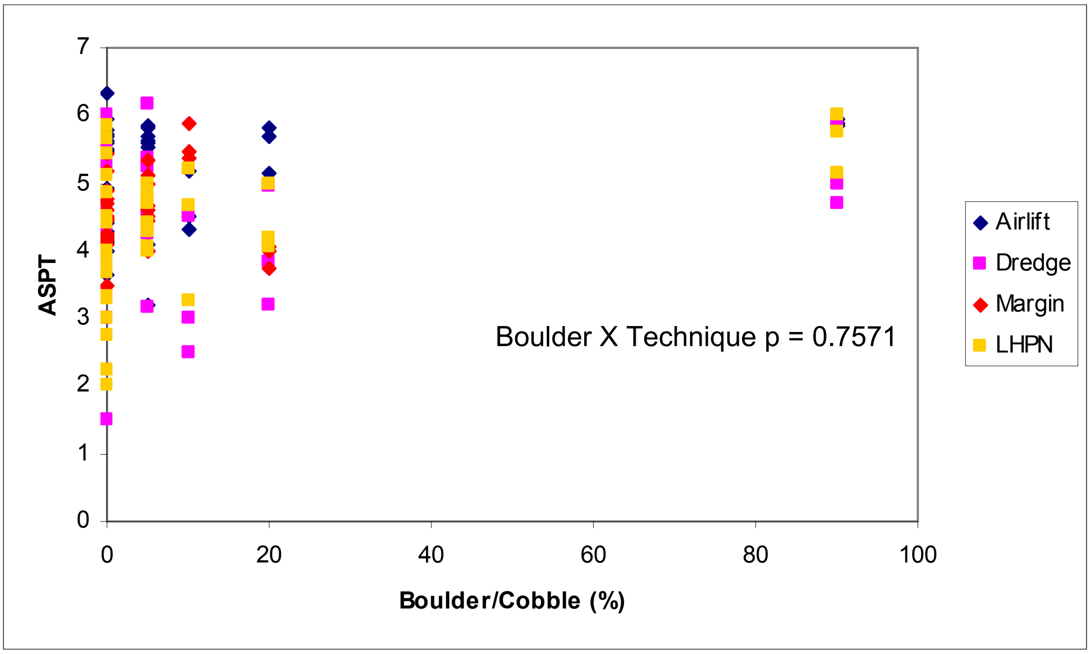
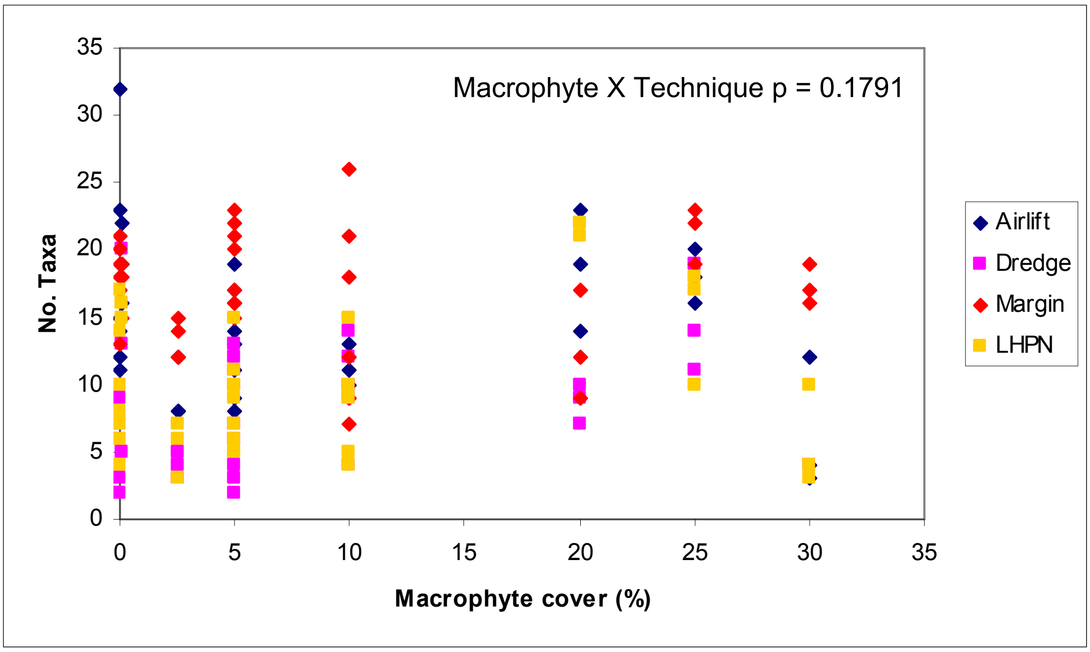


Figure 22. Influence of the proportion of macrophyte cover on relative performance of the four deep water techniques tested, in terms of a) number of BMWP scoring taxa, and b) ASPT. Results of interaction between technique and proportion of macrophyte cover from Ancova shown; this interaction indicates differences in relative performance if significant.

a)



b)

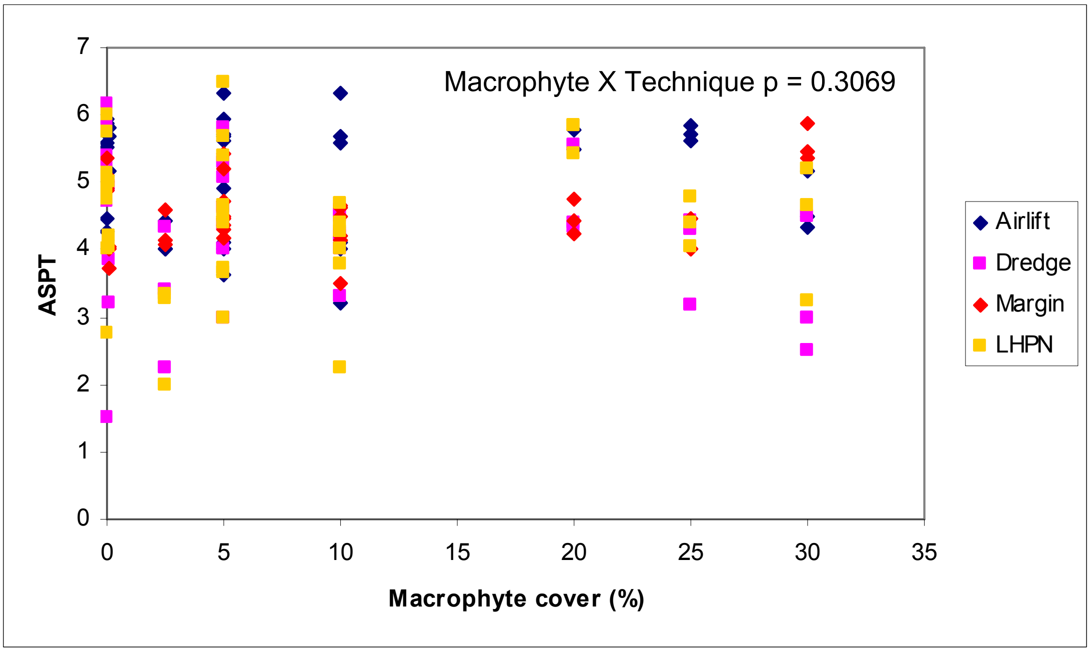
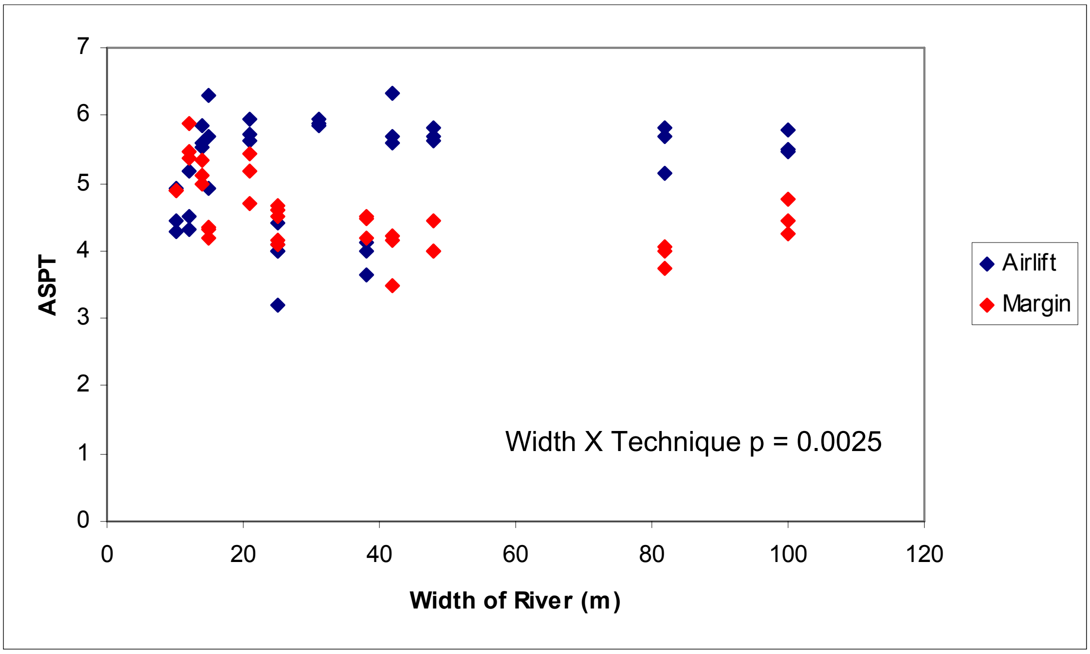


Figure 23. Influence of a) the width of the river channel and b) the depth of the centre of the river channel on relative performance of the four deep water techniques tested in terms of ASPT. Results of interaction between technique and width and depth from Ancova shown; this interaction indicates differences in relative performance if significant. NB only airlift and margin shown for clarity; there was no significant influence of width or depth on the relative performance of the margin or long-handled pond net.

a)



b)

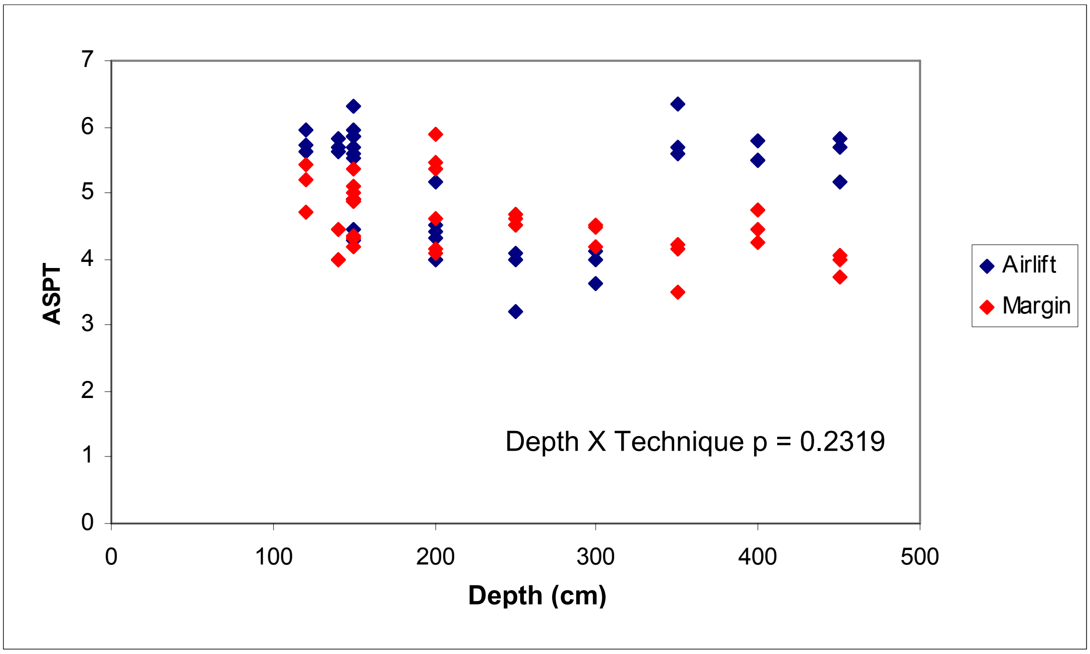
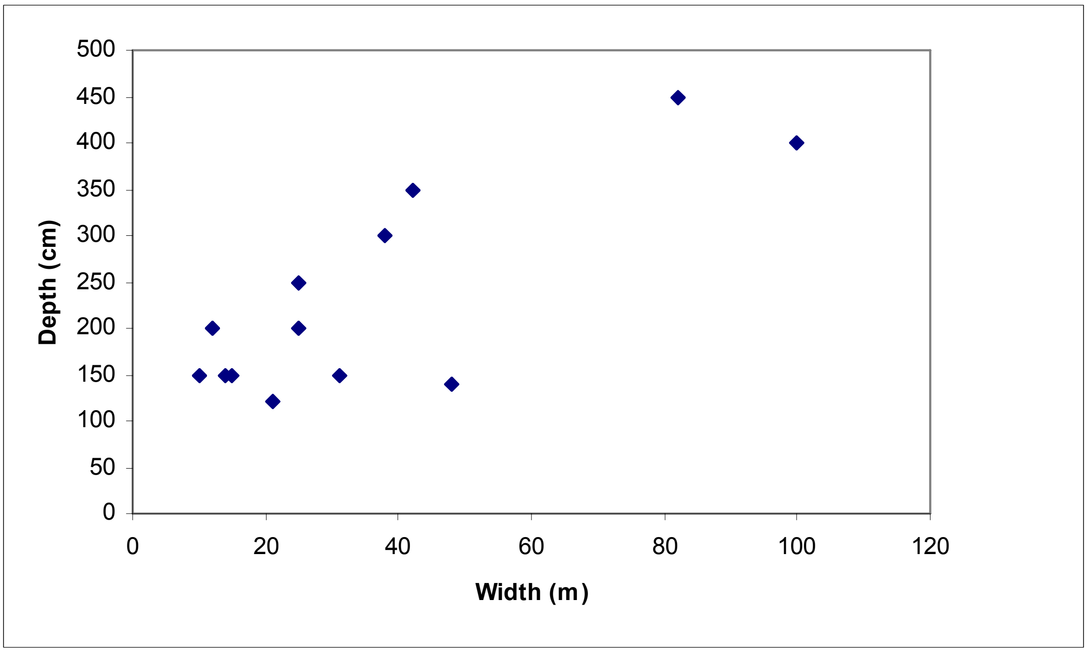


Figure 24. Relationship between the width of the river channel and the depth of the centre of the river channel.



3.8 Taxonomic Composition

For a sampling technique to be effective, the technique needs to provide a representative sample of the river fauna. If all techniques provide a similar representative sample of the fauna, a standard technique can be selected on its performance and suitability relative to other techniques. However, if techniques sample different portions of the fauna, consideration will have to be given to how well they represent the fauna before they can be considered. In such circumstances it may be necessary to adopt a combination of techniques.

A partial Cannonical Correspondence Analysis (i.e. with the influence of differences among the sites removed) indicated substantial differences in the macroinvertebrate taxa that were sampled by the different techniques, particularly between the samples collected from the margin and with the airlift (Figure 25). Marginal samples tended to contain more taxa associated with the air-water interface (e.g. Hydrometridae, Gerridae, Notonectidae,) or with vegetation (e.g. Libellulidae, Nepidae) whereas the airlift samples tended to contain taxa associated with deeper water (e.g. Astacidae, Leuctridae, Molannidae, Ephemeridae). The dredge and long-handled pond net samples contained a more average community which was characterised by a partial lack of both the marginal and deep water components of the fauna.

Discriminant analysis indicated a clear segregation between the samples collected from the margin, identified by the occurrence of Corixidae, Gerridae, and Hydrophilidae, and those collected from the river channel. The samples collected with the airlift were identified by the occurrence of Astacidae, Leuctridae, Ephemeridae, Psychomyiidae, Glossiphoniidae, Gammaridae and Sphaeriidae and those collected with the long-handled pond net and light dredge by a lower frequency of the former taxa and a higher frequency of Dendrocoelidae (Figure 26).

Figure 25. Influence of the four deep water techniques tested on the macroinvertebrate fauna present in the samples as shown by the results of a partial CCA ordination where site was used as a covariable (i.e. these are relative differences when the differences among sites have been taken into account). Centroids for the four techniques (▲) and the scoring taxa (Δ) are shown. Increasing proximity between taxa and techniques indicates increasing association with that technique; taxa that are equidistant between two techniques are equally associated with both. Total sum of eigenvalues = 1.93, both first axis (F-ratio = 13.45, p ≤ 0.002) and all canonical axes (F-ratio = 5.80, p ≤ 0.0020) are significant.

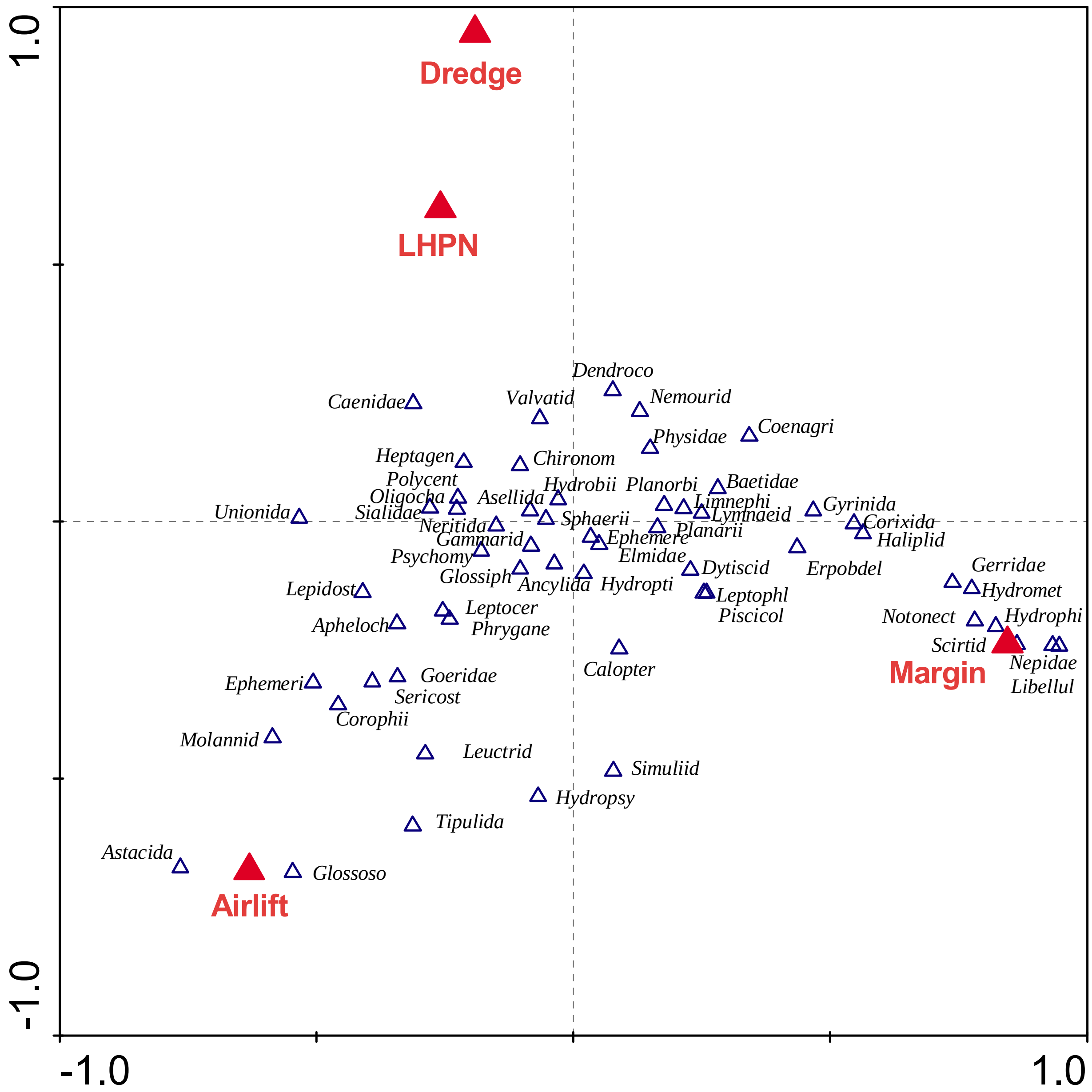
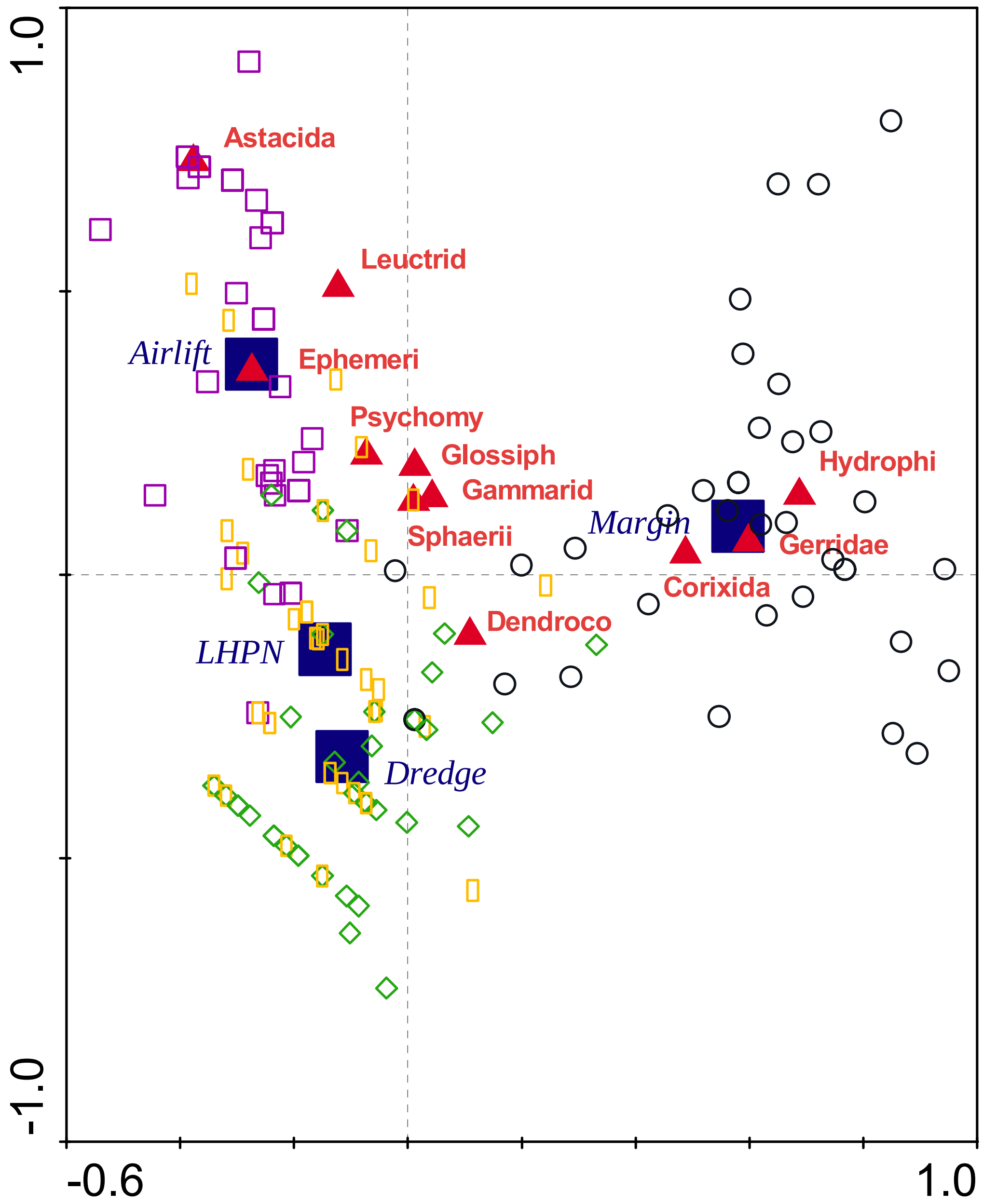


Figure 26. Results of a partial discriminant analysis used to show which taxa are significantly good ($p < 0.05$) at separating the samples collected by the four techniques after the influence of site has been removed (site used as a covariable). Centroids for the techniques (■), taxa (▲), and samples by technique (Airlift ▯, Dredge ◊, Margin ○, Long-Handled pond net ▯) are shown. Proximity of taxa centroids to technique centroids indicates how good the taxa are at identifying the samples collected with that technique; taxa that are equidistant between two technique centroids are equally good at separating both techniques. Separation of all four techniques was significant ($p < 0.05$) using the 11 taxa shown. Taxa selected using Monte Carlo permutations and forward selection (Bonferroni corrected). The 2 axes shown explain 43.6% of the variance between the techniques. Total sum of eigenvalues = 2.997.



3.9 Comparison with Standard RIVPACS Kick Sample

It is desirable for the samples collected with the recommended deep water technique to be comparable with those collected with the standard shallow water technique in order to provide a continuous transition between deep and shallow rivers. There are several advantages if this is the case.

1. It will not be necessary to develop independent models for deep rivers as they can be integrated into shallow water models.
2. Classification of a site as deep or shallow, in terms of the sampling technique to be used, will not influence the ecological status of the site.
3. Deep water reference sites can be classified along with shallow water reference sites, potentially reducing the number of deep water reference sites required.

At the two sites where conditions allowed (see Table 2), samples were collected with a standard RIVPACS kick sample, comprising a 3 minute kick and a 1 minute search where the water surface is sampled and rocks, plants, logs or other submerged objects are examined and all attached invertebrates removed and incorporated into the sample, from the same reach as the samples collected with the deep water techniques.

3.9.1 Sample processing

For a potential technique to be suitable for sampling invertebrates in deep rivers, as well as being easy to use, safe and practical in the field, it should be efficient in terms of the time it takes to process the samples produced. This will influence the time and cost involved in the assessment of the site. In order to compare this aspect of the deep water sampling techniques to the standard RIVPACS kick sample used in shallow rivers in the UK, a comparison was made of the time it took to sort the samples (influenced by the volume and composition of the sample matrix and the number of individuals) and the total time to process the samples (further influenced by the number of taxa and ease of identification).

The time it took to sort the samples collected with the airlift or from the margin was not significantly different to the time it took to sort a standard RIVPACS kick sample (Figure 27). The samples collected with the light dredge and long-handled pond net took significantly less time to sort than those collected with the three other techniques. There was a significant difference between the two sites in the time it took to sort the samples, but there was no interaction between technique and site.

Despite the mean total time to process the samples collected with the airlift being nearly 40 minutes longer than the mean time to process a standard RIVPACS kick sample, there was no statistically significant difference in the total time it took to process the samples collected with the airlift, from the margin or with a standard RIVPACS kick sample (Figure 28). The samples collected with the light dredge and long-handled pond net took significantly less time to process than those collected with the other techniques. Again there was a significant difference between the two sites in the time it took to process the samples, but no interaction between site and technique.

Figure 27. Comparison of the time taken to sort the samples collected by the four deep water techniques tested with samples collected by a standard RIVPACS kick sample. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference.

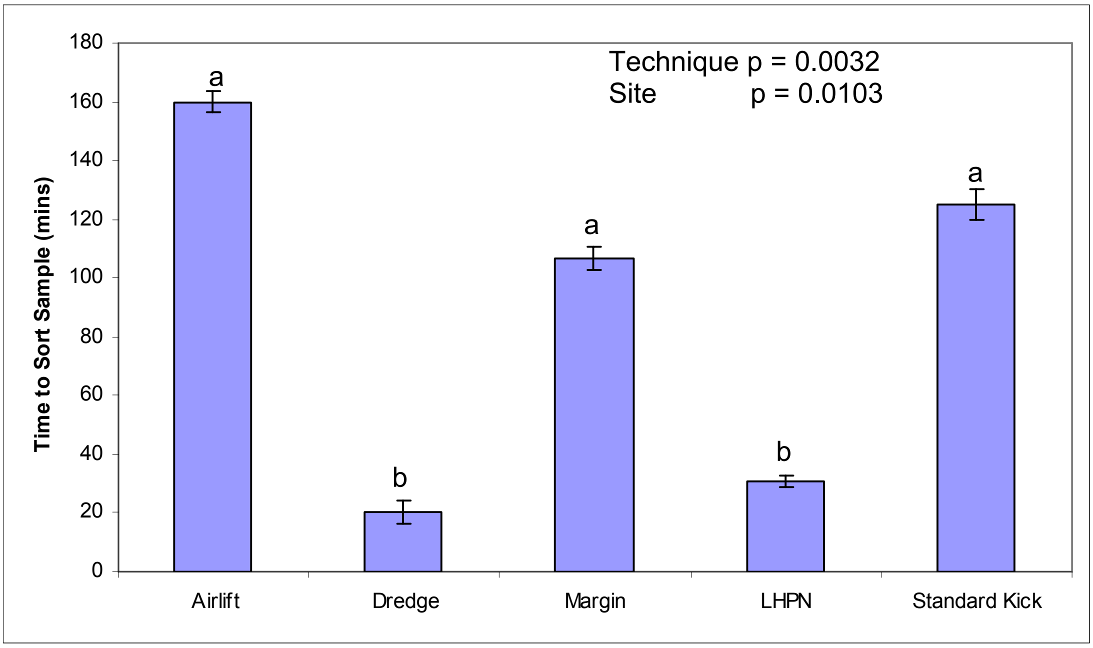
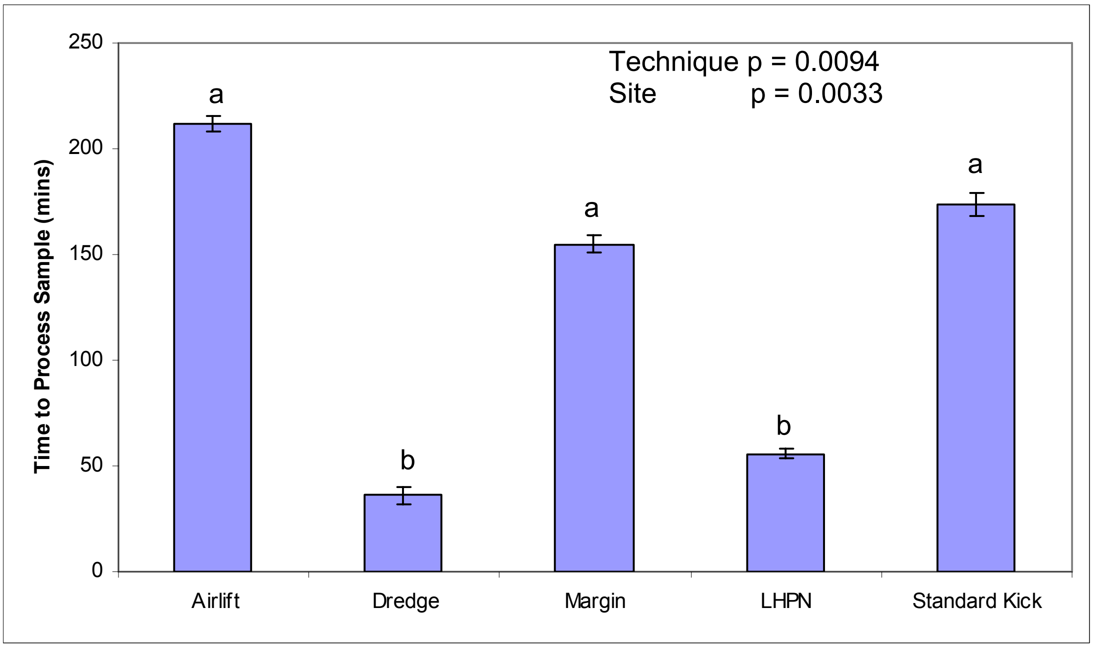


Figure 28. Comparison of the time taken to process the samples collected by the four deep water techniques tested with samples collected by a standard RIVPACS kick sample. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference.



3.9.2 Sample scores

For a deep water sampling technique to be comparable to standard shallow water techniques, the technique needs to provide an equivalent representative sample of the fauna, and to perform equivalently for key biotic indices.

Number of BMWP scoring taxa

The number of BMWP scoring taxa in the samples collected with the airlift and from the margin was not significantly different to that in the samples collected with a standard RIVPACS kick sample (Figure 29a). However, the samples collected with the light dredge and long-handled pond net contained significantly less scoring taxa than the standard RIVPACS kick sample. There was some statistical overlap between the samples collected with the long-handled pond net and those from the margin, possibly as consequence of the lower number of replicates collected from the margin (Figure 29b): due to low water levels, margin samples were only collected from one of the sites where it was possible to collect a standard RIVPACS kick sample.

Total BMWP score

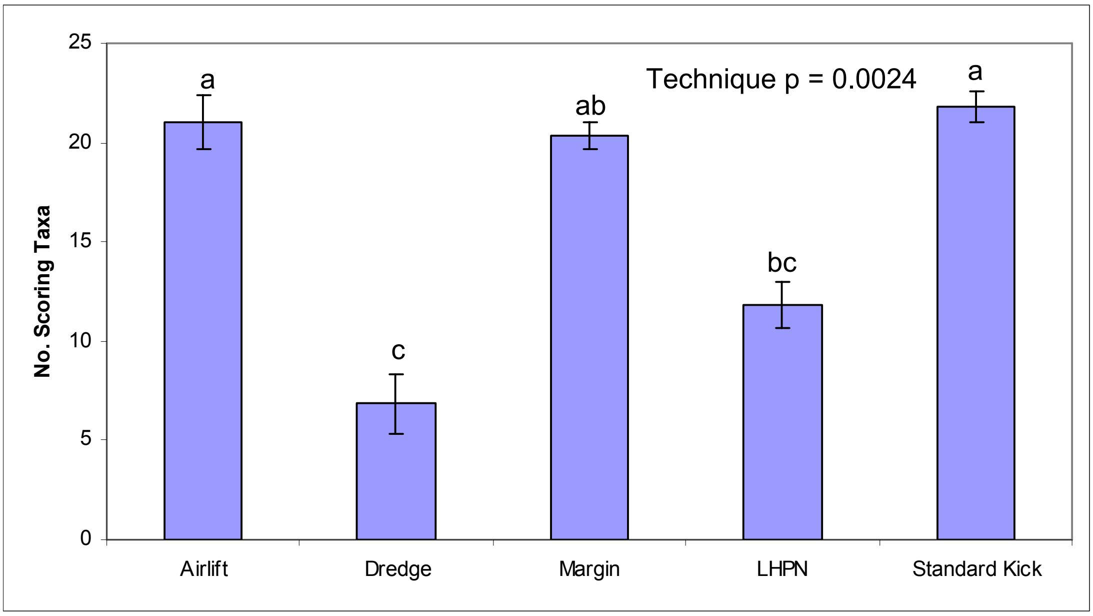
The total BMWP score of the samples collected with the airlift and from the margin was not significantly different to that of the samples collected with a standard RIVPACS kick (Figure 30a). The samples collected with the light dredge and long-handled pond net had a significantly lower total BMWP score than the standard RIVPACS kick sample. There was some statistical overlap between the samples collected with the long-handled pond net and those from the margin, again possibly as consequence of the lower number of replicates collected from the margin (Figure 30b).

APST

There was no significant difference in the ASPT of the samples collected with the deep water techniques from those collected with the standard RIVPACS kick sample (Figure 31), probably due to high variance relative to the mean values. The ASPT of the light dredge was particularly volatile as it was based on an average of very few taxa (mean 7 taxa, one sample contained only 3 taxa).

Figure 29. Comparison of the number of BMWP scoring taxa in the samples collected with the four deep water techniques tested with samples collected with a standard RIVPACS kick sample at the sites where it was possible to collect kick samples. a) the influence of technique, and b) the influence of technique by site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference, multiple letters indicate overlapping groupings of means. NB Due to low flow conditions, samples were only collected from the margin of one of the sites.

a)



b)

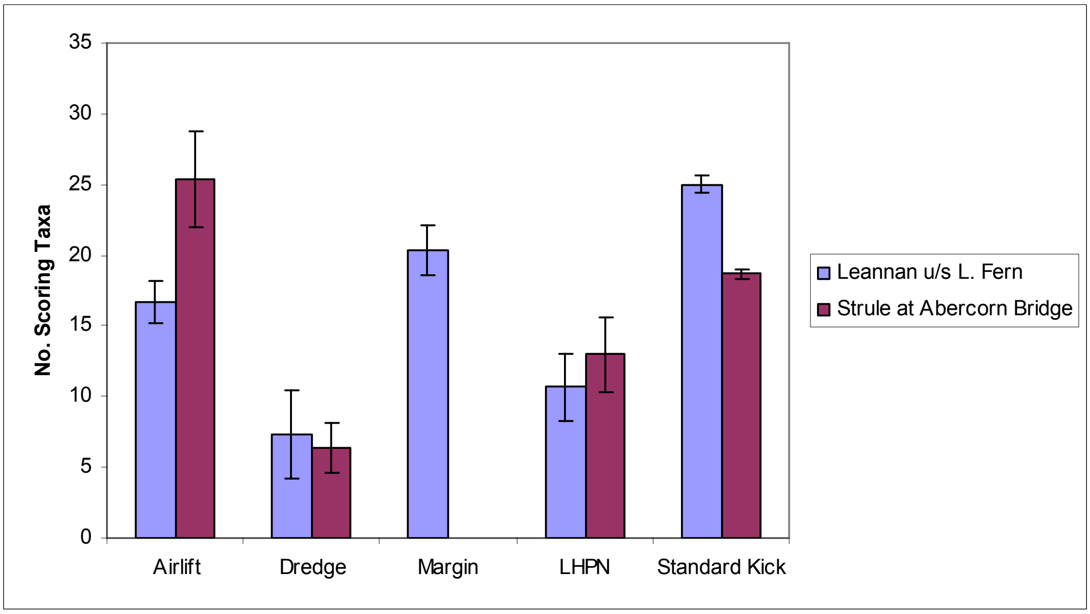
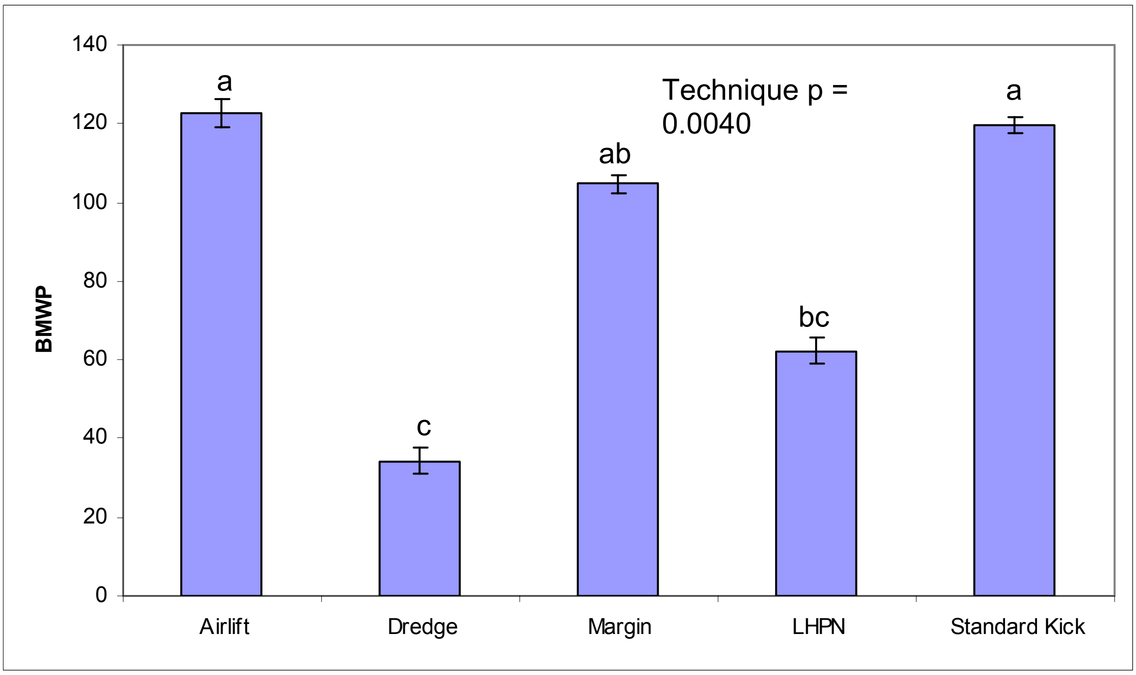


Figure 30. Comparison of the total BMWP score in the samples collected with the four deep water techniques tested with samples collected with a standard RIVPACS kick sample at the sites where it was possible to collect kick samples. a) the influence of technique, and b) the influence of technique by site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference, multiple letters indicate overlapping groupings of means. NB Due to low flow conditions, samples were only collected from the margin of one of the sites.

a)



b)

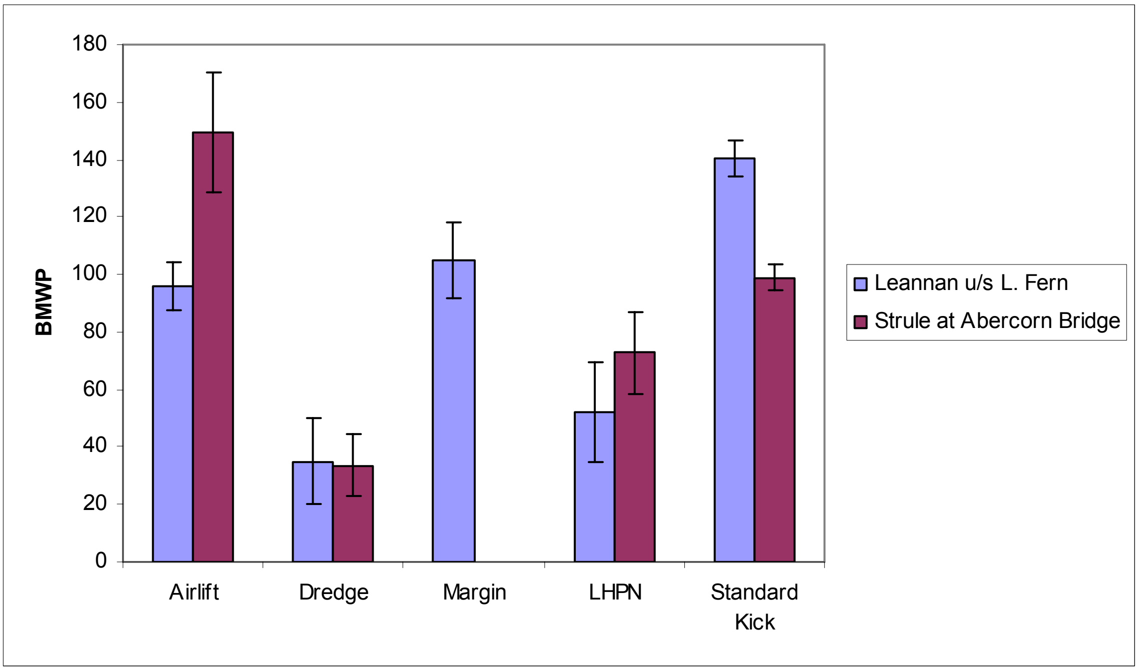
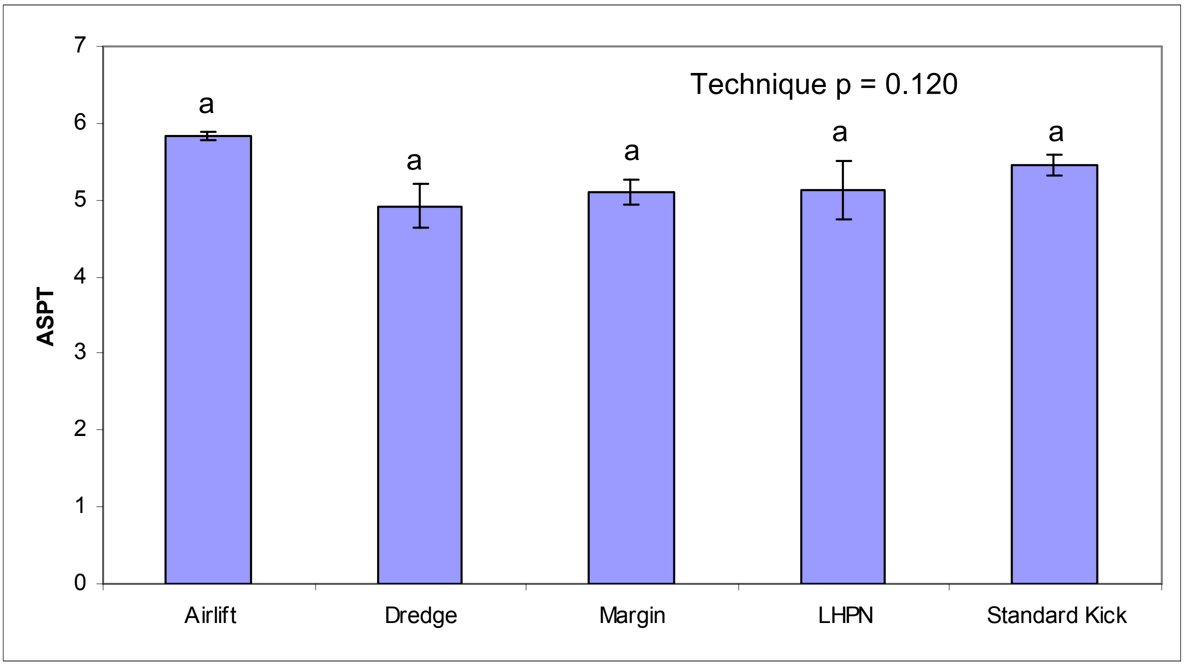
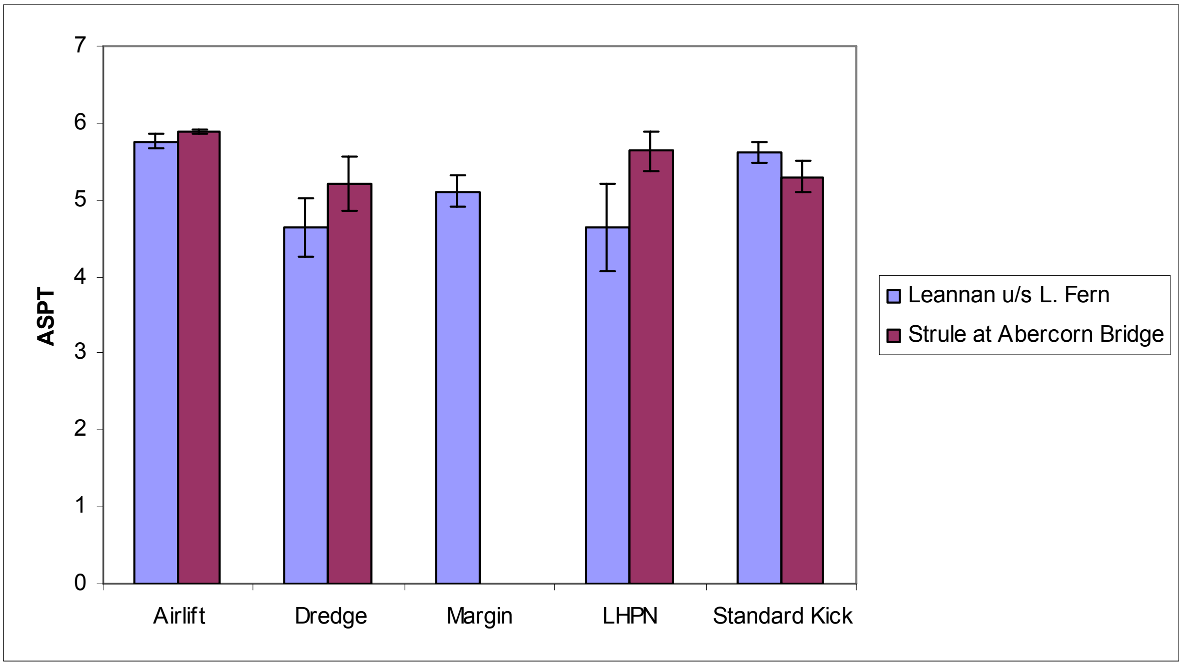


Figure 31. Comparison of the ASPT in the samples collected with the four deep water techniques tested with samples collected with a standard RIVPACS kick sample at the sites where it was possible to collect kick samples. a) the influence of technique, and b) the influence of technique by site. Mean values shown ±SE. Different letters indicate significant differences among mean values as identified by Tukey's test, shared letters indicate no significant difference. NB Due to low flow conditions, samples were only collected from the margin of one of the sites.



b)



3.9.3 Taxonomic composition

For a deep water sampling technique to be comparable to standard shallow water techniques, the technique needs to provide an equivalent representative sample of the fauna.

In terms of taxonomic composition, the samples collected with the airlift were most similar to those collected with the standard RIVPACS kick sample (Figure 32). The centroids for the airlift and the standard RIVPACS kick sample, were closer together than any other pair of centroids and closely associated with the majority of the macroinvertebrate taxa.

The samples from the margin were characterised by Hydrometridae, Corixidae, Gyrinidae and Scirtidae, and to a lesser extent by Haliplidae, Hydrophilidae and Gerridae, whereas the samples collected with the dredge and long-handled pond net were characterised by a lack of taxa, with the exception of Piscicolidae in the samples collected with the long-handled pond net.

Discriminant analysis showed a similar separation of the techniques, with the samples collected with the airlift and the standard RIVPACS kick sample being most similar (Figure 33). The samples collected from the margin were identified by the occurrence of Hydrometridae, the sample collected with the airlift, Lepidostomatidae, Polycentropodidae, Ancylidae, Psychomyiidae, and the samples collected with the light dredge and long-handled pond net were identified by a lack of taxa associated with the standard RIVPACS kick sample, namely Erpobdellidae, Lepidostomatidae, Polycentropodidae, Ancylidae and Psychomyiidae. Dytiscidae and Gerridae appeared to be associated with the separation of the samples collected with the standard RIVPACS kick, airlift and from the margin. It should be noted that a margin sample was collected from only one of the two sites, as the water level had dropped below the level of the marginal vegetation, leaving it dry. It was considered that a sample collected from the water margin would not be representative of the technique, or comparable to margin samples collected from other sites where the habitat available to the fauna had not suffered such disruption; any marginal samples collected at this site would be more akin to those collected with the long-handled pond net elsewhere.

3.9.4 Summary

The four techniques were ranked according to their similarity to a standard RIVPACS kick sample, thus enabling an average rank position to be established (Table 12). Ranks were shared where there was no statistically significant difference among the techniques. Ranks do not reflect the magnitude of the difference between the technique and a standard RIVPACS kick sample, merely the techniques' position relative to one another where a rank of 1 indicates most similar to a standard RIVPACS kick sample.

	Technique			
	Airlift	Dredge	Margin	LHPN
Sample processing	1	3	1	3
Metrics	1	3	1	3
Composition	1	3	4	2
Average rank	1	3	2	2.66

Table 12. Ranking of the field sampling techniques (airlift, dredge, margin and LHPN) with respect to their comparability to a standard RIVPACS kick sample in terms of sample processing time, metric scores (BMWP, No Taxa, ASPT), taxonomic composition and overall. Rank of 1 is the most comparable to a standard RIVPACS kick sample.

Figure 32. Influence of the four deep water techniques tested and standard RIVPACS kick sample on the macroinvertebrate fauna present in the samples as shown by the results of a partial CCA ordination where site was used as a covariable (i.e. these are relative differences when the differences among sites have been taken into account). Centriods for the five techniques (▲) and the scoring taxa (Δ) are shown. Increasing proximity between taxa and techniques indicates increasing association with that technique; taxa that are equidistant between two techniques are equally associated with both. Total sum of eigenvalues = 0.93, both first axis (F-ratio = 3.52, p ≤ 0.002) and all canonical axes (F-ratio = 2.44, p ≤ 0.0020) are significant.

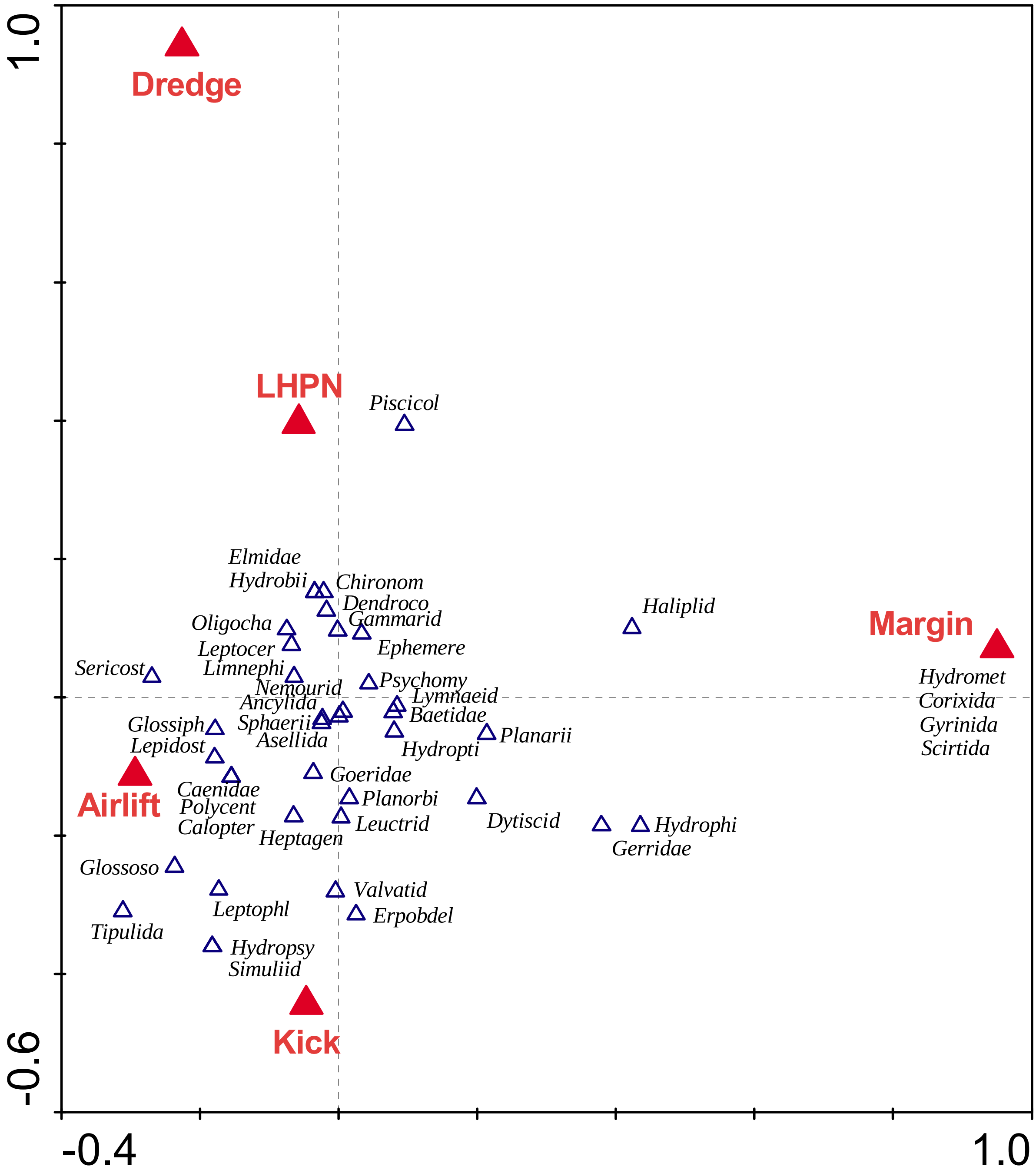
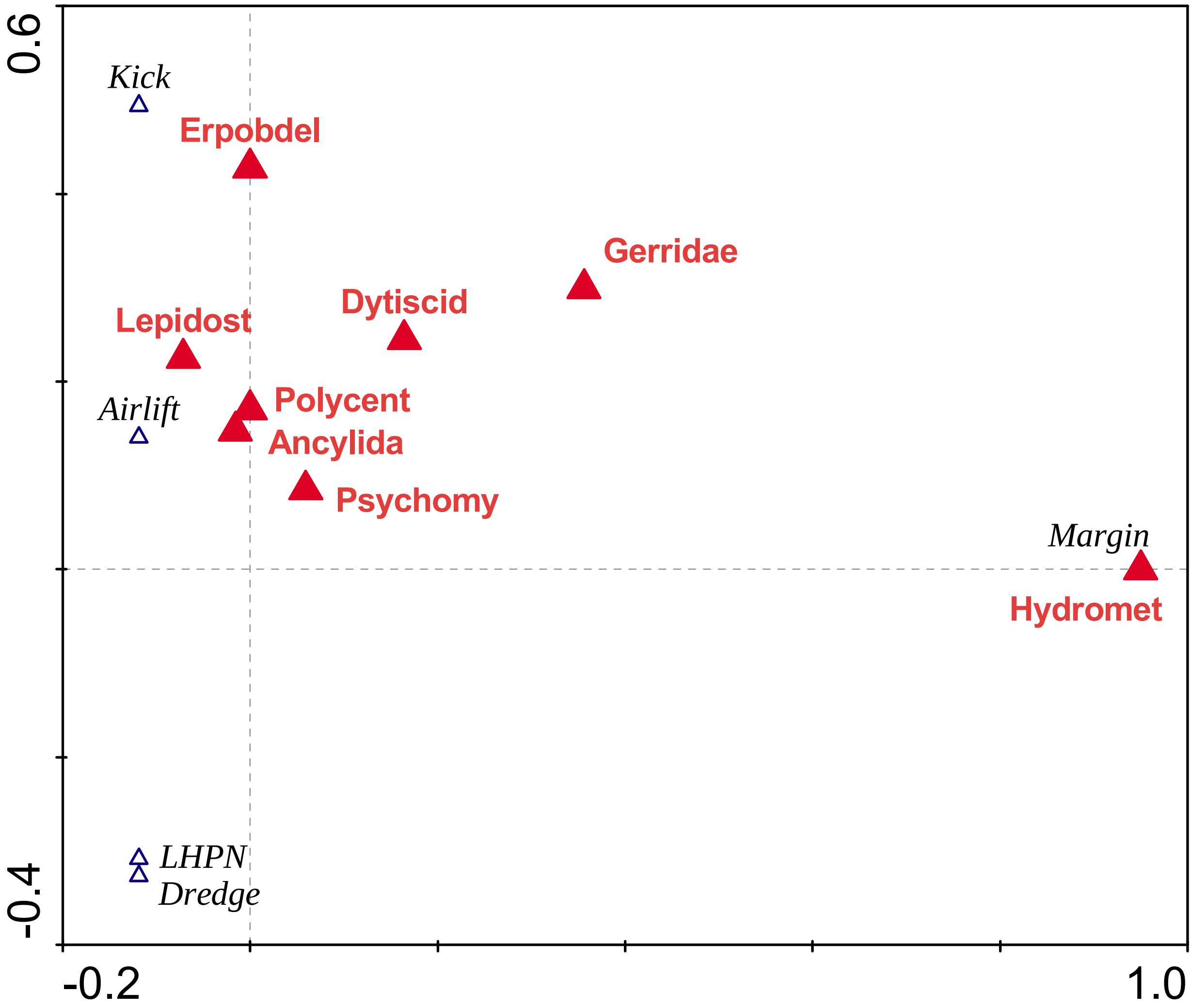


Figure 33. Results of a partial discriminant analysis used to show which taxa are significantly good ($p < 0.05$) at separating the samples collected by the four techniques and the standard RIVPACS kick sample after the influence of site has been removed (site used as a covariable). Centroids for the techniques (Δ) and taxa (▲) are shown. Proximity of taxa centroids to technique centroids indicates how good the taxa are at identifying the samples collected with that technique; taxa that are equidistant between two technique centroids are equally good at separating both techniques. Separation of the four techniques from the standard RIVPACS kick sample was significant ($p < 0.05$) using the 8 taxa shown. Taxa selected using Monte Carlo permutations and forward selection (Bonferroni corrected). The 2 axes shown explain 41.0% of the variance between the techniques. Total sum of eigenvalues = 3.900.



3.10 Comparison with historic data

In order to test the comparability of the deep water techniques tested to techniques currently used, the data from the field trial was compared to historic data collected by EHS at sites where this data was available. The techniques used and season that the samples were collected in were used as explanatory variables, together with the organisation that collected the data (EHS or CEH). As in all other analyses, site was used as a covariable to remove differences among sites, and reveal differences among techniques. Direct comparison of samples will be influenced by CEH restricting the area sampled by each technique to approximately 1.5 m^2, whereas a larger area was sampled by EHS (3 minute kick/sweep sample plus a 1 minute search where the water surface is sampled and rocks, plants, logs or other submerged objects are examined and all attached invertebrates removed and incorporated into the sample). As such, the number of taxa and BMWP will be larger in the samples collected by EHS also.

The results from the CCA ordination of the full dataset reveal a separation between samples collected by EHS and those collected by CEH consistent with a difference in season (Figure 34). A number of taxa occurred in samples collected in spring (e.g. Siphlonuridae) and autumn (e.g. Chloroperlidae) which were only collected by EHS: all the CEH samples were collected in the summer.

Samples collected from the margin alone and the margin combined with a kick sample or a long-handled pond net sample, were distinct from other samples. The centroid for the long-handled pond net was intermediate between the centroids for the kick sample and the sample collected from the margin. The dredge and airlift separated along a different axis associated with Goeridae, Physidae, Dendrocoelidae among others, probably influenced by CEH alone collecting samples using these techniques. Dreissenidae was fitted passively as this taxa may be a recent invasion to one of the sites (not recorded in the EHS data).

Analysis of the summer data alone (to remove seasonal effects) again indicated that the samples collected from the margin (or samples combined with samples collected from the margin) were distinct from those collected from the river channel, whether collected by EHS or CEH (Figure 35). The samples collected with the techniques employed solely by CEH seemed to segregate near to the kick samples collected by EHS. There was considerable overlap between samples collected by CEH and EHS.

To summarise, there was no systematic difference between the samples collected with techniques tested by CEH and the samples collected by EHS as part of their routine monitoring. The differences caused by EHS employing different techniques on different occasions (a practical response to variable conditions at some sites) resulted in as much variation as the difference between EHS and CEH.

Figure 34. Relationship between the macroinvertebrate fauna present in the samples collected with the four deep water techniques tested by CEH and samples collected by EHS using kick samples, a long-handled pond net, a marginal sweep or a combination of these techniques as shown by the results of a partial CCA ordination where site was used as a covariable (i.e. these are relative differences when the differences among sites have been taken into account). Centroids for the techniques, organisation either CEH or EHS, and season that the samples were collected (▲) and the scoring taxa (Δ) are shown. Increasing proximity between taxa and techniques indicates increasing association with that technique; taxa that are equidistant between two techniques are equally associated with both. NB Dreissenidae were fitted passively as this taxa may be a recent introduction to some sites. Total sum of eigenvalues = 1.30, both first axis and all canonical axes are significant (p ≤ 0.0020).

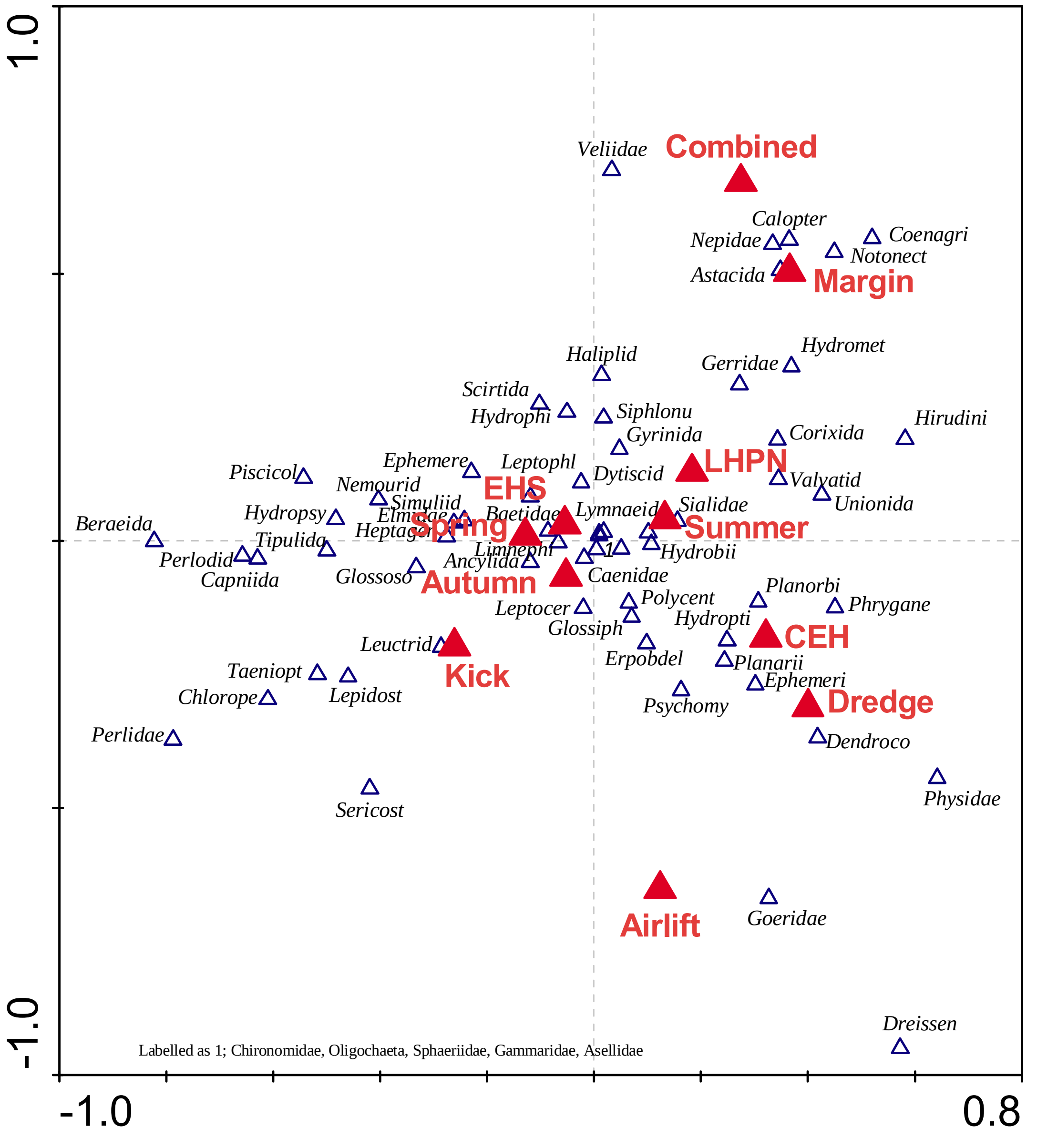
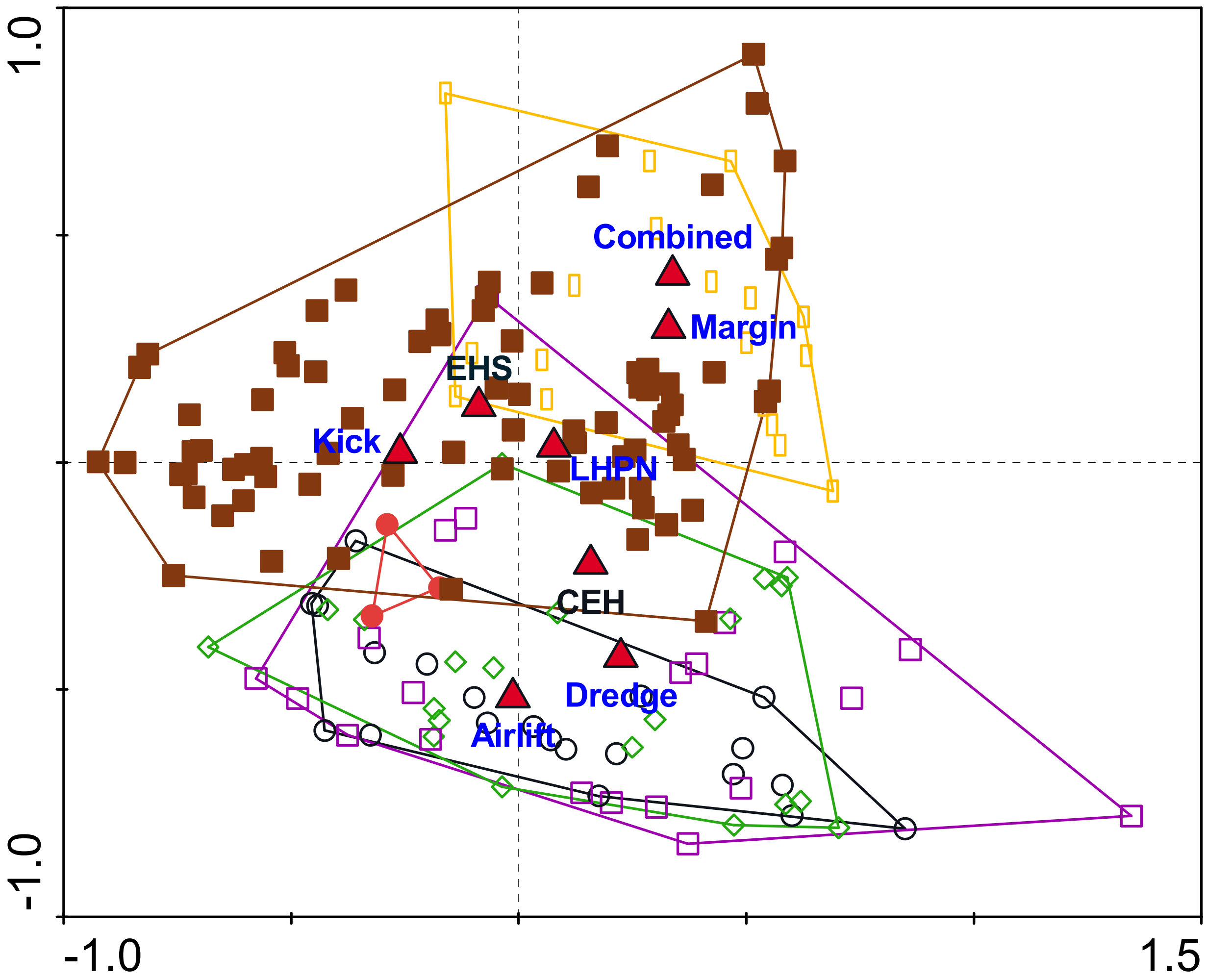


Figure 35. Relationship between the macroinvertebrate fauna present in the samples collected with the four deep water techniques tested by CEH and samples collected in the summer by EHS using kick samples, a long-handled pond net, a marginal sweep or a combination of these techniques as shown by the results of a partial CCA ordination where site was used as a covariable (i.e. these are relative differences when the differences among sites have been taken into account). Centroids for the techniques, and the organisation either CEH or EHS (▲) are shown, as are samples scores. Samples scores are colour coded, EHS all techniques (■), CEH LHPN (□), CEH airlift (○), CEH dredge (◊), CEH margin (▯) and CEH kick (●) and envelopes drawn around the classes. Taxa are not shown for clarity. Total sum of eigenvalues = 1.45, both first axis and all canonical axes are significant ($p \leq 0.0020$).



4 DISCUSSION

The choice of a standard methodology for the sampling of deep rivers must take into account how efficient the technique is in terms of the time and effort required to collect and process the samples, how effective the technique is at providing an adequate sample of the macroinvertebrate fauna present, how precise the technique is in terms of the uncertainty associated with each sample, how comparable the technique is to existing methodologies for sampling shallow rivers, and how suitable the technique is in terms of health and safety. In this report we discuss all these aspects of the methodologies tested before synthesising these results to produce our recommendation for the technique that should be adopted as standard for sampling macroinvertebrates in deep rivers.

4.1 How Efficient are the Techniques in Terms of the Time and Effort Required to Collect and Process the Samples?

From the survey of the workers' opinion it is clear that all the techniques are equally difficult to use in the field, and the only factor that discriminated between the techniques was the requirement of a boat to collect an effective sample. The airlift always requires a boat to collect a sample and a boat is required to collect a sample from the margin of narrow, deep rivers as they tend to be engineered or deeply incised and have steep banks. The use of a boat has clear implications for the time and manpower required to collect a sample, together with health and safety implications.

There were differences among the techniques in the amount of time it took to sort and process the samples, with the airlift, which often produced large samples, taking longer to sort and hence process than the other three techniques.

Overall it appears that the light dredge and long-handled pond net are the most efficient techniques in terms of the time and effort required to collect and process the samples, and the airlift the least efficient.

4.2 How Effective are the Techniques at Providing an Adequate Sample of the Macroinvertebrate Fauna Present?

In order to perform a bioassessment, the macroinvertebrate community is reduced to and summarised through one or more metrics, whose value can be compared to that expected if the site was of reference quality. It is crucial that the technique recommended for sampling macroinvertebrates from deep rivers provides a sample that performs well in terms of the metrics used. Here we used the number of BMWP scoring taxa, the total BMWP score and the ASPT to assess differences among the techniques.

The light dredge and long-handled pond net performed poorly relative to the airlift, in terms of all the metrics used to assess the macroinvertebrate community, particularly when the substrate comprised a high proportion of boulders. The light dredge and long-handled pond net also performed poorly compared to the margin samples in terms of total BMWP score and number of BMWP scoring taxa. The margin samples had the highest number of BMWP scoring taxa, and equal highest total BMWP score with the airlift. Importantly, the airlift had a significantly higher ASPT than all the other techniques, indicating that this technique sampled the more sensitive, high scoring taxa more effectively. It is important that the sensitive taxa are sampled, as these taxa will show most rapid response to changes in water quality.

It is clear that the samples collected at the margin sampled different components of the fauna to those collected from the river channel. There was a marked difference in taxonomic composition between the samples collected at the margin and those collected from the river channel, found in both the current field trial and in the comparison with historic EHS data. Furthermore, there was little correlation between the metric scores of the samples collected from the margin and those collected from the river channel. The fauna of the margins seems to be responding to different pressures to the fauna of the river channel. Perhaps this was because the fauna that characterised the samples collected from the margin tend to live at the air-water interface or be associated with vegetation and may be less sensitive to instream influences and be determined by habitat structure at the margin. The difference between the river channel and the margin increased with increasing width of the river channel, in terms of ASPT of the airlift and the margin samples, indicating an increasing divergence in the community sampled. It is possible that in narrow rivers representatives of the mid-channel fauna are present in the margins and caught by both techniques, whereas in wider rivers there is more spatial segregation between the margins and channel, together with the occurrence of sensitive, deep river taxa in the channel.

It is not necessary that the “highest score” should determine which technique is recommended provided that a model is developed using a standard technique that samples a subset of the fauna that is sensitive to the pressure of interest. However, it should be noted that the sample from the margin sampled different components of the community, which is less sensitive (in terms of ASPT) and responding to different drivers to the samples from the river channel.

The dredge and long-handled pond net appeared to sample a subset of the taxa collected by the airlift, being characterised by a lack of (obligate) deep water taxa. Neither of these techniques are effective at providing an adequate sample of the macroinvertebrate fauna present. The airlift provided the most adequate sample of the river channel fauna.

4.3 How Precise are the Techniques in Terms of the Uncertainty Associated with Each Sample?

For a sampling technique to be effective, sampling variability within a site and time period needs to be small relative to the real differences between sites in their biota and in their values for key biotic indices. In other words, samples should be repeatable within a site and discriminate between sites as far as possible. This has implications for detection of change and hence the number of samples that must be taken to achieve an adequate confidence of change, with consequences for the time and effort required to make a sufficiently precise assessment.

The airlift was the most precise of the techniques tested. More than 75% of the total variance was due to differences among sites for all three key metrics tested; implying that less than 25% was due to within site sampling variation. Furthermore, there was little/no effect of operator (0-4% of total variance).

Within site variance was so great among the samples collected with the light dredge that little, if any, of the variance could be attributed to differences among sites (0% BMWP, 23% Taxa, 5% ASPT). Operator effects accounted for a substantial and significant proportion of the variance for BMWP (64%) and number of scoring taxa (42%). The light dredge has such high sampling variance and low repeatability that it is effectively useless for assessing and discriminating the ecological status of sites and need not be considered further.

For both the samples collected at the margin and those collected with the long-handled pond net, between 40% and 81% of the total variance in the key metrics tested across the study sites was attributable to differences between sites; implying that between 19% and 60% was due to within site sampling variation.

In terms of precision, the airlift outperformed all the other techniques.

Sampling precision has implications for confidence of class. The technique with the lowest within site sampling variation will have the greatest confidence of class.

4.4 Which Technique has the Most Cost Effective Precision?

For a technique to be suitable for use in deep rivers it should be cost effective. The airlift has been shown to have the lowest ‘percentage within-site sampling variance’ and thus the highest statistical precision and repeatability of results amongst the four techniques assessed. However, a single airlift sample has been shown to take a longer time to sort and process and, therefore, costs more per sample. It is possible to combine these two aspects, namely sampling precision and sample processing time costs, to estimate the relative cost effectiveness of each technique.

Based on these calculations for each technique and index, it is evident that the increased costs in processing each airlift sample are outweighed by increased precision. To achieve a sampling variance of less than 20% across all three metrics tested (BMWP, number of taxa, ASPT) will take an estimated average of 534 minutes for the airlift, compared to 735 minutes for the margin sample and 714 minutes for the long-handled pond net. The airlift has the second most cost efficient precision for each of the individual metrics.

Obviously these comparisons still ignore any differences in costs associated with collecting the samples in the field. They also ignore any differences in the metric values achieved with the different techniques; it has been shown previously that for the airlift taxon accretion curves flattened out after fewer replicates than for the long-handled pond net and at higher number of taxa (Bass *et al.* 2000).

4.5 How Comparable are the Techniques to Existing Methodologies for Sampling Shallow Rivers?

For a technique to be suitable for use in deep rivers it should ideally be comparable to the methodology used for shallow rivers. There are several benefits in having this comparability.

1. It will not be necessary to develop independent models for deep rivers as they can be integrated into shallow water models.
2. A necessarily subjective, stepped boundary between deep and shallow rivers is avoided, such that the classification of a site as deep or shallow, in terms of the sampling technique to be used, will not influence the ecological status of the site.
3. Deep water reference sites can be classified along with shallow water reference sites, potentially reducing the number of deep water reference sites required for a RIVPACS-type module.

Two comparisons were made between the techniques tested here for sampling deep rivers and the techniques currently in place. The first was a direct comparison between a “standard kick sample” incorporated into the sampling strategy where conditions allowed, and the second was a comparison with historic data collected by EHS.

In terms of the time to process the samples, faunal composition and key metrics the samples collected with the airlift were the most similar to the standard RIVPACS kick sample. The light dredge and long-handled pond net collected a similar fauna to the kick and airlift, but less effectively, missing parts of the fauna and producing smaller, and lower scoring samples. The samples from the margin sampled a different fauna to the kick and airlift.

The samples collected by CEH for this field study compared well with those collected by EHS. Most of the differences were the result of CEH only sampling in one season, and possibly by sampling in a smaller area than would be standard practice for EHS. EHS also combined marginal and kick/long-handled pond net samples. Nevertheless, there is segregation of samples collected from the margin (either on their own or in combination with kick/long-handled pond net samples) and samples collected within the river channel, irrespective of who collected them, and considerable overlap between the samples collected by CEH and EHS.

There was no clear segregation of any technique tested by CEH from those used by EHS, so it is not possible to use this criterion to identify a technique that should be excluded from consideration. It was evident, however, that samples collected from the margin segregated from other samples irrespective of whether they had been collected by CEH or EHS.

4.6 How Suitable are the Techniques in Terms of Health and Safety?

There are obvious implications with respect to health and safety of the collection of any sample from deep water, but particularly with the use of the airlift or collecting a sample from the margin with the aid of a boat. We are not qualified or tasked to assess these in detail, but we are aware that they potentially include one or more of the following risks (applicable techniques given in brackets);

- Steep banks (margin, light dredge, long-handled pond net).
- Over-stretching (margin, long-handled pond net).
- Carrying a boat to and from the site (airlift, margin from boat)
- Gaining access to the water with a boat (airlift, margin from boat).
- The use of a boat (airlift, margin from boat).
- The use of equipment from a boat (airlift, margin from boat).
- Carrying heavy equipment (airlift).
- Use of compressed air (airlift).
- Throwing heavy objects (dredge).

These risks and others will have to be taken into consideration in implementing the recommendations of this report.

5. RECOMMENDATIONS

5.1 Choice of Deep Water Sampling Methods

- **The airlift is recommended for the routine monitoring of benthic macroinvertebrates at sites with extensive deep water habitats**

The airlift produces samples that are more precise, more representative and have higher metric values than the other techniques. Whilst obtaining the highest metric value is not necessarily a criterion for selection, it indicates that the technique is robust and more sensitive (in terms of high-scoring taxa and thus ASPT) than the other techniques tested. The airlift is less affected by environmental conditions than the other techniques tested and performs well when coarse sediments are present and in wide rivers. Deep water taxa are more effectively sampled than with the other techniques. The samples collected with the airlift are more similar to a standard RIVPACS kick sample than those collected with the other techniques. Whilst more effort is required to process each sample collected with the airlift, less samples are required to achieve the same precision and same confidence of site quality. The airlift is the most (or marginally second to the long-handled pond net) cost-effective in terms of sample processing time to achieve adequate precision and confidence of class and, hence, the ability to detect change of status. In a previous study (Bass *et al*. 2000) the airlift had a higher taxon accretion rate (a reflection of the number of samples required to adequately describe the macroinvertebrate community) than other techniques tested; in particular combining samples collected with the long-handled pond net could not achieve the same level of taxonomic richness as was possible with the airlift samples.

The light dredge has no power to detect differences among sites, implying that it also cannot detect change within sites. Thus, it is effectively useless for this purpose. On this fact alone we recommend that the light dredge is not considered for routine monitoring. The poor performance of the light dredge does not reflect on other dredges, but use of heavier dredges has been discounted on health and safety grounds. It appears that the light dredge is unable to penetrate the river sediment effectively.

The samples collected from the margin contained a large but partly different, less sensitive (i.e. lower average BMWP score per taxon), component of the fauna than those collected by the airlift. The component of the fauna found in the margin did not respond to differences among sites in the same way as that found in the river channel, and appeared to be sensitive to different pressures. The samples collected from the margin were least representative of the site as a whole in wider rivers.

The long-handled pond net was considerably more variable, less representative and lower scoring than the airlift, often being close to the measures obtained with the light dredge. The long-handled pond net did not sample obligate deepwater taxa effectively, and did not perform well when the substrate was coarse. In a previous study, it was shown that the long-handled pond net required more samples than the airlift to achieve an adequate description of the taxa present at a site and, as was shown here, did not recover as many taxa as the airlift (Bass *et al*. 2000). The long-handled pond net was not comparable to a standard RIVPACS kick sample.

5.2 Sampling Logistics

- **To permit the effective assessment of river quality at deep water sites, sampling activity should target deep water habitats and margin habitats**

The contrasts in fauna were greatest between the samples collected in the river channel (airlift, dredge and long-handled pond net) and those collected from the margins. To provide a more complete sample of the community in a river, consideration should be given to combining samples collected with the airlift with those collected from the margin, in the same way as a 3 minute kick sample is combined with a 1 minute search/sweep in a standard RIVPACS kick sample.

It is recommended that macroinvertebrate samples from deep waters are collected from a boat. This is the only practical way to collect representative samples with an airlift, and provides access to marginal areas that are otherwise difficult to access. It is recognised that use of an airlift and a boat has associated manpower, and health and safety issues. However, any sampling of deep waters will involve increased risks compared to shallow waters, and some of these may be avoided by the use of a boat. A boat is required to collect a sample from the margin of narrow, deep rivers as they tend to be engineered or deeply incised and have steep banks. Given the implications in terms of manpower, a compromise may be possible through less frequent sampling of deep water sites (e.g. alternate years).

5.3 Recommendations for Future Development of Tools for Macroinvertebrate Monitoring at Deep Water Sites

- **Deep water habitats should be integrated into existing shallow water models**

In terms of the time to process the samples, faunal composition and key metrics the samples collected with the airlift were the most similar to the standard RIVPACS kick sample. The light dredge and long-handled pond net collected a similar fauna to the kick and airlift, but less effectively, missing parts of the fauna and producing smaller, and lower scoring samples. The samples from the margin sampled a different fauna to the kick and airlift.

The use of the airlift to sample deep water sites may enable deep waters to be classified with shallow waters. This has implications in that it will not be necessary to develop new independent models to assess deep waters as they can be integrated into shallow water models. It should be possible to assess deep water sites with the Q system with little modification. In RIVPACS, addition of some deep water reference sites and reclassification of reference sites into modified TWINSPAN site groups will be necessary, but the integration of shallow and deep sites will reduce the number of deep water reference sites needed to achieve the same level of group discrimination and precision of O/E in a RIVPACS-type module.

GLOSSARY

The following terms are used within this report:

ASPT	Average Score Per Taxon (for a sample).
BAMS	Biological Assessment Methods
BMWP	Biological Monitoring Working Party (defined taxa and scores).
BMWP Score	BMWP total score for a sample.
CEH	Centre for Ecology and Hydrology
EA	Environment Agency (England and Wales)
EPA	Environmental Protection Agency (Ireland)
EHS	Environment and Heritage Service (Northern Ireland)
IFE	Institute of Freshwater Ecology (now CEH)
IRTU	Industrial Research and Technology Unit (now EHS)
Ntaxa	Number of BMWP scoring taxa present.
Q-Value	EPA Quality Rating System (defined taxa and scores).
RIVPACS	River Invertebrate Prediction and Classification System.
SEPA	Scottish Environment Protection Agency

REFERENCES

Bass, J. A. B., Wright, J. F., Clarke, R. T., Gunn, R. J. M. & Davy-Bowker, J. (2000) Assessment of sampling methods for macroinvertebrates (RIVPACS) in deep watercourses. Environment Agency R&D Technical Report E134, 57pp.

Clarke, R. T., Furse, M. T., Gunn, R. J. M., Winder, J. M. & Wright J. F. (2002) Sampling variation in macroinvertebrate data and implications for river quality indices. *Freshwater Biology*, **47**, 1735-1751

Dines, R. A. & Murray-Bligh, J. A. D. (2000) Quality Assurance and RIVPACS. In: Wright, J. F., Sutcliffe, D. W. and Furse, M. T. (Eds), Assessing the biological quality of freshwaters; RIVPACS and other techniques. Freshwater Biological Association, Ambleside, 71-78.

Elliott, J.M., Tullett, P.A. & Elliot, J.A. (1993) A new bibliography of samplers for freshwater benthic invertebrates. FBA Occasional Puiblication No 30, 92pp.

Jones, J. I., Bass, J. A. B. & Davy-Bowker, J. (2005) Review of methods for sampling invertebrates in deep rivers. INTERREG, 46pp.

McGarrigle, M. L., Lucey, J. & Clabby, K. J. (1992). Biological assessment of river water quality in Ireland. In: Newman, P.J., Piavaux, M.A. and Sweeting, R.A.(Eds) *River Water Quality – Ecological Assessment and Control.* 371-393, Commission of the European Communities, EUR 14606 EN-FR, Luxembourg, 751pp.

Murray-Bligh, J. A. D., Furse, M. T., Jones, F. H., Gunn, R. J. M., Dines, R. A. & Wright, J. F. (1997). *Procedure for collecting and analysing macroinvertebrate samples for RIVPACS.* Institute of Freshwater Ecology & Environment Agency, 155pp.

National Biology Technical Group (2000) BTG Working Document 38, BT001, October 2000.

Rayson, M. (2000) Physiological Assessment of Deep Water Sampling Techniques by Biologists in the Environment Agency. Report to the Environment Agency by Optimal Performance Ltd, 19 pp.

Wright, J. F., Clarke, R. T., Gunn, R. J. M, Blackburn, J. H. & Davy-Bowker, J. (1999) Testing and further development of RIVPACS – Phase 3. Development of new RIVPACS methodologies . Stage 1. Environment Agency, 138 pp.

APPENDIX 1

Site Photographs

River Blackwater upstream of Blackwater Town

River Blackwater at Moy

Clogh River at Glarryford

River Erne at Rosscor Bridge

River Finn at Wattle Bridge

River Leannan upstream of Lough Fern

River Main at Dundermot

River Moy at Arran Bridge

River Owencarrow at New Bridge

River Shannon at Hartley Bridge

Sillees River at Carr Bridge

River Strule at Abercorn Bridge

APPENDIX 2

Table of taxa occurrence in the replicate samples collected by the different methods. Numbers represent how many of the replicates collected with each method contained that taxon.

	Blackwater Moy				Blackwatertown				Clogh				Erne				Finn			
	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin
Planariidae													3	2	3	2		1	1	3
Dendrocoelidae													1	2		1		1	1	2
Neritidae																				
Valvatidae													2	2	3	1	3	1	1	
Hydrobiidae	3	1	2	3	3	3	3	3	2			3	3	3	3	3	3	3	3	3
Physidae	3	2	2	3		2	1	3					3	2	3	3			1	3
Lymnaeidae				2				2				3	2	2	3	3		1	1	3
Planorbidae				2	1							3	3	2	3	3	1	1	2	3
Ancylidae	1	1	2	3	3								2		1		1			2
Unionidae	3		1		2	2	1										1	2	2	
Sphaeriidae	3	1	1	1	3		1		3		3	3					1	3	3	3
Oligochaeta	3	2	3	2	3	3	3	2	3	2	3	3	3	3	2	2	3	2	3	1
Piscicolidae																				
Glossiphoniidae	1		1						3			1	2	1	1	3	3	3	2	3
Erpobdellidae														1	2	3				3
Astacidae	2				2															
Asellidae	1	2	1	3	2		1		3		2	3	3	3	3	3	3	3	3	3
Corophiidae																				
Gammaridae	3	1	2	3	2		1	3	3	1	1	2	3	2	3	3	2	3	3	3
Baetidae				3				2								3				
Heptageniidae		1	1																	
Leptophlebiidae												1								
Ephemeridae													3	1	1					
Ephemerellidae									2	2	2	3	1							
Caenidae													1	1	1			1	1	
Nemouridae						1														
Leuctridae													1							
Coenagriidae		3		3														1		3
Calopterygidae								1												
Libellulidae																				
Hydrometridae								1												2
Gerridae				3		1		3		1		3				2				2
Nepidae																				1
Aphelocheiridae																				
Notonectidae				2				3											1	3
Corixidae		1		3		1	1	3				1			2	3				1
Haliplidae				1			1	2				3				3				1
Dytiscidae				3				3	2	1	1	3	2	1	3					3
Gyrinidae								2		1		1				2		1		1
Hydrophilidae				3				2				3				2				3
Scirtidae																				
Elmidae	1			2	2	1		3	1	1	1	3	1							
Sialidae	1								3		2	2					2	2	3	
Glossosomatidae																				
Hydroptilidae					1					1		2		1		2				
Psychomyiidae				2									2		1					
Polycentropodidae				1	1	1			3	1		2	3	3	3	2	3	3	3	3
Hydropsychidae																				
Phryganeidae																	1		1	
Lepidostomatidae													3	1	1					
Limnephilidae									2		1	3		1	1				1	2
Goeridae													2							
Sericostomatidae													3	1	1					
Molannidae																				
Leptoceridae									3		2	3	1					2	1	2
Tipulidae									1											
Simuliidae									1											
Chironomidae	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

c

	Garavogue				Leannan					Main				Moy				Owencarrow			
	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin	Kick	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin
Planariidae			1	2				1								2	2				
Dendrocoelidae																	1				
Neritidae	2	1	2											3	2	1	2				
Valvatidae	3	1	3	1										1	3	3	3				
Hydrobiidae	3	2	3	2	3	3	3	3		1			1	3	3	3	3	1			1
Physidae	2	1		1					3						3	3	3				
Lymnaeidae	3	1	3	1		1	2	3					3	3	3	3	3	1	1		3
Planorbidae	3	2	3	2					3				2	1	1	2	3				
Ancylidae		1	1				2	1				1	3	1	1						
Unionidae	1								3												
Sphaeriidae	3		3	2	3	1	2	3		3		3	2	3	1	3	3	1	1	1	2
Oligochaeta	3	2	3	2	3	2	3	2	3	3			1	3	3	3	3	3	3	2	3
Piscicolidae									3	1			2								
Glossiphoniidae	3		2	2	3	1	1			3			1		2	2	3				1
Erpobdellidae									3								3				
Astacidae																					
Asellidae	2	1	1		3	1	1	2		3	2		2	2	3	2	3				
Corophiidae									2												
Gammaridae	3	1	3	3	3	2	1	3		3	1	1	3	3	1	1	3				1
Baetidae	1	2	3	1	1	1	1	3	3				1		2		1		2	2	3
Heptageniidae		1	2	1	1				3					1							1
Leptophlebiidae									2				1								
Ephemeridae	3		2						1					3							
Ephemerellidae			1		2	1	1	3		3	3	1	3	1			1		1	2	3
Caenidae	2	2	3						3					1							
Nemouridae			3		1		1	1												1	3
Leuctridae					1			1	1	3				3				3			2
Coenagriidae									3						1	2	1	1		2	3
Calopterygidae																1					1
Libellulidae																					3
Hydrometridae								3													
Gerridae								3			1		2				2		1		2
Nepidae									3												1
Aphelocheiridae														3	2	2	1				
Notonectidae																					
Corixidae			2	3				2					1				3			1	3
Haliplidae		1	1	1	1		1	3					2		3	1	2				1
Dytiscidae	1			3				3		2	1	2	3	2	2	2	3	1		1	3
Gyrinidae	1	1		2				2	3			1	1				1			1	1
Hydrophilidae		1		2				3					3								3
Scirtidae								1	2												
Elmidae			3	1	3	3	3	3		3	3	2	3	3	2	3	3	1			1
Sialidae	1								3	3	1	1	1		1			1			1
Glossosomatidae					1																
Hydroptilidae				1			1	2	2	1		1	1	2			1				
Psychomyiidae			3		2		1	2	3												
Polycentropodidae		1							1	3	2	2	1	1	1						
Hydropsychidae													1	1							
Phryganeidae	3								3								1				
Lepidostomatidae		1	2		3	1		1						1							
Limnephilidae					3	1	1	2	3				1		1	1				1	3
Goeridae	2		3	1	1		1	1	2												
Sericostomatidae	3		2	1	3	1	1		1	2			1					1			1
Molannidae	3		1						2												
Leptoceridae	3	1	3		3	1	2	2		3	1		2	3		1		1			
Tipulidae					2				2	3											
Simuliidae									3					2			3				
Chironomidae	3	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

	Shannon				Sillees				Strule			
	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Margin	Airlift	Dredge	LHPN	Kick
Planariidae						1		2	2			
Dendrocoelidae						1			1	1		1
Neritidae												
Valvatidae								2	1			1
Hydrobiidae	3	2	2	3	3	1	1	1	3	2	3	3
Physidae												
Lymnaeidae				1			1	2	3			1
Planorbidae					1	3	1	3	2		1	2
Ancylidae									3	1	2	3
Unionidae							1					
Sphaeriidae	3	3	3	3	1	2	3	2	3			1
Oligochaeta	3	3	3	2	2	1	2	3	3	2	1	3
Piscicolidae											1	
Glossiphoniidae			1	1	2		1	1	2		2	2
Erpobdellidae		1		1					2			3
Astacidae												
Asellidae	3	2	2	3	3	3	3	3	3		2	3
Corophiidae	2	1										
Gammaridae	3	2	1	3	3	1	1	1	3	2	3	3
Baetidae						1	1	3	3		3	3
Heptageniidae					2	2	1		3		2	3
Leptophlebiidae							1		1			
Ephemeridae	3				3	1	3					
Ephemerellidae						1		1	3	3	2	3
Caenidae					1			1	1			
Nemouridae												
Leuctridae	1				2	1		1	3		2	3
Coenagriidae												
Calopterygidae					1				1			
Libellulidae												
Hydrometridae						1		2				
Gerridae								3				
Nepidae				1				3				
Aphelocheiridae	2											
Notonectidae				2				3				
Corixidae			1					3				
Haliplidae				1				1				
Dytiscidae				1	2		1	2	2		2	3
Gyrinidae	1	1		1		1		3				
Hydrophilidae								3				1
Scirtidae												
Elmidae	1					1		1	3	2	3	3
Sialidae		2			1							
Glossosomatidae									2			
Hydroptilidae									3	1	2	3
Psychomyiidae	2								3	1	3	3
Polycentropodidae	2				2	2	1		1			
Hydropsychidae									1			
Phryganeidae	2	1	1	1	1		1					
Lepidostomatidae									2			
Limnephilidae		1		1								
Goeridae									3			1
Sericostomatidae									2		1	
Molannidae												
Leptoceridae	2	1	1		3	1	1		3	1	1	1
Tipulidae								2				
Simuliidae					1				1			
Chironomidae	3	3	3	3	3	3	3	3	3	3	3	3