Replicate sampling programme : Proposal Ralph Clarke (CEH) 11/7/02

It is important to have quantitative estimates of the effects of sampling variation on the value of any biotic index used to assess the ecological status of a river site. Sampling methods and derived indices which are very prone to high levels of variation between replicate samples will tend to provide less reliable estimates of ecological quality ratios and ecological status for a site; and have less power and confidence to detect changes in ecological quality.

"An index of ecological quality or status is of little value without some knowledge of its levels of uncertainty"

This is why the STAR project incorporated a replicate sampling component. Replicate samples are needed for each country's sampling method and the standard AQEM and RIVPACS sampling methods, so that in Workpackage 11 we can derive and compare estimates of the effects of sampling variation using each sampling method on the various biotic indices used to assess and compare site quality.

The original intention in the STAR Project plan was for the replicate sampling programme to be carried out in Workpackage 6 during the two sampling workshops (at La Bresse in April 2002 and Dorchester in September 2002). It soon became clear and agreed that it was not possible to fit in a replicated sampling study for macroinvertebrates within the La Bresse sampling workshop as this would be dominated by the initial field training in the different sampling methods for macroinvertebrates, macrophytes and phytobenthos and surveying methods for habitats. At La Bresse replicate samples were taken for diatoms.

The original agreed project plan was that 3 replicate macroinvertebrate samples would be taken at each of five sites in both the spring and autumn workshops; this was to be done for each of six sampling methods: AQEM, RIVPACS, Nordic standard, French IBGN, Italian IBE, Dutch EBBEOSWA.

This would have amounted to 15 spring and 15 autumn samples giving of 30 samples for each of 6 sampling methods and a total of 180 samples. Each of the 10 partners involved in the replicate sampling was to have taken the samples they took back to their own lab to process and identify using the appropriate methods.

It is proposed that these replicate samples are now taken as part of each country's agreed sampling of sites in their own country under Workpackage 7 (core streams types 1 and 2) and/or Workpackage 8 (additional stream types). The replicate samples will be taken at the same time as the samples for Workpackages 7 and 8 are taken.

Ten partner countries are involved in the sampling programme: UK, Germany, Denmark, Sweden, Czech Rep, Austria, France, Italy, Portugal and Greece. Therefore each country has obligations to take (at least) 180/10 = 18 replicate samples as part of the replicate sampling programme.

In Workpackages 7 and 8, each country will be taking one sample by the AQEM method and one sample by their own 'national' or RIVPACS sampling method in each of two seasons at a range of sites (see Table 1 below). These samples will be referred to as the "Main samples". We need estimates of the effects of sampling variation for each sampling method to compare this with sampling variation using the AQEM method. Therefore we need replicate samples

using both the 'national' (or RIVPACS) method and AQEM method at a range of stream types, stresses and ecological status.

By taking the replicate samples at the same time as a site is already being sampled once as part of Workpackages 7 and 8, each additional replicate sample will provide one extra 'degree of freedom' for estimates of sampling standard deviations.

It is proposed that each partner country sample take a minimum of one (but preferably two) additional replicate samples using each of the two agreed sampling methods for their country at each of 6 of their sites in each of the two sampling seasons (spring 2003 + summer or autumn 2002).

If only one additional replicate sample is taken for each sampling method, this will mean that a total of 24 replicates samples (2 sampling methods x 6 sites x 2 seasons) will need to be taken and processed by each partner.

The 6 sites should be selected to cover the stream types or types being sampled in that country (e.g. 3 sites from each of Core types 1 and 2). Within each selected stream type, at least one site should be of 'high' ecological status and at least one site of 'moderate' or 'poor' status. We will have to assume that 'bad' sites are consistently 'bad' when sampled by any method.

Table 1 gives the proposed choice of site combinations for replicate sampling in each country partner.

Table 1: Selecting a subset of sites in Workpackages 7 and 8 for replicate sampling (numbers in brackets = agreed total number of sites being sampled in Workpackages 7 and 8.)

Country	Stream type	Sampling method			No. of sites to have an additional replicate sample for each sampling method in both seasons (spring 2003 and summer or autumn 2002)			total number of replicate samples
		AQEM	RIVP.	Other	'High' status	'Good' status	'Moderate' or 'Poor'	
UK	Core 2	(12)	(12)		1	1	1	12
	Addition.	(12)	(12)		1	1	1	12
Germany	Core 1	(10)	(10)		1		1	8
	Core 2	(10)	(10)		1		1	8
	Addition.	(10)	(6)		1		1	8
Denmark	Core 2	(10)		(10)	2	2	2	24
Sweden	Core 2	(10)		(10)	1	1	1	12
	Addition.	(14)		(6)	1	1	1	12
Czech Rep	Core 1	(14)		(14)	1	1	1	12
	Addition.	(10)		(7)	1	1	1	12
Austria	Core 1	(10)	(10)		1	1	1	12
	Addition.	(10)	(5)		1	1	1	12
France	Addition.	(10)		(10)	2	2	2	24
Italy	Addition.	(20)		(20)	2	2	2	24
Portugal	Addition.	(10)	(10)		2	2	2	24
Greece	Addition.	(10)	(10)		2	2	2	24

It is up to the partner to choose whether the selected sites not of 'high' status are chosen because they considered to be subject to organic or habitat (or toxic or acidity) stresses or impairment; and this needs to be recorded.

Most partners countries are already sampling the same set of sites by both sampling methods in each season. Taking the replicate samples at a different subset of 6 sites in the two seasons would give replicate variance estimates for a larger number of sites. However, we consider that it is best to take the replicate samples at the same set of 6 sites in both seasons. This will make it easier to compare variation between seasons. Importantly it will also enable us to assess the effects of sampling variation on site assessments based on either the combined season sample or the average (or minimum) of the biotic index values for the two seasons' samples from a site.(Currently in the UK, national survey assessments on based on RIVPACS O/E ratios for number of BMWP taxa and ASPT based on combined spring and autumn samples.) Combined season samples, and index values based on the average of two or more replicate samples, are likely to be less prone to the effects of sampling variation and have greater power to detect impairment, especially chronic stress.

The two samples for a site in one visit should ideally be taken by ***different*** trained people so as to include any inter-person effects of the sampling method (i.e. its consistency in application between people).

For AQEM sampling both people taking replicate samples should make their own independent assessment of the percentage cover of the substratum/microhabitat types within the site and choose their own allocation and position for the 20 AQEM sampling units. This site assessment is all part of the AQEM sampling method. The AQEM substratum/microhabitat cover estimation forms for both 'the Main sample' and 'the Replicate sample' should be kept as they may be analysed at a later date to analyse habitat assessment contribution to AQEM inter-sample variation.

If only one person trained to sample is available for a replication site, then it is still necessary for that person to take a replicate sample in addition to the main sample. This will be used to assess the susceptibility of the sampling method to the effects of within-site spatial heterogeneity in the macroinvertebrate community.

In the UK, CEH has previously carried out a similar replicate sampling programme across a wide range of types and qualities of site. By taking 3 samples per site visit, two samples by one person and one sample by a second person, we were able to assess the effect of using different people on sampling variation. This may be important in assessments of the potential change in ecological quality between years when different personnel were used. Using RIVPACS sampling method , spatial variation was far more important than inter-person differences, but this may not be so for other sampling methods.

Therefore, for STAR, it would be very useful if partner countries were able to take two replicate samples by a second person, to supplement the standard single sample taken for WP 7/8. This would not necessarily have to be done for all 6 sites; if fewer than 6 sites, choose 'high' status sites which are likely to be the most diverse and variable.

Some of the replicate samples will be selected for the general audit and must be retained in the same manner as your Workpackage 7 and 8 samples.

There will also be a request for any existing information and estimates of the effects of sampling variation using 'national' methods on variation in the biotic indices to be used within STAR. This may be helpful in Workpackage 11.

Outputs

Replicate sample data from all countries will contribute towards the overall assessment and synthesis of the effect of sampling variation using the AQEM method. Those countries using RIVPACS replicate sampling will provide valuable estimates of the effect of sampling variation based on RIVPACS across Europe.

The replicate samples using a 'national' sampling method will be used to provide estimates of the effects of sampling variation on biotic indices based on that method and provide contrasts with estimates for other methods.

"Sample number" code revision

It is suggested that for the purpose of the replicate sampling programme:

- the first sample taken, which is the sample to be used as part of Workpackages 7 and 8, is referred to as 'the main sample'.
- the second sample taken is referred to as 'the replicate sample'
- if a third sample is taken it is referred to as 'the second replicate sample', and so on.

In the AQEM/STAR site protocol manual, it is proposed that each macroinvertebrate sample collected within the STAR programme is given a unique "sample number" code, using the following coding system:

digit 1 : country single letter abbreviation
digits 2-3 : stream type number in the country/ecoregion
digits 4-7 : sampling site number (assigned by partners to sites in their own country)
digit 8 : season (1=spring, 2=summer, 3=autumn, 4=winter).

It is recommended that the following is added to the "sample number" code:

digit 9 : replicate number (1 = the main sample, 2 = the replicate sample
3 = the second replicate sample (if taken))

To ensure standardisation, the ninth digit should also be recorded as a '1' for samples from STAR sites for which no replicate sampling is undertaken.