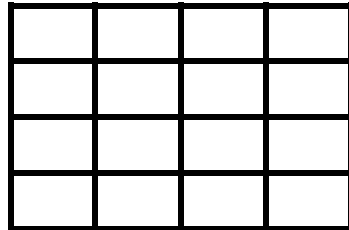


## **RIVPACS sorting and recording**

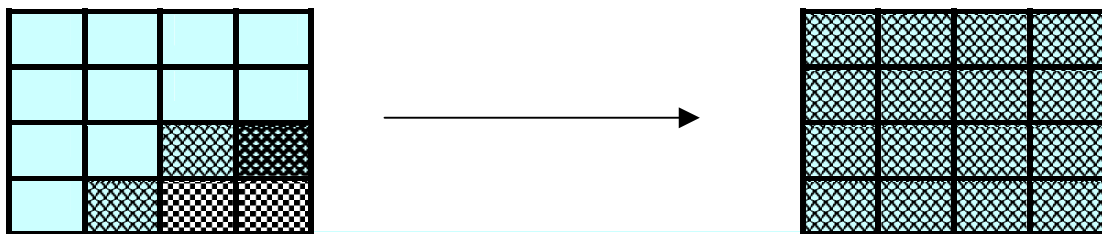
- 1) Samples are sorted in white bottomed plastic trays of approximate size 25cm by 30cm. This size is not critical – other sizes can be used.
- 2) Trays are marked into 16 equal sized cells using a permanent marker. Ideally the trays should be translucent and the lines drawn on the outside using a blue marker. If the trays are not translucent then the lines should be drawn on the inside. Lines are drawn in the following pattern:



- 3) The sample to be picked is washed through a 500 $\mu$  sieve to remove all traces of preservative or fixative. This is best done in a fume cupboard.
- 4) The washed sample, including inorganic and organic material plus the animals, is tipped into a bowl and covered with water.



- 5) A very small aliquot (amount) of sample material is transferred to the sorting tray, which now contains enough water to cover the portion of sample to be picked. The material can be decanted from the bowl or a portion can be transferred by hand or by spoon. The amount of each aliquot taken from the bowl for sorting should be small and a complete sample may have more than fifty separate portions of material, each sorted separately.
- 6) The material to be sorted is spread as evenly as possible on the bottom of the tray. This is done by hand or with forceps. The material should be spread thinly and there should be much more white surface of the tray showing than material to be sorted. If this is not the case then you have put too much sample material in the tray.



The diagram above shows the aliquot of sample (in black) before it has been spread evenly over the bottom of the tray. Material is concentrated in one corner in this example.

The diagram above shows the aliquot of sample (in black) after it has been spread evenly over the bottom of the tray.

- 7) The most difficult bit. The person sorting must decide what proportion of the total sample they intend to pick. Two main factors need to be considered.

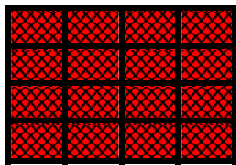
Firstly, our original rule was that no RIVPACS sample should take more than two hours to sort. We gradually became aware that this was not always possible but we would still regard four hours as being a maximum time. What this means is that you are more likely to pick all of a small sample but more likely to pick a small proportion of a large sample.

Secondly, the number of specimens in the sample should be considered. As far as possible account should be taken of all taxa present. If Chironomidae are very common but very few other taxa are common then the fraction picked should be larger than if most taxa are common (see also paragraph 17 below). The first tray of material sorted will give some idea of whether the sample has a lot or a few animals. This first tray should have slightly more material than usual to give you a better idea of the number of animals present and this portion is better picked out by hand than decanted. Picking a portion by hand, rather than decanting, is best for this first portion because it will give you a better representative part of the sample. Decanting only removes the lighter portions. You can also look in the bowl containing the main part of the sample and examine this to get a quick impression of the number of specimens present.

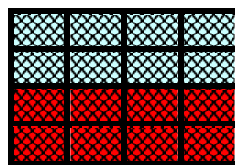
There are no rules about the number of specimens to be removed from the sample but the number, including those recorded on tally counters could reach >1000

The commonest proportion of a sample to be picked in detail is a quarter (25%) but bigger or smaller proportions are also picked.

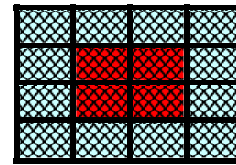
- 8) It is our general rule that the precise area of the tray to be picked in detailed should be varied for each tray. This reduces bias in the spreading of the material. Some examples of the area picked in detail are shown in red below:



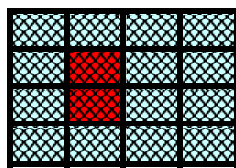
Whole sample sorted in detail



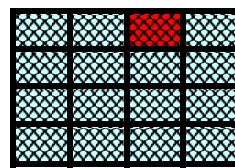
Half of the sample sorted in detail



Quarter of the sample sorted in detail

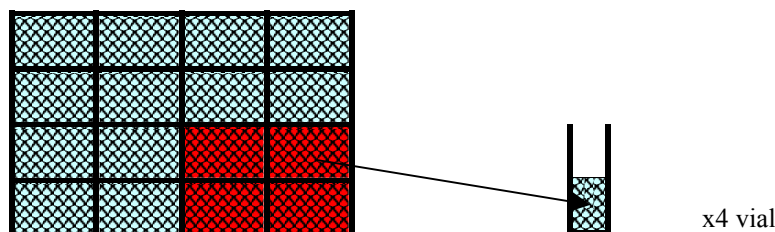


Eighth of the sample sorted in detail

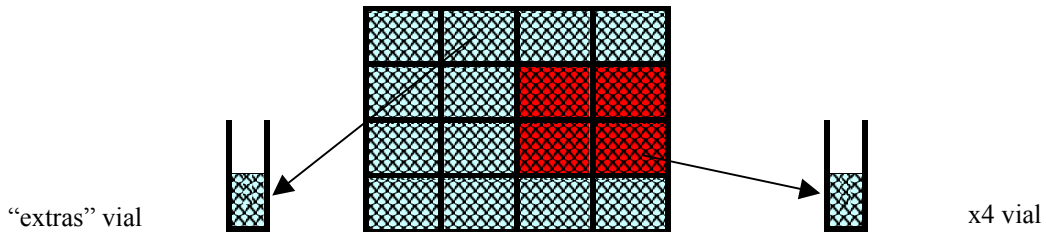


Sixteenth of the sample sorted in detail

- 9) All of the animals from the proportion of the tray picked in detail are put in a vial containing alcohol and glycerine (but see the >50 specimens rule below). The following example shows a sample in which a quarter of the tray is picked in detail. The vial in which the specimens are put is called the x4 (times four) vial.



- 10) After the chosen (red) fraction has been sorted in full and **all** the specimens present have been removed, the whole of the blue portion is **quickly** scanned (searched) to look forward any new (or apparently new) species that have not been found in the red portion. One specimen only (sometimes two if you think that the first specimen is too small or damaged to be easily identified) is removed from the tray and put in a new vial containing alcohol and glycerine). The vial in which the specimens are put is called the “extras” vial. We recommend that, before finishing sorting a tray, the tray is agitated slightly (a small movement of the tray to make the contents change their position). This often makes specimens missed in the first quick scan become visible on a second scan.

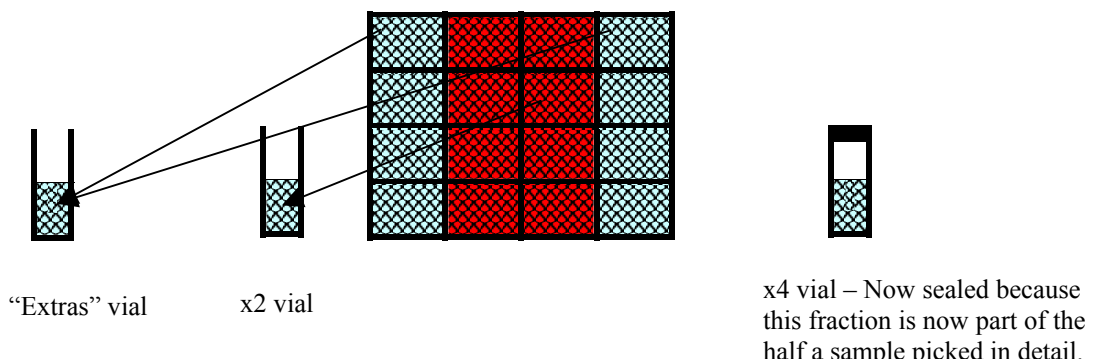


- 11) Once sorting of the tray is finished, all the material in the tray is passed through a ca 500 $\mu$  sieve and the retained portion is retained as the first part of the re-constituted sample for auditing. The contents of all the sorted trays together make the audit sample.
- 12) This process is repeated with the next small portion of the main sample. After the red portion has been fully sorted, the remaining blue portion is quickly scanned for any new (or apparently new) species that have **not been found so far in any of the trays previously searched** (an exception is that a second specimen can be removed if the specimen removed from an earlier tray was small or damaged and not easily identifiable). One (or two) specimens of this new species is removed from the tray and placed in the “extras” vial..

This process continues until the whole of the sample has been transferred to the sorting tray and sorted. In the later trays it is unusual to find any more specimens in the **blue** portion that need to be removed. Most species will have been found in earlier trays. However, the **red** portion is always sorted in full.

- 13) Paragraphs 1)-12) show the most common pattern. However, there are some exceptions to this routine. These are given in the following paragraphs.

- 14) It is possible that the initial decision to pick a quarter of the sample was found to be a mistake. If so, it is permitted to change the proportion. For example, you would do this if you discovered that specimens were not as common as you first estimated. Thus, it is possible to change from picking a quarter of a sample to picking half a sample. In this case a new vial would need to be created which is the x2 (times two) vial. All specimens picked in detail from the red half a sorting tray must be put in the x2 vial. Any new single taxa (exceptionally two taxa) still being picked from the blue half of the tray must go in the “extras” vial. The situation is now as follows:



- 15) It is also possible to switch from a quarter picked in detail to the whole tray picked in detail. In this case all specimens in the tray are removed and put in the x1 vial. The x1 vial is not the same as the “extras” vial. Specimens in the “extras” vial are only counted if no specimens of that species are found in any red section of the tray when sorting is completed.
- 16) Changes can also be made in the opposite direction so, for example a sample which you start to pick in full can be changed to only a quarter sample picked if there are more specimens in the sample than you first thought.
- 17) You can even pick different portions in detail for different taxa but remember that you must be careful to always put sorted specimens into the correct vial. Suppose you had a sample with very few insects, snails *etc.* but lots of Oligochaeta. You could pick the whole tray in detail for most groups and these specimens would go in the x1 pot. However, you could pick only a quarter of the tray in detail for the worms. Then every worm removed would be placed in the x4 pot.
- 18) It is of course possible to pick a specimen of, say, *Hydropsyche saxonica* from the blue part of a sample tray because you had not then found it in the red section and then, later, find several specimens in the red section. This sort of thing is very common and probably happens in most samples. In this case the specimen of *Hydropsyche saxonica* in the “extras” vial is no longer counted because of its later occurrence in the red section of the tray. Remember, even if you have already found and removed the species from the blue section, all specimens found in the red section must be removed (or counted – see paragraph 20 below).
- 19) Every vial used for a sample must be fully labelled with the sample details and the proportion of the sample that has been picked for that vial (e.g. x4).
- 20) As a rule, we only pick **approximately** fifty similar specimens from a sample. Similar specimens might mean a family in one case (e.g. Tubificidae) or a genus in another (e.g. *Leuctra*). After this, all specimens of these taxa will be left in the tray, even if they are in the red portion, but each specimen will be “clicked” on the appropriate tally counter. Here “clicked” means counted. It is the noise made when the tally counter button is pressed to add one specimen to the total. Each tally counter should have a temporary label to indicate which taxon is being “clicked”. The number of specimens of each “clicked” taxon must be recorded on the appropriate sample data sheet.
- 21) Once the sample is finished, the specimens in each vial are identified and their identity, number of specimens found and the vial’s multiplication number are recorded on a standard recording sheet. This is done for each vial that you use. In the following, deliberately complex example an “extras”, an x2 and an x4 vial were used. In addition, 84 specimens of *Leuctra* spp. were “clicked” (counted) from the quarter of the tray (= x4) picked in detail. After identification the number of specimens of Leuctridae in each vial was as follows:

Extras vial: *Leuctra moselyi* (1 specimen)  
*Leuctra geniculata* (1 specimen)

x2 vial *Leuctra fusca* (17)  
*Leuctra hippopus* (13)  
*Leuctra nigra* (4)

X4 vial *Leuctra geniculata* (1)  
*Leuctra fusca* (37)  
*Leuctra hippopus* (16)  
*Leuctra nigra* (6)

The first thing that happens is that the specimen of *Leuctra geniculata* found in the “extras” vial is not counted because this species was later found in the red section. However, the specimen of *Leuctra moselyi* is counted (multiplication factor 1) because it was not later found in a red section.

The number of specimens of each species is therefore recorded on the data sheet as follows:

Leuctridae	<i>Leuctra moselyi</i> (1x1) <i>Leuctra geniculata</i> (1x4) <i>Leuctra fusca</i> (17x2) (37x4)
Total = 645	<i>Leuctra hippopus</i> (13x2) (16x4) <i>Leuctra nigra</i> (4x2) (6x4) <i>Leuctra</i> spp. (84x4)

If required the 84 clicked specimens can be allocated to individual species in the same proportions as the identified specimens from the same portion of the sample. Thus the x4 pot contained the 60 specimens (In this case the approximately fifty similar taxa remove actually turned out to be 60 specimens. This does not have to be accurate, approximately 50 is fine). The proportions of each species amongst these 60 were as follows

<i>Leuctra geniculata</i> (1)	=	1/60 %	=	1.67%
<i>Leuctra fusca</i> (37)	=	37/60 %	=	61.67%
<i>Leuctra hippopus</i> (16)	=	16/60 %	=	26.67%
<i>Leuctra nigra</i> (6)	=	6/60 %	=	10.00%

Therefore the allocation of the clicked specimens to taxa is as follows:

<i>Leuctra geniculata</i>	=	1.67% of 84	=	1
<i>Leuctra fusca</i>	=	61.67% of 84	=	50
<i>Leuctra hippopus</i>	=	26.67% of 84	=	22
<i>Leuctra nigra</i>	=	10.00% of 84	=	8
		TOTAL =		81

Due to rounding errors, the new total is 81 specimens. Because the new total must equal 84, the estimated number of the commoner species must each be rounded up by 1 to give the following revised estimates.

<i>Leuctra geniculata</i>	=	1
<i>Leuctra fusca</i>	=	51
<i>Leuctra hippopus</i>	=	23
<i>Leuctra nigra</i>	=	9
	TOTAL =	84

Therefore the revised estimate of the numbers of each species of *Leuctra* in the x4 portion of the sample is as follows:

<i>Leuctra geniculata</i>	1 + 1	=	2
<i>Leuctra fusca</i>	37 + 51	=	88
<i>Leuctra hippopus</i>	16 + 23	=	39
<i>Leuctra nigra</i>	6 + 9	=	15
	TOTAL =	144	= 60 + 84

The new line on the data sheet would therefore read:

Leuctridae	<i>Leuctra moselyi</i> (1x1) <i>Leuctra geniculata</i> (2x4) <i>Leuctra fusca</i> (17x2) (88x4)
Total = 645	<i>Leuctra hippopus</i> (13x2) (39x4) <i>Leuctra nigra</i> (4x2) (15x4)

The example given above is very complex to show how each problem is dealt with. For most families/species in most samples the estimation of numbers is very much simpler than this.

The taxonomic level of identification for RIVPACS samples should be the same as for STAR/AQEM samples. RIVPACS also requires estimates of the numbers of specimens of each family. In the above example, the estimated number of Leuctridae in the sample was 645.