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Rapid Assessment of Macroinvertebrate Samples

Report to The Environment Agency
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University of Hull

S. C. Jarvis, N. V. Proctor, W. A. Musk

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For further information please contact:

Institute of Estuarine & Coastal Studies (IECS)
The University of Hull
Cottingham Road
Hull
HU6 7RX
UK

Tel: +44 (0)1482 465667 or 465661
Fax/Tel: +44 (0)1482 465001

E-mail:
iecs@hull.ac.uk

Web site:
http://www.hull.ac.uk/iecs

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1. Introduction

The implementation of the Water Framework Directive and EC Habitats Directive requires that the ecological status of all transitional and coastal waters be described. Traditional methods of sampling and laboratory analysis are unavoidably labour intensive and hence costly, and often involve a considerable delay between the commissioning of the survey and production of the report. The development of rapid assessment techniques that will provide repeatable and relevant data in a timely and cost effective way is therefore of considerable importance.

This report details results of some possible approaches which may be adopted in the laboratory to achieve the above aims. The data originate from samples taken during an Environment Agency field workshop held on board the CSV *Vigilance* in the Fal - Ruan estuary complex in September 2002. The workshop was held as part of the Environment Agency Research and Development project (E1-116), as part of the integrated UK & Republic of Ireland approach to assessment of transitional and coastal waters. Funding was provided by JNCC for the follow-up laboratory analysis.

The objectives were:

- to assess whether a subsample extracted during a pre-determined time period could be representative of the whole sample. This was addressed by Timed Sorting Analysis (TSA);
- to assess how an 'expert view' of a sieved sample assessed in the field relates to a full sample analysis (EVA - Expert View Analysis);
- to evaluate whether a sample analysed by an expert in the laboratory with basic magnification equates to the more usual microscope aided identification. This was tested using a protocol termed Restricted Laboratory Analysis (RLA);
- to validate these objectives with complete full analysis following NMBAQC protocols.

These methods were devised by Environment Agency and JNCC staff. IECS was contracted to perform the laboratory analyses.

2. Materials and Methods

2.1 Field methods

The samples used for this project were taken from six sites within the Fal Estuary system. Tresilian samples were taken by coring ($0.01m^2$), Malpas, Greatwood and Messack sampling was by Day Grab ($0.1m^2$) and the samples from Restronguet Creek and Ruan Creek were taken with either a Van Veen grab ($0.05m^2$) or a core ($0.01m^2$). All sampling and field processing was carried out by staff of the Environment Agency, JNCC, EHS and English Nature in mid-September 2002.

Samples for Timed Sorting Analysis (TSA), Whole Sample Analysis (WSA) and Restricted Laboratory Analysis (RLA) were immediately fixed in the field using 4% formalin and later transferred to IMS. Expert View Analysis (EVA) samples were sieved (1.0 mm) in the field and examined live for 15, 20 or 30 minutes by staff who were classified as either expert, intermediate or beginner. Animals identified and enumerated in this way were then removed to a separate labelled container and preserved for laboratory analysis. The remainder of the sample (residue) was similarly preserved. It was decided not to continue with the WSA (effectively all Greatwood and Tresilian samples) and these samples have been stored in case of future need.

At Messack the sediment consisted of subtidal gravel with shell fragments. At Restronguet Creek and Ryan Creek the substratum was estuarine muds/fine sands.

2.2 Laboratory methods

In the laboratory, samples for RLA, TSA, and residues for EVA were washed under fume extraction hood through a 0.5 mm or 1.0 mm sieve (as previously designated) to remove traces of fixative. The samples were then examined (approximately 0.25 l at a time) under a layer of water in white trays using a fluorescent 1.5x illuminated magnifier. Animals were removed using watchmakers forceps ("picking") and stored by taxonomic group in appropriately labelled containers under 70% industrial methylated spirits (IMS). With TSA samples, picking was restricted to either 15 or 30 minutes before proceeding with the full extraction. In each case the *whole* sample was examined during a 15 minute period to extract as much of the fauna as possible. This meant that, for 30 minute analyses, the samples were effectively scanned twice (for 15 minutes on each occasion) before being sorted in the usual way. These time-limited fractions were stored and analysed separately.

The invertebrates removed and identified in the field during EVA were washed as above before laboratory identification.

Identification of invertebrates was carried out using Olympus SZ30 zoom microscopes with 10x and 20x eyepieces, giving a maximum magnification of up to 80x. An additional 2x objective was occasionally used to increase the potential magnification to 160x. Compound microscopes were used for further magnification, up to 1000x. The macrofaunal animals were then identified to species level, wherever possible, using standard taxonomic keys and

dissection, when necessary. Oligochaetes were cleared in lactophenol prior to microscopic examination.

For RLA treatment the sorted animals were identified and counted as far as possible using a 1.5x desk magnifier. This work was then checked "blind" (i.e. without reference to these data) by a second member of staff, but this time using microscopes as described above.

A reference collection of taxa encountered during the study was compiled.

2.3 Statistical analysis

Univariate sample statistics (Shannon diversity index) and the variables sample species richness (S) and total abundance (A) were computed to compare samples under different treatments. Cluster analysis (using PRIMER™) was also used to investigate the differences between the various sample processing methods. All cluster analyses were conducted on untransformed data using the Bray - Curtis similarity measure (Bray & Curtis, 1957) and group average cluster mode. Further details of statistical analyses are given where appropriate in the text.

A Biotic Coefficient (Borja *et al*. 2000) was also calculated to compare the various treatments. This method assigns each taxon to one of five groups depending on its known ability to tolerate organic pollution. Group I species are very sensitive to organic enrichment and are confined to unpolluted conditions. Group II species are relatively indifferent to enrichment and their populations fluctuate independently of low levels of organic pollution. In Group III species are tolerant to excess organic matter but their abundance increases in response to organic enrichment. Group IV consists of "second-order" opportunistic species and Group V of "first-order" opportunistic species which are adapted to reduced sediments. The scores for each sample are converted to a continuous index (Biotic Coefficient) using a weighted percentage of the whole sample score (see Borja *et al.* 2000 for details).

Throughout this report sample numbers are preceded with a two-letter prefix indicating the site of origin (ME = Messack; RE = Restronguet Creek; RU = Ruan Creek).

3. Results

3.1 Timed sorting analysis (TSA)

TSA was carried out on a total of 16 samples, 12 from Messack and two each from Restronguet Creek and Ruan Creek. Eleven samples from Messack were processed with a 1.0 mm mesh for a fixed period of 15 minutes (and also 30 minutes in three cases). Two of these were also analysed using a 0.5 mm screen (ME6 and ME8). The remaining Messack sample and the Restronguet and Ruan Creek samples were processed on a 0.5 mm sieve only for both 15 and 30 minutes. The 1.0 mm samples are summarised in Table 3.1.1 and the 0.5 mm samples in Table 3.1.2. These five samples were also used in RLA.

Limiting the sorting time resulted in less information being extracted from the sample. On average, for the 1.0 mm sieve fractions, a fifteen minute sorting period constituted 3.4% of total sorting time (n = 11) i.e. about 97% reduction in sorting effort. During this time it was possible to extract a mean of 8.8% of the fauna (n = 11) and 38.2% of sample species richness (n = 11).

Where two sorting periods had been applied to the same sample sieved on a 1.0 mm mesh (samples 2b, 2c & 2d), a doubling of sorting time did not result in a commensurate increase in either total fauna extracted or sample species richness. The proportion of fauna extracted was increased from a mean of 5.0% to one of 7.3% (n = 3) and the mean proportion of species per sample (species richness) increased from 24.5% to 30.1% (n = 3).

Sieving with a 0.5 mm mesh (samples ME6 and ME8) more than doubled the total sorting time (see Table 3.1.1) and reduced the mean proportion of fauna extracted in timed sorting periods from 7.1% to 2.5% (n = 2) and the mean proportion of species from 46.1% to 35.4% (n = 2). However, it should be noted that the two size fractions were supplied (and therefore sorted) separately and the 0.5 mm fraction was not examined in the TSA. These data were subsequently added to the 1.0 mm fraction to give the 0.5 mm sample statistics as shown in Table 3.1.1. Usual practice would have been to sort the whole sample as one unit as was carried out with the 0.5 mm samples shown in Table 3.1.2. In this case a doubling of the sorting time from 15 to 30 minutes increased the mean proportion of fauna extracted (N) from 29.2% to 54.2% (n = 5) and the mean proportion of species extracted (S) from 54.8% to 74.7% (n = 5) (Table 3.1.3).

Table 3.1.2 summarises the TSA analyses using 0.5 mm mesh size. Two samples were of much higher abundance (ME7 and RU10c) resulting in a lower proportion of species and abundance being extracted during the fixed sorting times.

Figure 3.1.1 graphically displays some of the data from TSA. The generally higher percentages shown in the lower graph are a consequence of the reduced abundance in these samples.

Table 3.1.1. Extraction efficiency for 1.0 mm sieve fraction of TSA samples achieved in predetermined sorting periods (15 mins unless otherwise indicated). Samples ME6 and ME8 also show data for 0.5 mm sieve fraction

Sample	Total N	Total S	Total sorting time (hrs)	TSA as proportion of total sorting time (%)	Fauna extracted N (%)	Species extracted S (%)
ME2b	1140	69	9.5	2.6	61 (5.4)	14 (20.3)
30 mins				5.2	93 (8.2)	16 (23.2)
ME2c	1184	66	14.7	1.7	78 (6.6)	18 (27.3)
30 mins				3.4	107 (9.0)	19 (28.8)
ME2d	1576	81	6.6	3.8	48 (3.0)	21 (25.9)
30 mins				7.6	72 (4.6)	31 (38.3)
ME5	1668	80	10.4	2.4	130 (7.8)	32 (40.0)
ME6	1653	73	11.2	2.2	101 (6.1)	32 (43.8)
0.5 mm			22.1	1.1	101 (2.5)	32 (34.0)
ME8	1295	66	8.2	3.0	106 (8.2)	32 (48.5)
0.5 mm			18.5	1.3	106 (2.5)	32 (36.8)
ME9	1775	74	12.5	2.0	106 (6.0)	28 (37.8)
ME10	438	39	6.3	5.2	58 (13.2)	17 (43.6)
ME11	425	24	4.8	5.2	58 (13.6)	9 (37.5)
ME12	320	39	10.4	2.4	57 (17.8)	21 (53.8)
ME13	810	51	3.4	7.0	77 (9.5)	21 (41.2)

Table 3.1.2. Extraction efficiency for 0.5 mm sieve fraction achieved in TSA samples (15 mins unless otherwise indicated)

Sample	Total N	Total S	Total sorting time (hrs)	TSA as proportion of total sorting time (%)	Fauna extracted N (%)	Species extracted S (%)
ME7	1355	60	18.4	1.4	94 (6.9)	30 (50.0)
30 mins				1.6	179 (13.2)	37 (61.7)
RE8	14	6	0.7	37.5	5 (35.7)	3 (50.0)
30 mins				75.0	10 (71.4)	5 (83.3)
RE3a	17	9	1.0	25.0	10 (58.8)	6 (66.7)
30 mins				50.0	16 (94.1)	9 (100.0)
RU7a	65	14	1.0	25.0	18 (27.7)	9 (64.3)
30 mins				50.0	40 (61.5)	10 (71.4)
RU10c	443	14	13.8	1.8	75 (16.9)	6 (42.9)
30 mins				3.6	136 (30.7)	8 (57.1)

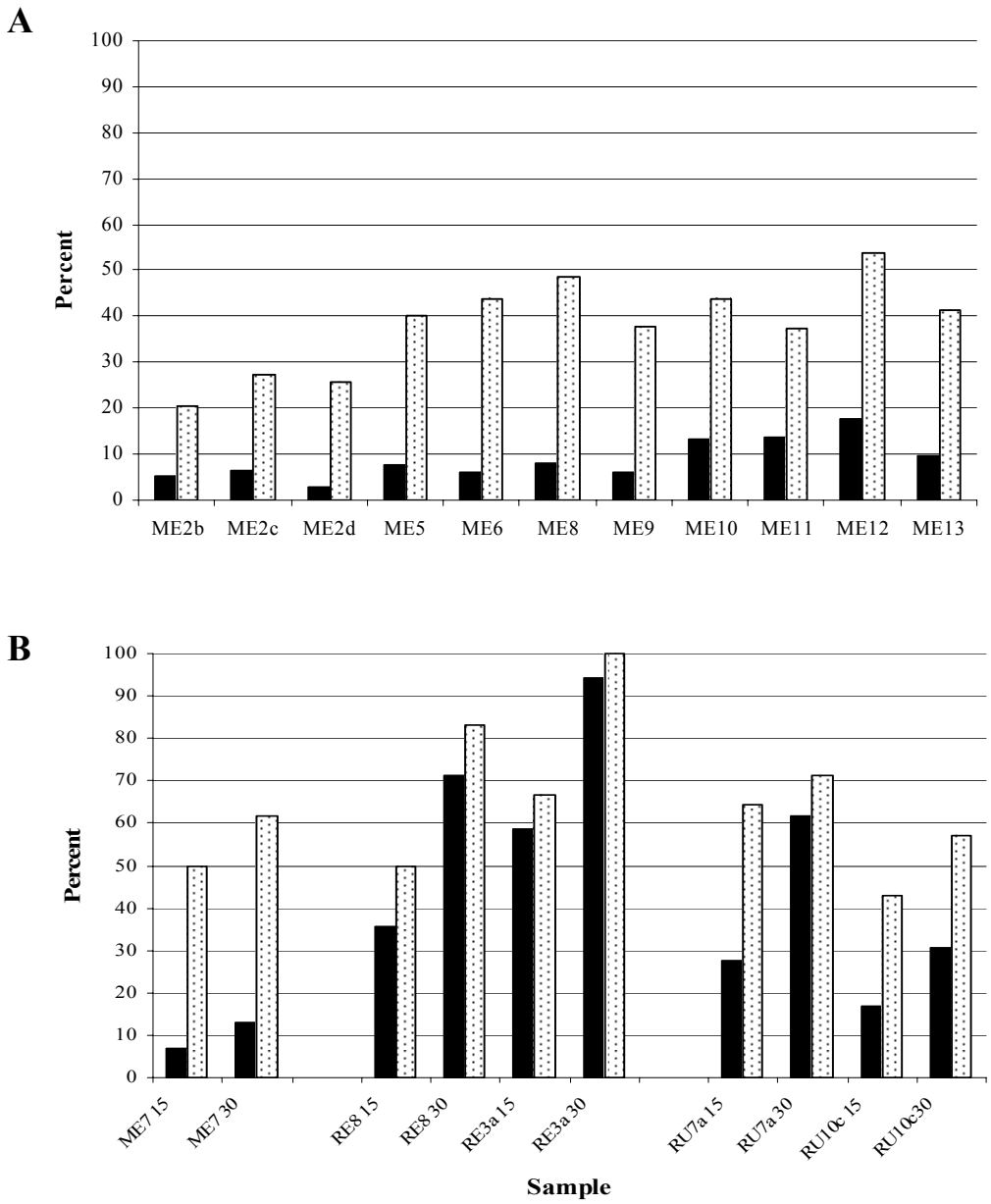

Figure 3.1.1. A Proportion of total individuals (N = solid bars) and total sample species richness (S = stippled bars) extracted from 1.0 mm mesh samples in 15 minute sorting period (see Table 3.1.1 for data). B Proportion of total individuals (N = solid bars) and total sample species richness (S = stippled bars) extracted for 0.5 mm mesh samples 15 and 30 minute sorting times (see Table 3.1.2). Sorting times indicated after sample number

Table 3.1.3. Summary of mean percent fauna (N) nd mean percent species (S) extracted by Timed Sorting Analysis

	N		**S**	
	15 min. sort	**30 min. sort**	**15 min. sort**	**30 min. sort**
1.0 mm mesh	8.8 (n = 11)	7.3 (n = 3)	38.2 (n = 11)	30.1 (n = 3)
0.5 mm mesh	29.2 (n = 5)	54.2 (n = 5)	54.8 (n = 5)	74.7 (n = 5)

The species extracted from each sample during the restricted sorting period were ranked in order of abundance (Table 3.1.4). This revealed that three taxa were consistently picked more frequently: cirratulid polychaetes, the polychaete genus *Nephtys* and the bivalve *Abra alba*. These rankings did not coincide with the most abundant taxa found after full sample analysis, indicating that animals were picked for their conspicuousness and not on account of their actual abundance. However, within this subset of conspicuous fauna an attempt was made while sorting to achieve a representative collection of fauna.

Table 3.1.4. Top ten ranked species for each 1.0 mm sieve fraction (whole sample) and respective TSA data (singletons omitted). TS15 prefix = 15 minute sorting time; TS30 prefix = 30 minute sorting time; WS = whole sample

Sample ME 2b					
TS15		TS30		WS	
Phoronis sp. Indet	18	*Phoronis sp. Indet*	25	*Mediomastus fragilis*	361
Abra alba	12	*Abra alba*	17	*Phoronis sp. Indet*	233
Chaetozone gibber	12	*Chaetozone gibber*	14	*Abra alba*	107
Melinna palmata	4	*Melinna palmata*	9	*Melinna palmata*	48
Mediomastus fragilis	3	*Mediomastus fragilis*	7	*Chaetozone gibber*	43
Sthenelais boa	3	*Nephtys kersivalensis*	5	*Protocirrineris sp.*	35
Nephtys kersivalensis	2	*Sthenelais boa*	3	*Caulleriella alata*	35
		Aphelochaeta marioni	3	*Aphelochaeta marioni*	31
		Ampelisca tenuicornis	2	*Praxillella (affinis)*	25
		Praxillella (affinis)	2	*Mysella bidentata*	20

Sample ME 2c					
TS15		TS30		WS	
Abra alba	26	*Abra alba*	32	*Mediomastus fragilis*	320
Chaetozone gibber	10	*Phoronis sp. Indet*	14	*Phoronis sp. Indet*	203
Nephtys kersivalensis	7	*Chaetozone gibber*	12	*Abra alba*	145
Phoronis sp. Indet	6	*Mediomastus fragilis*	9	*Chaetozone gibber*	68
Aphelochaeta marioni	5	*Nephtys kersivalensis*	7	*Aphelochaeta sp. A (unico-key)*	48
Praxillella (affinis)	4	*Praxillella (affinis)*	7	*Melinna palmata*	46
Ampelisca tenuicornis	4	*Aphelochaeta marioni*	6	*Caulleriella alata*	34
Mediomastus fragilis	3	*Ampelisca tenuicornis*	4	*Praxillella (affinis)*	33
Cirriformia tentaculata	3	*Cirriformia tentaculata*	3	*Aphelochaeta marioni*	27
Scoloplos armiger	2	*Scoloplos armiger*	2	*Monticellina dorsobranchialis*	26

Sample ME 2d					
TS15		TS30		WS	
Abra alba	7	*Abra alba*	8	*Mediomastus fragilis*	617
Nephtys kersivalensis	6	*Nephtys kersivalensis*	6	*Aphelochaeta sp. A (unico-key)*	124
Chaetozone gibber	5	*Mediomastus fragilis*	6	*Protocirrineris sp.*	114
Mediomastus fragilis	4	*Melinna palmata*	6	*Chaetozone gibber*	96
Aphelochaeta marioni	3	*Chaetozone gibber*	5	*Phoronis sp. Indet*	54
Cirriformia tentaculata	3	*Aphelochaeta marioni*	3	*Abra alba*	45
Liocarcinus arcuatus	3	*Cirriformia tentaculata*	3	*Melinna palmata*	45
Praxillella (affinis)	2	*Liocarcinus arcuatus*	3	*Monticellina dorsobranchialis*	38
Phtisica marina	2	*Crepidula fornicata*	3	*Caulleriella alata*	34
Notomastus sp. (latericeus)	2	*Sylllidia armata*	3	*Praxillella (affinis)*	27

Sample ME 5			
TS15		WS	
Abra alba	36	*Mediomastus fragilis*	697
Nephtys kersivalensis	14	*Phoronis sp. Indet*	208
Cirriformia tentaculata	13	*Cirriformia tentaculata*	110
Phoronis sp. Indet	12	*Aphelochaeta marioni*	67
Mediomastus fragilis	12	*Tubificoides ?galiciensis?*	62
Melinna palmata	6	*Abra alba*	54
Liocarcinus arcuatus	3	*Chaetozone gibber*	53
Nematonereis unicornis	3	*Phtisica marina*	30
Sthenelais boa	2	*Microdeutopus anomalus*	29
Tapes (Tapes) decussatus	2	*Tanaopsis graciloides*	26

Sample ME6			
TS15		WS	
Melinna palmata	13	*Mediomastus fragilis*	565
Abra alba	10	*Melinna palmata*	168
Cirriformia tentaculata	9	*Chaetozone gibber*	164
Nephtys kersivalensis	8	*Cirriformia tentaculata*	93
Ampithoe ramondi	6	*Aphelochaeta marioni*	73
Scoloplos armiger	5	*Tubificoides benedii*	57
Mediomastus fragilis	5	*Abra alba*	49
Tanaopsis graciloides	4	*Tubificoides ?galiciensis?*	49
Euclymene (oerstedii)	4	*Galathowenia/Myriochele*	38
Aphelochaeta marioni	4	*Galathowenia/Myriochele*	38

Sample ME8			
TS15		WS	
Cirriformia tentaculata	28	*Mediomastus fragilis*	537
Nephtys kersivalensis	13	*Cirriformia tentaculata*	110
Abra alba	10	*Tubificoides benedii*	109
Tubificoides benedii	7	*Phoronis sp. Indet*	109
Cheirocratus sundevallii	5	*Chaetozone gibber*	43
Heteromastus filiformis	4	*Tubificoides ?galiciensis?*	39
Caulleriella bioculata	4	*Aphelochaeta marioni*	37
Mediomastus fragilis	4	*Abra alba*	30
Aphelochaeta marioni	3	*Microdeutopus anomalus*	30
Chaetozone gibber	3	*Monticellina dorsobranchialis*	29

Sample ME9			
TS15		WS	
Abra alba	22	*Mediomastus fragilis*	596
Nephtys kersivalensis	14	*Tubificoides benedii*	239
Cirriformia tentaculata	9	*Melinna palmata*	207
Aora gracilis	6	*Cirriformia tentaculata*	111
Mediomastus fragilis	6	*Aphelochaeta marioni*	107
Melinna palmata	5	*Chaetozone gibber*	65
Megalomma vesiculosum	4	*Aora gracilis*	57
Platynereis dumerilii	4	*Abra alba*	41
Euclymene (oerstedii)	4	*Phoronis sp. Indet*	34
Ampithoe ramondi	4	*Tubificoides ?galiciensis?*	32

Sample ME10			
TS15		WS	
Abra alba	16	*Melinna palmata*	176
Melinna palmata	12	*Mediomastus fragilis*	57
Nephtys kersivalensis	6	*Chaetozone gibber*	55
Chaetozone gibber	6	*Abra alba*	39
Nephtys hombergii	5	*Tubifex tubifex*	22
Microdeutopus anomalus	2	*Microdeutopus anomalus*	14
		Nephtys kersivalensis	6
		Nephtys hombergii	6
		Tubificoides benedii	6
		Ampelisca tenuicornis	5

Sample ME11			
TS15		WS	
Melinna palmata	21	*Melinna palmata*	197
Abra alba	13	*Chaetozone gibber*	81
Chaetozone gibber	7	*Galathowenia/Myriochele*	35
Abra nitida	6	*Abra alba*	29
Nephtys kersivalensis	5	*Mediomastus fragilis*	26
Nephtys hombergii	3	*Euclymene (oerstedii)*	10
		Abra nitida	6
		Nephtys kersivalensis	5
		Aphelochaeta marioni	4
		Tubificoides ?galiciensis?	4

Sample ME12			
TS15		WS	
Aora gracilis	12	*Melinna palmata*	104
Nephtys hombergii	11	*Mediomastus fragilis*	43
Melinna palmata	9	*Aora gracilis*	32
Abra alba	5	*Chaetozone gibber*	31
Nephtys kersivalensis	3	*Aphelochaeta marioni*	23
Chaetozone gibber	2	*Nephtys hombergii*	11
		Nephtys kersivalensis	7
		Protocirrineris sp.	7
		Abra alba	6
		Microdeutopus anomalus	6

Sample ME13			
TS15		WS	
Melinna palmata	14	*Chaetozone gibber*	233
Chaetozone gibber	11	*Melinna palmata*	192
Euclymene (oerstedii)	9	*Mediomastus fragilis*	108
Abra alba	8	*Galathowenia/Myriochele*	67
Cirriformia tentaculata	5	*Protocirrineris sp.*	22
Nephtys hombergii	4	*Aphelochaeta marioni*	18
Ampelisca tenuicornis	4	*Euclymene (oerstedii)*	15
Aphelochaeta marioni	4	*Aora gracilis*	13
Galathowenia/Myriochele	4	*Tubificoides ?galiciensis?*	13
Ampelisca brevicornis	2	*Cirriformia tentaculata*	12

The results of multivariate analyses for the 1.0 mm sieve fraction from Messack are shown in Figure 3.1.2. Two main groups of data (with subgroups designated by capital letters) can be seen in the dendrogram (Figure 3.1.2), one with all the TSA data (A+B+C) and the other containing the whole sample analyses (D+E). These two "treatments" (partial analysis by restricting sorting time and full analysis) produced sufficiently dissimilar sample data for them to be separated unambiguously. Analysis of whole samples creates two clear groups (D and E) whereas TSA of the same samples identifies three less defined groups (A, B and C). TSA in effect removed samples ME2b, ME2c and ME2d from their association with ME5, ME6 ME8 and ME9 to form their own cluster (C). Sample ME12 did not appear in any cluster under TSA analysis but was grouped with samples ME10, ME11 and ME13 when the whole dataset was analysed (Cluster E).

The 15 minute and 30 minute data in samples ME2b, ME2c and ME2d are sufficiently similar to group each sample together within cluster C indicating that there was no qualitative difference between the 30 minute and 15 minute sort results.

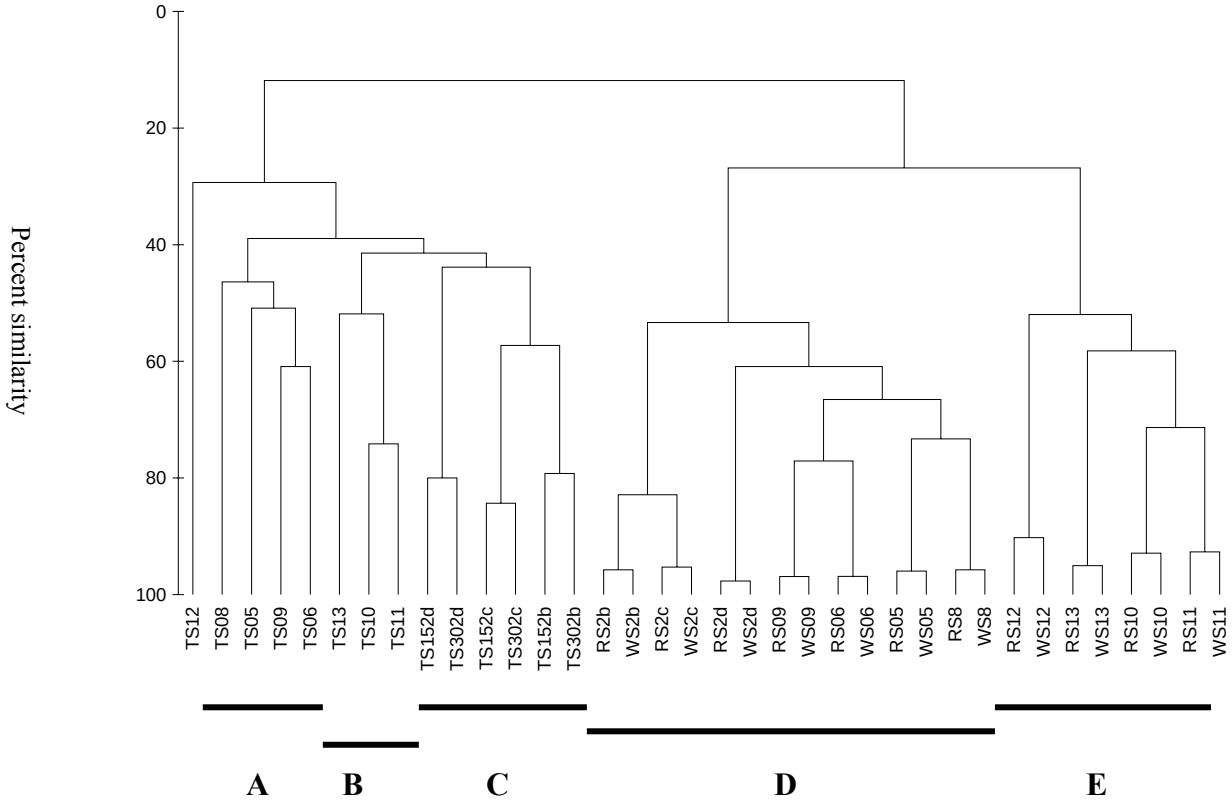

Figure 3.1.2. Cluster analysis using untransformed 1.0 mm mesh sample data. Each sample (Messack) number is prefixed as follows: TS = Timed sample (15 mins); TS15 = Timed sample 15 mins; TS30 = Timed Sample 30 mins; RS = Sample Residue; WS = Whole sample (TS + RS)

Samples processed through a 0.5 mm screen were also subjected to cluster analysis and the resulting dendrogram can be seen in Figure 3.1.3. In each case the whole sample data were closely associated with their respective TSA data. The high abundance samples (RU10c and ME7) clustered together and in each one the 15 and 30 minute sorting times were more similar to each other than to the full data. In contrast, although the low abundance samples again formed discrete clusters, in this instance the 30 minute data were closer to the full data than to the 15 minute subset.

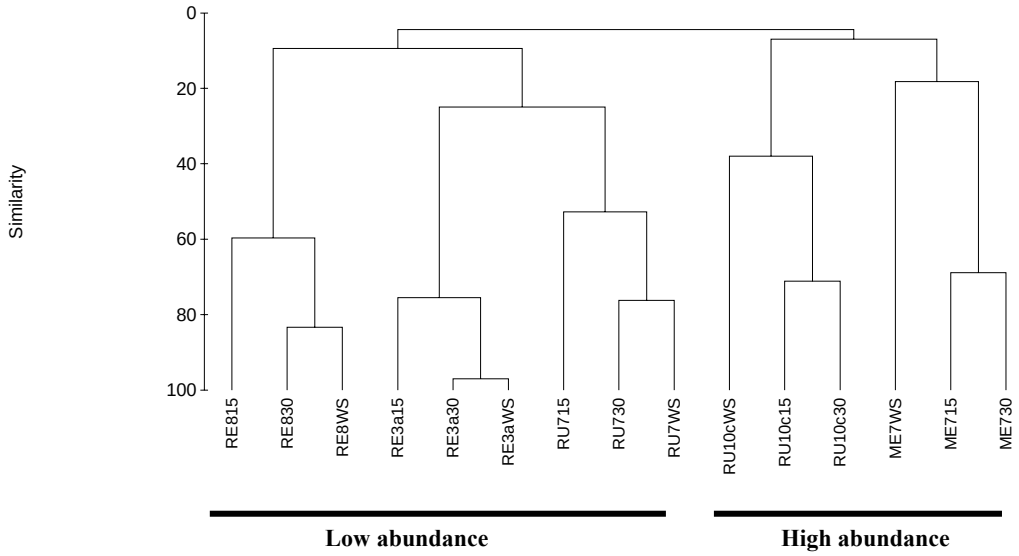

Figure 3.1.3. Dendrogram showing results of cluster analysis of samples analysed for TSA using a 0.5 mm mesh. Sorting times of 15 and 30 minutes. The -15, -30 and -WS suffixes denote 15 minute sort, 30 minute sort and Whole Sample data respectively

Borja's Biotic Coefficient (Borja *et al.* 2000) was calculated for 1.0 mm sieved samples. This revealed very little difference between pollution classifications for sub-samples identified after time-restricted sorting and for those analysed in full. Only ME9 was re-classified following full analysis. In this case the data from the timed sorting indicated a slightly polluted environment whereas the full dataset indicated further degredation and a classification of moderately ("meanly") polluted (see Table 3.1.5). In 9 of the 11 samples compared, analysing the full sample resulted in a higher Biotic Coefficient.

Table 3.1.5. Results from Borja's Biotic Index analysis for Messack samples processed on 1.0 mm sieve. BC = Biotic coefficient

	Timed sorting (15 mins)		Whole sample analysis	
	BC	Pollution classification	BC	Pollution classification
ME2b	2.02	Slightly polluted	2.22	Slightly polluted
ME2c	2.46	Slightly polluted	2.32	Slightly polluted
ME2d	2.41	Slightly polluted	2.96	Slightly polluted
ME5	2.38	Slightly polluted	2.74	Slightly polluted
ME6	2.33	Slightly polluted	3.21	Slightly polluted
ME8	3.08	Slightly polluted	3.26	Slightly polluted
ME9	2.28	Slightly polluted	3.31	Moderately polluted
ME10	2.56	Slightly polluted	3.09	Slightly polluted
ME11	2.87	Slightly polluted	2.91	Slightly polluted
ME12	1.55	Slightly polluted	2.61	Slightly polluted
ME13	3.14	Slightly polluted	3.05	Slightly polluted

The TSA samples processed on a 0.5 mm screen produced a more variable situation (Table 3.1.6). RE8 and RU10c gave the same results (slightly polluted and heavily polluted respectively) regardless of time spent extracting the fauna. The other samples were designated as slightly polluted on the basis of a 15 minute timed subsample, but would be classed as moderately polluted following full analysis.

Table 3.1.6. Results from Borja's Biotic Index analysis for samples processed on 0.5 mm sieve. BC = Biotic coefficient

	Timed sorting (15 mins)		Timed sorting (30 mins)		Whole sample analysis	
	BC	Pollution classification	BC	Pollution classification	BC	Pollution classification
RE3a	3.30	slight	3.94	moderate	4.06	moderate
RE8	1.87	slight	1.50	slight	1.85	slight
RU7	3.25	slight	3.35	moderate	3.75	moderate
RU10c	5.42	heavy	5.44	heavy	5.37	heavy
ME7	3.18	slight	3.14	slight	3.50	moderate

3.2 Expert view analysis (EVA)

3.2.1 Laboratory check of field - derived data

This section effectively constitutes a quality control check of the expert field analyses. Comparison with the whole sample as subsequently determined in the laboratory is reported in Section 3.2.2.

Various techniques were used in the field to identify and enumerate part of or the complete sample. These included a restriction on the time for analysis (15, 20 or 30 minutes), splitting into different size fractions (1.0 mm, 0.5 mm) and the use of staff with varying degrees of experience (beginner, intermediate or expert). The results are summarised in the following sections according to the sampling location. This is followed by a general overview of these results.

3.2.1.1 Messack

Identification of the fauna in samples ME8, ME9, ME10, ME11 and ME13 was not attempted in the field. In these cases only one taxon was identified and this, together with animals removed during a 15 or 20 minute in-field "picking" session, was analysed by staff at IECS. Primary community variables are shown in Table 3.2.1. Field data for samples ME2b-15 min, ME2d and ME6 are presence/absence so comparisons of abundance are not possible. Sample 2a was split into two fractions; data from field identification are (mostly) numeric and a comparison of these is shown in Table 3.2.1 and included in the general overview. In this sample additional animals were extracted in the field but not identified and these are shown in Table 3.2.1 on a separate line.

Table 3.2.1. Summary statistics for Messack field identification. Proportion of total species richness (S) and Abundance (A) are calculated wherever possible

			No. of taxa (S)			Abundance (A)		
Lab ref	**Level of experience**	**picking time (mins)**	**Field**	**Lab**	**%**	**Field**	**Lab**	**%**
ME2a	Expert	?	14	18	77.8	21	28	75.0
ME2a	n/a	n/a		33			93	
ME2b	Intermediate	15	4	14	28.6	n/a	61	
ME2b	Intermediate	30	1	10	10.0	n/a	32	
ME2d	Intermediate	15	5	21	23.8	n/a	48	14.6
ME2d	?	30	?	15		n/a	24	
ME6	Expert	?	12	32	37.5	n/a	101	9.9
ME8	Beginner	15	1	32	3.1	7	106	6.6
ME9	Expert	15	1	29	3.4	2	106	1.9
ME10	Expert	20	1	17	5.9	~100	58	
ME11	Expert	15	1	9	11.1	~100	58	
ME13	Expert	15	1	21	4.8	~100	77	

Note - IECS was supplied with sample for 2d (30 mins) but field data are not available

3.2.1.2 Malpas

The Malpas samples were analysed using a 0.5 mm screen. The 0.5 mm fraction was saved in the field and combined with the 1.00 mm fraction before laboratory analysis. In both samples the smaller fraction was noted as containing *Corophium*? and oligochaetes. Gross comparative statistics using combined size fractions are shown in Table 3.2.2. As with most Messack samples the field data were predominantly presence/absence and therefore not amenable to comparisons of abundance. The sample picking was not timed, the whole sample being worked up in the field.

Table 3.2.2. Summary statistics for Malpas field identification

			No. of taxa		Abundance	
Lab ref	**Level of experience**	**picking time (mins)**	**Field**	**Lab**	**Field**	**Lab**
M2	Intermediate	n/a	5	3	1	36
M5	Intermediate	n/a	5	5	<100	18

3.2.1.3 Restronguet Creek

Two samples were processed from this site (Table 3.2.3). One specimen of *Abra* sp. was found in field analysis of RE10 and three taxa were recorded as present in RE12. Picking time is not known for these sites.

Table 3.2.3. Summary statistics for Restronguet Creek field identification

			No. of taxa		Abundance	
Lab ref	**Level of experience**	**picking time (mins)**	**Field**	**Lab**	**Field**	**Lab**
RE10	Intermediate	unknown	1	3	1	18
RE12	Intermediate	unknown	3	4	0	13

3.2.1.4 Ruan Creek

Field data for three samples are available for comparison (Table 3.2.4). Most of the fauna from sample RU2 appears to be missing as only 1 bivalve was found in the laboratory analysis. Sample RU4 was picked initially by an intermediate level taxonomist, re-combined and then picked again by an expert. Large numbers of mysids were found in sample RU8 but not included in the container for analysis. IECS has analysed sample RU11 but no field data are available.

Table 3.2.4. Summary statistics for Ruan Creek field identification

			No. of taxa		Abundance	
Lab ref	**Level of experience**	**picking time (mins)**	**Field**	**Lab**	**Field**	**Lab**
RU2	Expert	?	4	1	25	1
RU4	Expert	15	8	8	123	106
RU4	Intermediate	15	6	n/a	14	n/a
RU8	Expert	?	4	3	17	15
RU11	?	?	?	1	?	9

3.2.1.5 General overview

The foregoing sections briefly compare samples worked up in the field with the same material analysed in the laboratory. The majority of samples produced non-count data thus preventing comparisons of numerical abundance. Those samples with numerical data are compared at the end of this section.

There were 11 samples in which field identified species richness (i.e. number of taxa) can be compared with laboratory data. Five of these were identified at expert level (ME2a, ME6, RU2, RU4 and RU8) and six at intermediate level (ME2b-15 min, ME2d, M2, M5, RE10, and RE12).

Expert level identification achieved a mean of 83% (n = 5) of species richness against that determined in the laboratory. Two expert level samples found fewer species than revealed by later laboratory analysis (see Table 3.2.1). These were Messack 2a and Messack 6 which found 78% and 37% of laboratory determined taxa respectively. Missed taxa were as follows:

- Polychaeta: *Praxilella* sp. (from sample 2a), *Sthenelais boa, Hypereteone foliosa, Aphelochaeta marioni, Chaetozone gibber, Mediomastus fragilis* and *Euclymene* sp (all from sample 6);
- Oligochaeta: *Tubificoides benedii* and *T. cf. galiciencis* (both sample 6);
- Crustacea: *Cheirocratus sundevallii* (sample 2a), *Apherusa ovalipes, Ampelisca sp. Ampithoe ramondi, Corophium sextonae, Astacilla longicornis, Leptochelia dubia, Tanaopsis gracilioides* (sample 6);
- Mollusca: *Moerella pygmaea, Venerupis senegalensis* (sample 2a);
- Phoronida: *Phoronis* sp. (sample 6).

The converse situation applies to the other expert level samples taken in Ruan Creek where more (or the same number of) species were identified in the field than in laboratory analysis

(see Table 3.2.4). However, most material from RU2 (*Nereis* sp., *Polydora* sp. and *Hydrobia ulvae*) was missing from the container (as can be seen by the lower abundance detected in the laboratory), thus indicating a problem created by sample handling in the field. In RU8 the presence of *Neomysis integer* was noted during fieldwork but none was included in the sample.

Expert workers were successful in identifying annelids such as *Nematonereis unicornis, Scoloplos armiger, Scalibregma* spp. *Melinna palmata, Cirriformia tentaculata* and *Polydora* spp. but, understandably, other taxa proved more difficult. There were four species of nereid polychaetes in the samples (*Neanthes irrorata, Perinereis cultrifera, Platynereis dumerilii* and *Hediste diversicolor*) but these were recorded as *Nereis* sp. Nephtyidae is a closely related family which is also difficult to identify to species level in the field. Two species were present in expert level samples (*N. hombergii* and *N. kersivalensis*). In one instance (RU4) a *Nephtys* sp. appears to have been mistaken for *Nereis* sp. Two species of sabellid polychaete (*Megalomma vesiculosum* and *Sabella pavonina*) were identified at family level only, reflecting the difficulty of identifying these in the field.

Small crustaceans (amphipods, isopods and tanaids) were problematical and this is reflected in the list of missing taxa shown above. In sample ME2a an amphipod was recorded as *Ampelisca* sp. but only *Cheirocratus sundevallii* was found during laboratory inspection. *Ericthonius* spp. were successfully identified in sample ME6 but these may have included two species - *Ericthonius punctatus* and *Aora gracilis*. *Melita palmata* was correctly assigned in sample RU4. The larger crustaceans should be easier to identify and in these samples the shrimp *Crangon* sp. was identified correctly in the field.

Molluscs (*Hinia reticulata, Littorina littorea, Hydrobia ulvae, Cerastoderma edule, Parvicardium exiguum, Abra alba, Scrobicularia plana* and *Tapes decussatus*) were correctly identified. In one Messack sample (sample ME6) *Mysia undata* had been misidentified as *Chamelea gallina.*

Intermediate level identification achieved a mean of 60% (n = 6) species richness when compared to laboratory analysis. One sample from Malpas (M2) had more field identified taxa than found in the laboratory with *Crangon*, *Cerastoderma* and a Sand Goby being absent from the sample container.

The taxa missed in the field were as follows:

- Polychaeta: *Pholoe balthica* (2b-15 min), *Pholoe inornata* (2d), *Sthenelais boa* (2b-15 min, 2d), *Eteone longa/flava* (2d), *Nephtys hombergii* (RE10), *Nephtys kersivalensis* (2b-15 min, 2d), *Nematonereis unicornis* (2b-15 min, 2d), *Aphelochaeta* spp. (2b-15 min, 2d), *Cossura longocirrata* (M5), *Caulleriella alata* (2d), *Cirriformia tentaculata* (2d), *Chaetozone gibber* (2b-15 min, 2d), *Monticellina* sp. (2d), *Mediomastus fragilis* (2b-15 min, 2d), *Notomastus latericeus* (2d), *Praxilella* sp. (2b-15 min, 2d), *Melinna palmata* (2b-15 min, 2d);
- Crustacea: *Ampelsica* spp.(2b-15 min, RE10), *Maera grossimana* (2b-15 min), *Cheirocratus* spp.(2d), *Phtisica marina* (2d), *Leptochelia dubia* (M2);
- Mollusca: *Abra alba* (2d);
- Phoronida: *Phoronis* spp. (2b-15 min).

No annelids were successfully identified to genus or species by intermediate level workers. *Perinereis cultrifera* was identified as *Nereis* spp. (sample 2d) and worms identified as juvenile *Nereis* spp. in the field were in fact juvenile *Nephtys* spp. (M5 and RE12). In the Malpas samples oligochaetes were recorded in both size fractions but were not found to be present under microscope examination. These were mostly *Tharyx* spp.

The few small crustaceans in the intermediate level samples were mostly missed. *Corophium* was recorded (sample M2) but cannot be safely assigned to species in the field. Three crabs from Messack sample 2d were thought to be *Carcinus maenas* but were found to belong to the closely related (and, as juveniles, morphologically similar) *Liocarcinus arcuatus*.

The molluscs *Crepidula fornicata* and *Parvicardium exiguum* were accurately identified in the field (Messack 2d). The bivalve genus *Abra* was correctly identified in Messack 2b-15 min and RE10. In the former sample this was subsequently identified as *A. alba* and in the latter as *A. nitida*. *Cerastoderma* sp. in sample RE12 was later assigned to *C. edule* and a juvenile *Cerastoderma* sp. in sample M5 was later ascribed to *Parvicardium ovale* in the laboratory. Phoronids were missed in Messack 2b-15 min.

Three expert level samples were amenable to basic numerical analysis but there was insufficient replication for significance testing between field-derived data and laboratory checks. Results can be seen in table 3.2.5.

Table 3.2.5. Comparison of field-derived data with subsequent laboratory analysis

	S	N	Shannon (log_2) (H′)	Bray-Curtis similarity
Messack 2a (Field)	14	25	3.67	41.5
Messack 2a (Lab)	18	28	4.01	
Ruan RU4 (Field)	8	123	1.51	95.0
Ruan RU4 (Lab)	8	115	1.42	
Ruan RU8 (Field)	3	17	1.25	0.0
Ruan RU8 (Lab)	3	15	1.29	

Species richness (S), and the diversity statistics H′ were similar for expert level field identification and subsequent laboratory analysis. These univariate measures do not retain the identity of the species involved and so qualitatively different samples may produce similar results. As an indication of how similar the analyses were in terms of species identified the Bray-Curtis similarity index was calculated (using untransformed data). Results were variable, RU4 (8 taxa) showing a high degree of concurrence but RU8 (only 3 taxa) showing no similarity.

3.2.2 Comparison of field - derived data with whole sample laboratory data

Field-derived data can be compared with whole sample data in six instances, two of which (Messack 2a and Ruan Creek RU4) have numerical data from field analysis. Data from all these samples are summarised in Table 3.2.6.

Table 3.2.6. Comparison of field data with whole sample analysis

Sample	H′	No. of taxa (S)	Abundance (N)
Messack 2a whole sample	3.84	72	1119
Messack 2a field data	3.67	14	21
Messack 2b whole sample	n/a	70	1140
Messack 2b field data	n/a	4	n/a
Messack 2d whole sample	n/a	84	1577
Messack 2d field data	n/a	5	7
Messack 6 whole sample	n/a	73	1653
Messack 6 field data	n/a	12	n/a
Malpas M2 whole sample	n/a	18	1599
Malpas M2 field data	n/a	5	n/a
Ruan RU4 whole sample	2.74	23	566
Ruan RU4 field data	1.51	8	123

Field data consistently underestimated sample species richness, on average finding 18.3% (n = 6) of whole sample species richness. Comparison of abundance data does not apply as there was no attempt to quantify abundance in the field.

Output from cluster analysis can be seen in Figure 3.2.1. Three main clusters were distinguished on the dendrogram. Whole sample data from Messack sites ME6, ME2b, ME2d and ME2a clustered at a similarity of 59.6 (group B). The remaining whole sample analyses paired at a similarity of 48.8 despite being from different locations (Cluster A). Two field samples from Messack clustered at similarity 53.8.

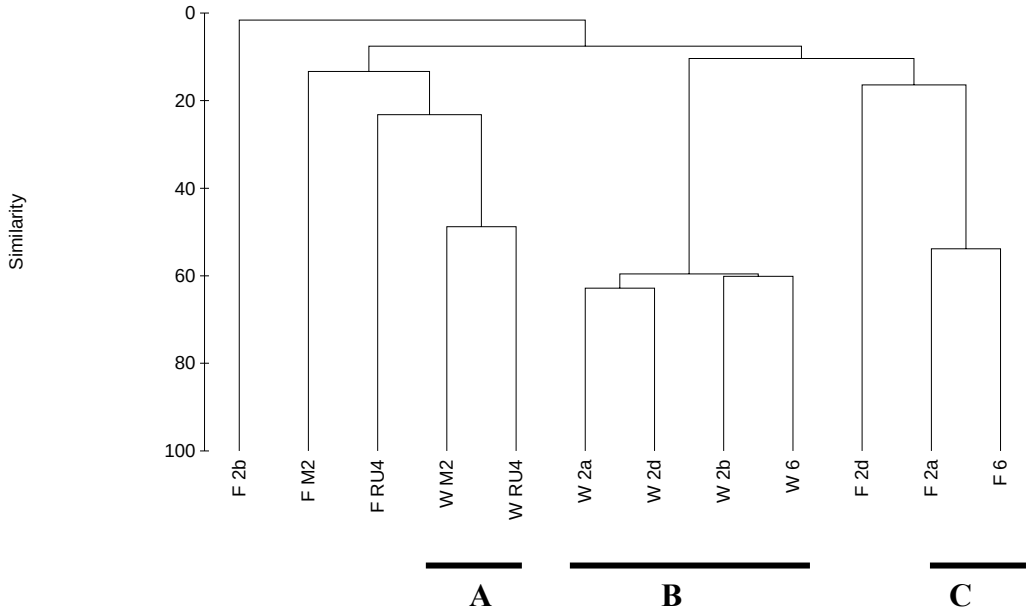

Figure 3.2.1. Dendrogram comparing field - derived data (F prefix) with laboratory analysis of the whole sample (W prefix). Bray-Curtis similarity on presence-absence data, group average clustering

Where numerical data were available for field identifications the Biotic Coefficient (Borja *et al.* 2000) was calculated and compared with the data from the full sample analysis. In each case the coefficient was higher when the full sample was analysed but in no case was the pollution classification altered (see Table 3.2.7).

Table 3.2.7. Results from Borja's Biotic Index analysis for data produced in the field and subsequently by laboratory analysis. BC = Biotic coefficient

	Identification in field		Laboratory analysis	
	BC	Pollution classification	BC	Pollution classification
ME2a	2.05	Slightly polluted	2.61	Slightly polluted
ME2d	2.78	Slightly polluted	2.96	Slightly polluted
RU4	2.96	Slightly polluted	3.18	Slightly polluted

3.3 Restricted laboratory analysis (RLA)

Results for the five RLA samples are given in Table 3.3.1. There was a large range in abundance and species richness, two samples (ME7 and RU10c) having at least 7x greater abundance than the rest. The extraction efficiency of the two sorting periods has been dealt with in section 3.1.

Table 3.3.1. Summary sample statistics for Restricted Laboratory Analysis showing results from analysis with 1.5x illuminator and subsequent microscopic analysis

		Microscope		Illuminator			
Sample	**Sorting time**	**N**	**S**	**N**	**%**	**S**	**%**
ME7	15 mins	94	30	95	> 100	21	70.0
	30 mins	179	37	180	> 100	31	83.8
	Whole sample	1355	60	587	43.3	45	75.0
RE3a	15 minutes	10	6	11	> 100	5	83.3
	30 minutes	16	9	17	> 100	6	66.7
	Whole sample	17	9	16	94.1	6	66.7
RE8	15 minutes	5	3	4	80.0	2	66.7
	30 minutes	10	5	9	90.0	4	80.0
	Whole sample	14	6	13	92.9	5	83.3
RU10c	15 minutes	75	6	75	100.0	5	83.3
	30 minutes	136	8	142	> 100	5	62.5
	Whole sample	443	14	593	> 100	10	71.4
RU7	15 minutes	18	9	15	83.3	7	77.8
	30 minutes	40	10	29	72.5	8	80.0
	Whole sample	65	14	49	75.4	10	71.4

In classification analysis the high abundance samples differed from the low abundance ones in the pattern of relationships between their various "treatments" or sub-components. The basic pattern was imposed by the initial sorting period. This can be seen in Figure 3.3.1 where high and low abundance samples resolved into separate groups (i.e. the high abundance cluster A + B and the low abundance cluster C + D). In both cases these clusters were defined with very low similarity. However, within the low abundance samples the longer sorting period converged towards the whole sample data (i.e. 30 minute data paired with whole sample data in the dendrograms) whereas in the high abundance samples the two restricted sorting periods were more similar to each other than the whole sample data. With high abundance samples the results of both sorting periods were sufficiently similar to cluster each sample as a single entity, i.e. as cluster A (ME7) and cluster B (RU10c) (see figure 3.3.1). However, in the case of samples with low species richness and abundance all illuminator analyses were combined as one group (Group C Figure 3.3.1) distinct from the microscope analyses which formed Group D. This implies that illuminator data from low abundance samples bear less resemblance to the actual sample data (i.e. microscope data) than in high abundance samples (ME7 and RU10c).

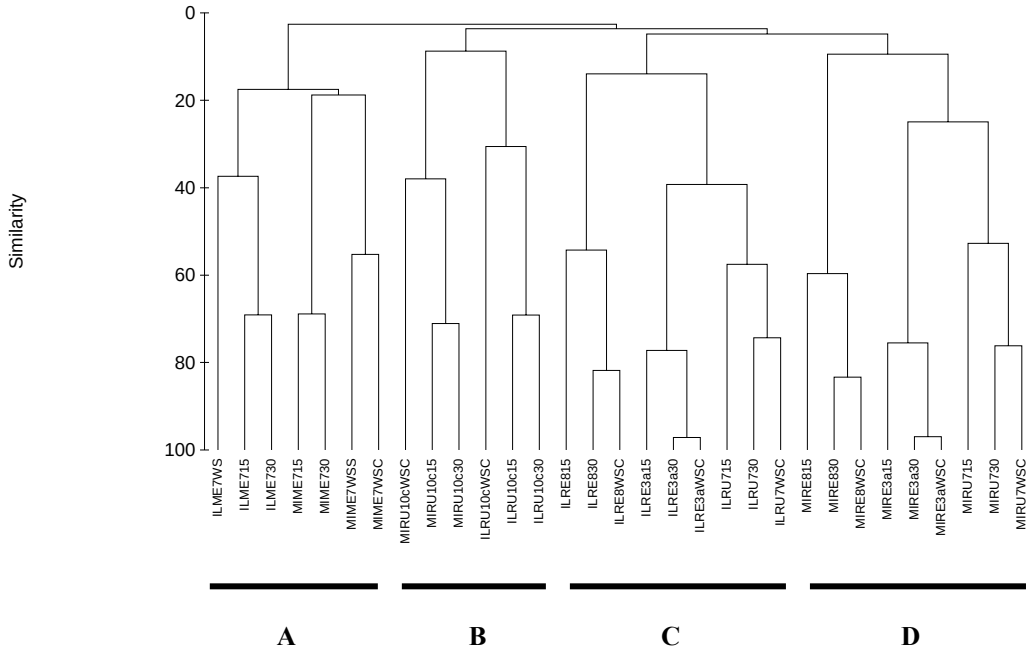

Figure 3.3.1. Cluster analysis of RSA samples. IL prefix designates data from magnifying illuminator and MI prefix denotes data from microscope analysis. "Treatments" are either 15 minute sort, 30 minute sort or whole sample analysis using compound microscope when required (15, 30 and WSC suffixes respectively). The suffix WSS in sample ME7 denotes that only a stereo microscope was used in production of the dataset

In many cases the use of a 1.5x magnifying lens prevented identification to species level and was therefore generally similar to identification at genus or family level. Examples of such "conservativeness" include the designation of *N. hombergii* and *N kersivalensis* as *Nephtys* spp (RE3a), *Tubificoides ?galiciensis* and *T. benedii*, as Oligochaeta spp (RE3a), *Aphelochaeta marioni*, *Caulleriella alata*, *Monticellina cf dorsobranchialis* as Cirratulidae (ME7) and *Polydora cornuta*, *Pygospio elegans* and *Streblospio shrubsolii* as Spionidae spp. (RU10c). For this reason microscope analysis invariably revealed more species than identification by illuminator (see Table 3.3.1). Samples containing large numbers of cirratulids proved difficult to analyse under low power because of the difficulty in distinguishing between tentacular filaments or branchiae and smaller worms.

Some species were sufficiently distinctive to identify with the magnifying lens: *Nematonereis unicornis, Melinna palmata, Abra alba* (ME7); *Abra nitida* (RE8); *Hydrobia ulvae, Cerastoderma edule* (RU10c); *Cyathura carinata* (RU7).

In sample ME7 a microscopic identification was carried out first using a stereo microscope only (MIME7WSS in Figure 3.3.1). This gave results similar to the subsequent analysis during which further taxonomic resolution was obtained with a compound microscope. The compound microscope allowed the different species of *Pholoe, Ampithoe* and *Corophium* to be identified and also gave higher precision in the identification of species in the Cirratulidae, Capitellidae and Tubificidae.

The Biotic Coefficients of samples, using magnifying lens and microscopes, are shown in Table 3.3.2. In each case, when the whole sample was analysed, the method of identification did not affect the assignment of pollution classification. However, with a 30 minute timed subsample the low power magnification data classified ME7 as "moderately polluted" when microscope analysis would have indicated only slight pollution. The same re-classification occurred in samples RE3a, RU7 and ME7 with the smaller, 15 minute subsample.

Table 3.3.2. Results from Borja's Biotic Index analysis for samples in which invertebrates were identified using a 1.5 x magnifier and then with full use of microscopes. Subsamples from 15 and 30 minute timed sorting are also shown. BC = Biotic coefficient

		Timed sorting (15 mins)		Timed sorting (30 mins)		Whole sample analysis	
		BC	Pollution classification	BC	Pollution classification	BC	Pollution classification
RE3a	Magnifier	3.67	Moderate	4.20	Moderate	4.31	Moderate
	Microscope	3.30	Slight	3.94	Moderate	4.06	Moderate
RE8	Magnifier	1.87	Slight	1.50	Slight	1.85	Slight
	Microscope	1.87	Slight	1.50	Slight	1.85	Slight
RU7	Magnifier	3.50	Moderate	3.37	Moderate	3.66	Moderate
	Microscope	3.25	Slight	3.35	Moderate	3.75	Moderate
RU10c	Magnifier	5.38	Heavy	5.52	Heavy	5.55	Heavy
	Microscope	5.42	Heavy	5.44	Heavy	5.37	Heavy
ME7	Magnifier	3.67	Moderate	3.37	Moderate	3.54	Moderate
	Microscope	3.18	Slight	3.14	Slight	3.50	Moderate

4. Discussion

Many published papers have addressed ways of streamlining the processing of marine and estuarine benthic samples (see, for instance, James *et al.* 1995; Thompson *et al*. 2003 and references therein). These generally adopt two approaches:

- to show that a large mesh (usually 1.0 mm) is as discriminative as a small mesh (0.5 mm), so enabling an investigator to do away with the extra time and effort involved with sorting and identifying smaller species and juveniles;

- to perform the analysis at a higher taxonomic level (e.g. genus or family) thus avoiding the necessity of identifying each individual to species level (e.g. Warwick, 1988; Somerfield & Clarke, 1995).

The choice of mesh size is primarily dictated by the purpose for which the data are being collected or the size of any specific target taxon (Kingston & Riddle, 1989; Bachelet, 1990; Schlacher & Wooldridge, 1996). Analyses at higher taxonomic levels give results which vary with the classification of the group of animals under consideration and the habitat type. There appear to be no ground rules for choosing an appropriate taxonomic level *a priori*.

The three methods reported here (TSA, EVA, RLA) are somewhat different in approach and, as far as is known, have not been previously described in the mainstream scientific literature. Time restricted sampling has been developed for lotic freshwater environmental assessment (Predictive System for Multimetrics or PSYM), but here the restriction is on sampling effort and not on sample processing (Anon. 2000). TSA and EVA address the problem by imposing a strict time limit on sample sorting (in the field and laboratory) and by placing constraints on identification by prohibiting the use of microscopes. The former creates non-random, fixed-size subsamples (as opposed to random, proportional subsamples) and the latter will tend to produce data at high taxonomic levels.

TSA greatly reduced the sorting time that would otherwise be needed to extract 95% of the fauna from the sample (the level stipulated in NMBAQC standards). Subsamples produced in this way generally did not resemble their 'parent' when the parent sample had high abundance and species richness. With these samples, the brevity of sorting time inevitably led to large differences between subsample size and sample size. This effect can be seen in the TSA of the Messack samples in Figure 3.1.2 where TSA samples formed a completely separate group (A + B + C) to the fully analysed samples, and in the high abundance cluster in Figure 3.1.3 where the time-restricted samples were more similar to each other than to their respective 'parent'. With a reduced sample abundance and fewer species, time-restricted subsamples more closely resembled the full sample (see here the low abundance cluster in Figure 3.1.3 where the 30 minute sort was closer to the 'parent' than the 15 minute sort). This is because a fixed sorting time will more closely approximate the actual time required for full sorting when there are fewer organisms to extract.

How closely a timed sample will come to resemble traditional data will depend on the species richness and abundance of the sample. In species-rich areas TSA may lead to differences in interpretation of community and habitat boundaries when compared to full analyses because a more restrictive analysis will produce different relative abundances and a

reduced species richness by overlooking rarer species. For example, in the Messack area, TSA effectively removed sample ME12 from Cluster E and samples ME2a, ME2b and ME2c from cluster D (Figure 3.1.2) producing clusters B and A respectively and thus altering the shape of a "traditionally" defined assemblage as it would have been mapped on the ground.

Borja's Biotic Coefficient was resilient to timed subsampling and only one sample sieved on a 1.0 mm mesh was re-classified (as more polluted) following a full analysis. With a smaller mesh three out of five samples were given more polluted status by full analysis. However, these were from different locations and so these re-classifications may not be a function of sieve size but may reflect differences in habitat type or pollution status.

Restricting time for sorting also has other practical implications. The sorter must have sufficient experience to be able to assess the sample and rapidly pick out a representative selection of fauna. Even with experience there is always the possibility that some taxa may be preferred over others, thus making it very difficult to achieve repeatability in future analyses. In this study most of the fauna appears to have been selected on the basis of conspicuousness (either size or an interest feature, such as an easily recognised tube) which has led in some cases to highly abundant animals being ignored (for instance *Tubificoides benedii* in MS9 and *Mediomastus fragilis* in ME10 and ME12 - see Table 3.1.4). Unfamiliar species may also be missed (e.g. *Protocirrineris* - ME2d). In any future investigations (and before analysing samples from a new area or habitat) it would be advisable to sort a series of abundant samples for successive periods of (say) 15 minutes until all the fauna has been removed. This would create data resembling a species - area curve from which the most acceptable sorting time could be estimated.

The field data of EVA have necessarily been treated in a more qualitative way. The potential for accuracy in the field was seen in a Ruan Creek sample (RU4) where a Bray – Curtis similarity of 95% with the laboratory check analysis was achieved. This degree of accuracy is unlikely to be seen in the majority of cases, (especially in estuarine muds) and will depend greatly on the experience of the field worker and the diversity of the sample. Field conditions are not always conducive to detailed observational work which may be hindered by poor lighting and adverse weather. Although in this case the field identification of selected animals was accurate, there was still little resemblance to the whole sample when analysed in the laboratory (Table 3.2.6). There were insufficient EVA samples with numerical data in this study to perform any meaningful analysis or to detect any trends. However, the exercise is worth repeating to determine the potential accuracy of field evaluation. As with laboratory TSA there will be problems with the repeatability of results (among individual workers, from year to year and from place to place) and also, because these are again non-random, fixed size subsamples, their representativeness will depend on the species richness and abundance of the 'parent' sample.

Accurate identification using low power magnification (either in the field or laboratory) is dependent on the experience of the biologist concerned. Basic invertebrate morphology is taught in universities but the emphasis has moved away from detailed comparative zoology with the result that biologists with strong taxonomic knowledge and skills are in short supply. Even someone experienced at microscope identification of preserved material may not be sufficiently competent on live animals (which are seldom studied) without appropriate training. The outcome here suggests that using a 1.5x magnifier in the laboratory was sufficient to identify relationships between samples (Figure 3.3.1) if sufficient expertise was available. The differences between high and low abundance samples as noted in Section 3.3

were probably the result of the statistical sampling effect already alluded to. Field identification was inconclusive and bore less resemblance to fully analysed samples (Figure 3.2.1). The Biotic Coefficient was again resilient to the identification method used. In this case the sample differences were due to the reduced taxonomic resolution achievable with low power magnification, and this did not result in major differences in Biotic Coefficient as congeners are frequently assigned to the same pollution category. For instance, all species of *Ampelsca* (and hence also the genus) are assigned to Group I (even though there is evidence that *A. sarsi* might be more resistant to oil spills than other species – Dauvin *et al.* 2003)

It would not be appropriate to draw firm conclusions about the use of a magnifier without further replication and attention to experimental design. For instance, in this exercise much of the microscope analysis of TSA samples was undertaken before trying to identify fauna with the magnifier in RLA. The operators therefore had prior knowledge of precisely which species to expect and this may have improved results considerably.

Some form of restricted sorting combined with a less rigorous (microscope - free) approach to identification may have potential as a rapid assessment tool in areas of low abundance and diversity (the limits of which are yet to be defined and which may be difficult to assess *a priori*). It may also be used in situations where abundance and diversity are higher but with the understanding that results will reflect traditional analyses less faithfully (although it is recognised that a rapid assessment analysis is not intended to accurately describe a community).

The main drawbacks of restricting analysis time and eliminating microscopes are poor repeatability or consistency and lack of statistical rigour. Repeatability may be improved by confining analyses to one laboratory or group of experts, or by instituting a training schedule or series of workshops (similar to those set up under the NMBAQC scheme) through which consistency could be improved. However, the simple imposition of standard sorting times will not produce random subsamples. The ultimate aim of TSA is to reduce the size of the sample to be analysed. This is better achieved through conventional, well-established subsampling techniques (e.g. Elliott, 1977) in which the subsample size could be adjusted according to the total sample abundance (or to habitat or biotope) so keeping the processing time down to a minimum. Analysed in the conventional way, these subsamples will provide accurate species level data free from bias introduced through timed sorting. This small sampling unit would probably be insufficient to estimate the populations from which it was taken but this is often also the case with the 'parent' sample (and any replicates).

An alternative approach to "sample volume" subsampling would be to investigate random sequence techniques as advanced by Cairns *et al*. (1968) and adopted for nematode work by Moore *et al.* (1987) In this technique a fixed number of randomly selected animals is removed from the sample. Each is compared with its predecessor and runs of similar species are then analysed statistically.

In many cases an experienced worker will have difficulty identifying animals with a 1.5x magnifying lens especially with difficult groups such as cirratulids and spionids, or when specimens are damaged. Often, only the family or genus level can be assigned tentatively. The quality of the data may be improved, however, with the use of a stereo microscope with which genus can often be rapidly determined without recourse to reference works or a compound microscope. Further investigation of scaled, random subsampling followed by genus (or family) identification using a stereo microscope may therefore be appropriate for rapid assessments.

5. References

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