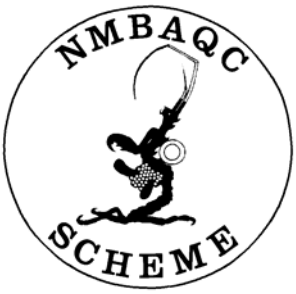

National Marine Biological Analytical Quality Control Scheme

Oligochaeta Questionnaire Report:
Including Provisional NMMP Standard Policy for Oligochaete Identification.

Client — NMBAQC Scheme
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Report NMBAQColigquest
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1. Introduction

The exercises of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme conducted over the past eight years have shown that there is little or no consistency in recording between laboratories (Worsfold & Hall, 2001). Oligochaetes are identified to a variety of taxonomic levels by the participants and standardisation is required. Ring test nineteen (RT19) was selected to target 'oligochaetes and similar fauna' and assess comparative levels of identification. In addition to the ring test, all participating laboratories were sent a questionnaire to enable the ring test results to be qualified and to gather general information on levels of oligochaete identification.

RT19 comprised twenty-five single specimens and was distributed to eighteen ring test participant laboratories on 17th January 2002. One cirratulid and two capitellid species were included in the ring test due to their oligochaete-like features. Nine Tubificidae species were distributed, including repeated taxa. They accounted for seventeen of the twenty-two oligochaetes. The remaining five oligochaete specimens were repetitions of two Naididae species. All oligochaetes distributed within RT19 were readily identifiable on gross morphological features. Unfortunately the original intention to send oligochaetes from a variety of habitats was hindered as no external expert could be appointed within the timescale required. This meant that the expert help required to assist in the compilation of enchytraeids and *Tubificoides pseudogaster* aggregate species was lacking. The three non-oligochaete ring test specimens were included to highlight the problems associated with laboratories that do not routinely identify oligochaetes beyond class and the potential problems of these laboratories not being able to distinguish between oligochaetes and some polychaetes. Habitat notes were provided for each specimen (sediment, salinity, depth and geographical location). The participating laboratories were given ten weeks to complete RT19. Results were received from ten of the eighteen participants.

This report reviews the questionnaire returns to give an overview of current approaches to oligochaete identification amongst the NMBAQC Scheme participants. Reference is made, where relevant, to the RT19 results. Recommendations for National Marine Monitoring Plan (NMMP) standardisation are given, where appropriate, as a precursor to standard operating procedures (SOPs). SOPs in marine biological sample collection and analysis were reviewed for the NMBAQC Scheme by Cooper & Rees (2000). However, that report focussed primarily on sampling methods and safety and did not deal with all issues concerning the fundamental requirements of processing of macrobenthos samples (Worsfold & Hall, 2001).

Few agencies or other organisations that commission samples for analysis of macrobenthos give clear guidelines as to the required treatment of samples. Laboratories that carry out sample analysis generally develop their own in-house practices. The practices are often not explicitly written down but become established through tradition. As the agencies requiring data do not give clear guidelines and as they often subcontract their sample analysis to more than one laboratory, it is important to evolve and maintain consistency of practice between laboratories.

Consistency is particularly important where data collected by different organisations are to be used for comparative purposes, as with the NMMP (Worsfold & Hall, 2001).

2. Methods

The RT19 data returns were scored and a ring test bulletin (RTB19 – Appendix I) was posted on the Scheme web site (www.nmbaqcs.org) and circulated to participating laboratories. RTB19 gave reasons for differences in identifications and participating laboratories were instructed to retain the RT19 specimens for review in light of these results. All specimens will subsequently be returned to Unicomarine Ltd., as they are part of an in-house museum collection and may be included in future ring tests to assess participant development.

On 2nd April 2002, a questionnaire (Appendix II) was sent, via email, to nineteen participants of the ring test component of the NMBAQC Scheme. Reminders for outstanding questionnaires were circulated on 11th and 16th April 2002. The purpose was to evaluate the expertise level, policy and techniques for oligochaete identification between different laboratories that carry out NMMP macrobenthos sample analysis and also to qualify the results obtained in RT19.

Section one of the questionnaire contained questions that were designed to determine how often oligochaetes are encountered and the current approaches for identification, including use of literature and reference specimens. The second section of the questionnaire comprised questions directly related to RT19 and was for completion only by laboratories that had supplied data for this exercise. The questions sought to ascertain the methods used, time taken and difficulty experienced in completing RT19. The questions from the questionnaire are quoted in the text below with question numbers in brackets.

2.1 Current Data - Quantity and Quality

The quality and quantity of oligochaete data residing on the NMMP database can be inferred by comparing current laboratory protocols for oligochaete identification and how often oligochaetes are encountered.

Two questions on the form (stated below) were concerned with the quantity and quality of oligochaetes encountered in participants usual samples:

> "How often do you encounter oligochaetes in your macrobenthic samples?" (Q.1A)
>
> "At what taxonomic level do you normally identify oligochaetes? Give qualifying comments (e.g. species but Enchytraeids to family)" (Q.1B)

Both questions gathered quantifiable data, with space provided for qualitative comments.

2.2 Importance of Oligochaete Identification

Many laboratories may have preconceived ideas regarding the importance of oligochaete identification and this may affect their approach. We asked laboratories to describe how important they considered oligochaete identification:

> "How important do you consider oligochaete identification? Add comments" (Q.1C)

The responses were to be free form to permit discussion.

2.3 Identification Tools - Experience, Methods, Training and Literature

The ability to correctly identify oligochaetes to species is often a combination of many factors, such as experience, training, access to and understanding of literature, availability of equipment and chemicals, access to verified reference specimens and an understanding of the ecological requirements of different oligochaetes. These can all be described as identification tools. It is important to discover and understand which tools are being applied to identify oligochaetes and which are not. Participants were asked to rank keys/tables, publication/descriptions, reference material, experience/memory and habitat information in order of importance:

> "Place the following identification aids in rank order of importance for oligochaete identification at your laboratory" (Q.1D)

The availability and relevance of literature is suspected to be a major problem for ecologists identifying oligochaetes, therefore the participants' opinions were sought:

> "Do you find your oligochaete literature adequate? Add comments" (Q.1E)

The level of training and experience in oligochaete identification greatly affects the identifiers confidence with oligochaetes and the literature. Two questions were associated with training and ranking experience:

> "Did you attend the 1994 Oligochaete workshop hosted by Unicomarine Ltd.? Add comments" (Q.1F)

> "How would you rank your experience with oligochaete identification?" (Q.1G)

Oligochaetes are often dismissed for detailed examination due to the time constraints involved with compound microscopy and clearing specimens. Hence the time available for identification may directly relate to the method of examination employed. Laboratories may have opted not to use compound microscopy and or clearing techniques and consequently decided to identify to either family or class on the basis of economics. Participants were asked to select which methods they normally use for oligochaete identification from stereomicroscopy of gross morphological features, compound microscopy of temporary mounts for chaetal examination and compound microscopy of permanent cleared mounts for examination of internal anatomy:

> "What methods do you normally use for oligochaete identification?" (Q.1H)

2.4 Ring Test

The second section of the questionnaire comprised eight questions relating to the completion of the ring test (RT19). This section was only to be completed by laboratories that provided data for the 'oligochaete and similar fauna' target ring test.

All ring test participants received accompanying habitat notes for each of the specimens to be identified. The usefulness of the habitat notes was investigated as the availability of literature and ecological notes for oligochaetes is often perceived to be poor:

> "Did you find the habitat notes supplied with the ring test useful? Give comments" (Q.2A)

All of the oligochaete ring test specimens could be identified using gross morphological features and examination of chaetal structures using a compound microscope. There should, therefore, have been no need for clearing procedures and subsequent examination of internal structures (penes and other reproductive systems). Participants were asked to provide specific details of any clearing undertaken:

> "Did you clear any of the ring test specimens? Give numbers, examples and reasons" (Q.2B)

The use of verified comparative material when identifying specimens is extremely helpful. The maintenance of reference material is considered to be a standard requirement for identification and is promoted as best practice. Participants were asked to detail which of the ring test specimens they identified with the aid of comparative specimens:

> "Did you use reference material to assist your identifications? Give examples" (Q.2C)

Oligochaetes are perceived by many ecologists as time consuming and difficult to identify to species. The resultant identifications can commonly be qualified with uncertainty. Several questions were asked to determine this information:

> "How long did the ring test take to complete?" (Q.2D)

> "How many people were involved in the ring test identifications?" (Q.2E)

> "How difficult did you find the ring test?" (Q.2F)

> "How many of the 25 RT specimens do you think you identified correctly to species?" (Q.2G)

Finally, we asked participating laboratories to provide any further comments relevant to the ring test and suggestions for future target ring tests:

> "Please use the space below if you have any further comments regarding the ring test, or suggestions for future target ring tests" (Q.2H)

3. Results

The questionnaire was sent to nineteen laboratories, including government organisations and independent consultancies. All laboratories provided returns, which would have included some from the same organisation. RT19 was sent to eighteen laboratories. Nine laboratories decided not to participate in the ring test due to time constraints, this was the highest number of abstaining laboratories for any of the ring tests to date. However, the questionnaire that followed the ring test was returned by all ring test subscribers and one non-subscriber.

The responses for sections 1 and 2 of the questionnaire are presented in Tables 1A-1H and 2A-2H, respectively. These appear in the same order and format as found in the original questionnaire.

3.1 Current Data – Quantity and Quality

All participating laboratories encounter oligochaetes in their macrobenthic samples (Table 1A). The majority of respondents (53%) stated that they 'often' encountered oligochaetes in their macrobenthic samples; three laboratories 'always' encountered oligochaetes; two laboratories stated that they 'rarely' encountered oligochaetes.

Several permutations of levels of oligochaete identification were received (Table 1B). Two laboratories identify their oligochaetes to class, one of which stated that they rarely encountered oligochaetes. Three laboratories stated that they identify their oligochaetes to family, one of which stated that they rarely encountered oligochaetes. One of the laboratories that gave family level identification as their standard stated that the level of identification would normally depend upon client requirements and existing data. One laboratory indicated that family level identification would be used apart from easily recognisable species, such as *Tubificoides benedii* and *Heterochaeta costata*. The majority of laboratories (74%) stated that they would identify to species, wherever possible. Half of these laboratories added that they would identify enchytraeids to family or genus. One laboratory identifies naids to family. One laboratory stated that they identify naids to species, but tubificids and enchytraeids to family.

3.2 Importance of Oligochaete Identification

When asked to consider the importance of oligochaete identification the participating laboratories gave a variety of responses (Table 1C). Responses ranged from 'extremely important' to 'not important'. Two laboratories did not respond to this question.

3.3 Identification Tools – Experience, Methods, Training and Literature

Table 1D shows the ranked scores for importance given to the primary identification tools by laboratories. Twelve laboratories selected 'keys and tables' as their most important aid for identification, one ranked it as their least important (however, this laboratory has possibly confused the ranking system); one laboratory selected 'publications and descriptions' as their most useful identification aid, five stated them as least useful; four laboratories selected 'reference material' as most important, five

reported that it was least useful; three laboratories chose 'experience and memory' as most important, one chose it as their least useful; one laboratory chose 'habitat data' as most important, six listed it as least important.

Table 1E shows the participating laboratories' responses on the adequacy of their oligochaete literature. Ten laboratories stated that their oligochaete literature was inadequate, nine laboratories commented that their literature was adequate for family or local identifications. The majority of laboratories suggested several possible improvements. A full list of comments provided by responding laboratories is provided in Table 1E.

The 1994 Oligocheate workshop, hosted by Unicomarine Ltd., was attended by thirty-five delegates from various organisations. The chief demonstrator, Mike Milligan (Center for Systematics and Taxonomy, Florida), led practical classes on clearing techniques and general identification of Oligochaeta, concentrating mainly upon tubificids and especially *Tubificoides* spp. The workshop was extremely well received and confirmed geographical records of U.K. oligochaetes were compiled. A portfolio of workshop notes was produced containing several significant items of literature (Baker, 1983; Baker & Brinkhurst, 1981; Brinkhurst 1971, 1982, 1985 & 1986; Brinkhurst & Baker, 1979; Erséus, 1982). Most of the workshop participants felt that the Tubificidae features table version 2 (Unicomarine, 1994) was of particular use, which was reflected in the questionnaire responses regarding the adequacy of literature (Table 1E). Eleven of the respondent laboratories have had either current or past staff that attended the 1994 Oligochaeta workshop. Eight laboratories did not have any attendees, past or present, at the workshop but two of these laboratories did state that they have the workshop literature. Several comments were given regarding the workshop and resultant literature (Table 1F).

Table 1G shows how each of the participating laboratories rated their experience with oligochaete identification. All laboratories rated their identification experience as either little or reasonable.

Two participating laboratories when identifying oligochaetes study only temporary slide preparations to examine chaetal structure using a compound microscope. A combination of chaetal examination and studying gross morphological features using a stereomicroscope are the methods used by 89% of laboratories. Four laboratories stated that they would normally prepare permanent slide mounts in order to identify their oligochaetes using internal anatomy. Three laboratories stated that they would clear a subsample of oligochaetes for species differentiation and one laboratory noted that they would clear as a final method for identification when other methods are ineffective. The comments given by responding laboratories regarding identification methods are listed in Table 1H.

3.4 Ring Test

Nine of the ten RT19 participants found the habitat notes supplied useful, one did not. There was a range of comments regarding how useful the habitat notes were. Full details and comments are listed in Table 2A.

Three of the ten participant laboratories cleared single differing oligochaete ring test specimens (Table 2B) in order to examine internal anatomy in the absence of conclusive external features. Therefore in total only three specimens from a potential two hundred and twenty were examined for internal anatomy.

Table 2C lists the participants use of in-house reference material during completion of the ring test. Seven out of the ten laboratories stated that they had either no or limited oligochaete reference material available. One laboratory did not give reasons for not using reference material.

Tables 2D and 2E detail how many members of staff participated in the ring test from each laboratory and how long (in total) the ring test took to complete. One laboratory did not give a time for ring test completion. The ring test took between six and twenty-seven hours to complete, with an average duration of over thirteen hours (approximately two working days). This equates to an average of less than two identifications per hour. The highest number of staff involved in the ring test from a single laboratory was five. The ring test was completed by single individuals at six laboratories. The average number of staff participating from a single laboratory was two.

Table 2F shows how difficult the participants rated the ring test. The responses are clearly skewed towards 'hard', with no respondent classifying the ring test as easy. When asked to predict the number of correct species identifications attained eight out of ten participants underrated their abilities (Table 2G). The average RT19 score achieved was 69% correct species identifications. Only two laboratories predicted their species identification scores to be above 60%. The average predicted score was approximately 53%. Two laboratories correctly predicted their scores.

Table 2H gives the participants responses for further comments. Comments were made by seven laboratories. The majority of comments received were ring test result qualifying comments.

4. Discussion

The questionnaire data shows that all NMBAQC Scheme ring test subscribing laboratories encounter oliochaetes in their macrobenthic samples and that the majority of these laboratories attempt to identify most oliochaetes to species. However, a number of laboratories showed variations in their identification policies towards tubificids, naids and enchytraeids. These variations, although minor in many cases, when examined as combined data from all laboratories would result in a significant loss of specific detail. Two laboratories that normally would not identify their oligochaetes beyond class, achieved the lowest number of correct identifications for RT19. One such laboratory identified the *Capitella capitata* specimen as *Tubificoides amplivasatus*. Under normal macrobenthic processing conditions how many specimens could potentially be assigned to the wrong class? Such problems can arise when entire faunal groups are not examined or understood in sufficient detail. Gaps in faunal knowledge must be bridged to achieve data comparability. A major problem confronting analysts of combined data from several laboratory sources is that of having to reduce each taxon to the lowest common denominator (i.e. highest

taxonomic level). For example, an entry of 'Tubificidae' could result in all tubificids being lumped to family. However the Tubificidae specimen could have simply been in poor condition with no discernible features beyond the family level. A recording system should be agreed to counter the discrepancy. Identification consistency is important if data from different laboratories is to be compared, as is the case with NMMP data. There is a need for a standard policy for NMMP oligochaete identification.

There was an overwhelming indication that RT19 was found by participants to be very challenging although most achieved better results than they expected. Single oligochaete specimens are rarely easy to identify. This, coupled with many laboratories' discomfort with oligochaete identification, was reflected in their difficulty ranking for this exercise. This lack of confidence with oligochaete identification was reiterated by the participants' low predictions of their RT19 scores. Those laboratories that do not routinely encounter or identify oligochaetes must be commended for their participation in RT19. Several supposedly more experienced laboratories decided not to participate. The inexperienced laboratories invariably achieved the lowest RT19 scores. They are, however, very likely to have achieved disproportionate gains in knowledge, as compared with more experienced laboratories, particularly those that did not participate. The majority (six out of ten) of laboratories provided RT19 data produced by solitary workers. The practice of solitary identifiers is not recommended. Even experienced staff function much better with an additional staff member with which to discuss their identifications. An element of quality control / assurance can be achieved by such practice.

The habitat notes appear to have been of limited use, primarily due to a lack of available ecological information. Records of habitats need to be kept for verified oligochaete taxa in order to build a better understanding of specific requirements and distributions.

The results sheet for RT19 required laboratories to list any items of literature that were consulted for identification of each specimen. Several sources of oligochaete literature were noted in the data received. These were Brinkhurst (1971, 1982 & 1985), Brinkhurst and Jamieson (1971), Erséus (1975) and the 1994 Oligochaete workshop notes (which contained several Tubificidae papers and a Tubificidae features table). Some laboratories utilised just a single text which, in most instances, greatly reduced their capability to identify specimens correctly. The majority of questionnaire respondents commented upon the inadequacy of oligochaete literature. Several laboratories stated that the literature was too subjective. The comments can be summarised as a majority desire for a single Oligochaeta text containing marine, estuarine and freshwater taxa, which includes whole animal diagrams and / or images, comparative diagrams of chaetae, detailed descriptions, ecological notes and less reliance upon internal anatomy for identification.

The use of reference material to aid identification is universally understood by participants of the NMBAQC Scheme to be best practice. However, many laboratories have either no or very limited reference collections of oligochaete taxa. A positive correlation between the amount of reference material available and each laboratory's performance was evident in RT19. Those laboratories with little or no reference specimens invariably achieved the lowest number of correct identifications. It must

also be noted that laboratories with larger oligochaete reference specimens are likely to be more familiar with identifying oligochaetes and are consequently capable of relatively high ring test scores.

The majority of laboratories identify their oligochaetes using gross morphological features and temporary slide preparations for chaetal examination. Several laboratories stated that they do not find the clearing of oligochaetes to be an efficient use of time and the use of Ammans Lactophenol also raises health and safety (COSHH) issues. Four laboratories use permanent cleared mounts for the examination of internal oligochaete anatomy. The method is rarely performed upon all specimens encountered and usually a 10% subsample is selected for identification to species by this method. One laboratory stated that the expert opinion was that oligochaetes could not be identified reliably to species without the internal anatomical examination of adult specimens, which influenced oligochaete identification policy significantly. Laboratories may identify their oligochaetes to higher taxonomic levels because they believe that without clearing oligochaetes species identification is unachievable and / or the process of clearing all oligochaetes is not economically viable. The net result is reduction in oligochaete data and a dismissive attitude towards uncleared oligochaetes identified to species.

The ring test has proven that, with experience, several common species, including most sexually immature specimens, can be identified consistently without resorting to internal examination. The clearing of oligochaetes, aside from COSHH concerns, is not conducive to full sample audits. Secondary biomass calculations cannot be conducted and initial biomass records, as well as abundance records, are commonly estimated from proportions attained from an examined subsample. Random subsampling of oligochaetes prior to detailed examination is not recommended, as less abundant taxa are often overlooked and bias towards larger specimens and hence species often occurs. All RT19 oligochaete specimens were identifiable without examination of internal anatomy. Hence, only 1% of the RT19 specimens were cleared for identification by the participating laboratories. Clearing is often used as a final identification tool in instances where other external features are inconclusive. Intertidal estuarine macrobenthic samples often contain a large proportion of juvenile (sexually immature) oligochaete individuals. Clearing techniques would not classify such specimens to species. However, with experience and an understanding of growth series and gross morphological features, many such individuals can be identified to species and a far greater quality of ecological data acquired.

When asked to give their opinions of the importance of oligochaete identification, several laboratories gave surprising questionnaire responses. Many laboratories directly related oligochaete identification importance to relative oligochaete abundance. One laboratory rated oligochaete species identification of little importance because of its limited interpretative use. The interpretative use of oligochaetes would undoubtedly improve if more comprehensive literature and records were available. Greater levels of identification expertise would, in turn, lead to better ecological knowledge. One laboratory, with a relatively high degree of oligochaete identification experience in comparison with most laboratories, described oligochaete identification as extremely important. They added that Oligochaeta are dominant fauna at several of their stations and estimates of species diversity can be seriously skewed by failure to include diversity within the Oligochaeta. Oligochaeta show species partitioning on

salinity, sediment, habitat, depth and organic enrichment (pollution) characteristics. Some laboratories persist in suggesting the short-sighted 'horses for courses' approach of only processing according to perceived immediate objectives. Such an approach has been dismissed for NMMP data (Worsfold & Hall, 2001). The knowledge and understanding of oligochaetes will improve with time unless ill-conceived 'horses for courses' policies are allowed to prevail. The cost and damage caused by environmental surveys necessitates that the resultant data be transferable, used to their full potential, and not processed according to imagined short-term objectives.

The RT19 scores achieved by participating laboratories were very good considering that only single specimens were available for examination and many laboratories had limited experience. Two laboratories achieved very high scores with only two taxonomic differences recorded. The poorest results were achieved by laboratories that encounter few oligochaetes of limited diversity, which they do not routinely identify beyond class or family. Hopefully, such laboratories, given training and better literature, will be capable of raising the standard of their oligochaete knowledge to meet the proposed NMMP oligochaete identification requirements, discussed later.

Differences in the taxonomic levels to which animals are identified reduce the comparability of data. Current quality control procedures (NMBAQC Scheme Own Sample audits) do not highlight the problems as identifications to higher taxonomic levels are taken to be correct. Reduction of data to the lowest common denominator (i.e. highest taxonomic level) is a poor short-term solution to the use of the data that will not ensure maximum benefit (Worsfold & Hall, 2001). Therefore a SOP for NMMP oligochaetes is proposed (Appendix III), to be posted on the Scheme web site (www.nmbaqcs.org). Comments are invited. The SOP has been devised using ring test and macrobenthic data studied over the duration of the NMBAQC Scheme coupled with the questionnaire data. Essentially, the SOP advocates the best identification possible for oligochaete taxa without resorting to clearing and internal examination. It is the first version and is subject to change should subsequent studies enable greater taxonomic detail using gross morphological features. A laboratory adopting the NMMP oligochaete SOP (Ver.1.1) can qualify their data as such and greatly improve the comparative value of their data. For example, 'Tubificidae' recorded by such a laboratory (due to poor condition or recognition of an unfamiliar taxon) should not cause all tubificid species to be combined to family.

Implementation of the oligochaete SOP must be accompanied by sufficient training opportunities to enable all NMMP laboratories to achieve the required standard of expertise. Scheme participants may use the Laboratory Reference (LR) exercise to verify their NMMP oligochaetes, if necessary.

5. Conclusion

Three proposals are given for the improvement of Oligochaeta records for the NMMP. These are the development of an Oligochaeta SOP, additional training and improved literature. Initiatives for these proposals are detailed.

1. Development of an Oligochaeta SOP.

- Adoption of an NMMP standard policy for oligochaete identification.

  - **NMMP Oligochaeta SOP Version 1.1 (provisional) – Appendix III.**

2. Additional Training.

- Use of NMBAQC Scheme taxonomic workshop and Laboratory Reference (LR) exercise to improve and disseminate knowledge of oligochaetes.

  - **NMBAQC Scheme workshop (provisionally March 2003, MBA Plymouth) to include Oligochaeta. NMBAQC Scheme LR exercise is now free form to allow submission of any UK taxa.**

3. Improved Literature.

- Improved oligochaete literature covering marine, estuarine and freshwater taxa, including diagrams / images of whole specimens and details of ecological preferences. Ongoing literature search on taxonomy regularly submitted to NMBAQC (required for all taxonomic groups – NMBAQC funding required).

  - **Literature updates and ecological notes to be distributed at NMBAQC Scheme workshop (provisionally March 2003, MBA Plymouth).**

Oligochaetes, like many faunal groups, first all appear alike (probably none more so than oligochaetes). However, with experience and training, differences in gross morphological features can be observed and habitat details recorded to improve our understanding. In truth, the economics of clearing has long been a convenient excuse for many laboratories not attempting to identify the oligochaetes encountered. Methods in pure taxonomy require great attention to detail but it is essential that practical (e.g. ecological) outlets for taxonomic research be considered. The logical progression from the anatomically verifiable definition of a species is to find pragmatic means of quickly recognising it to provide ecological information. The present report and provisional SOP represent progress to that end.

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Tables 1A - 1H. Data from section 1 of the oligochaeta questionnaire.

1A.) How often do you encounter oligochaetes in your macrobenthic samples?

	No. Labs.	LabCodes
1. Always	3	10,15&20
2. Often	10	03,06,08,12,13, 14,16,18,19&21
3. Occasionally	4	04,05,09&11
4. Rarely	2	02&07
5. Never	0	-

1B.) At what taxonomic level do you normally identify oligochaetes? Give qualifying comments (e.g. species but Enchytraeids to family)

	No. Labs.	LabCodes	Comments
Class	2	02&03	-
Family	3	06,07&19	Depends upon customer requirements and existing data(06); Except for easily identifiable species such as T.benedii and H.costata(19).
Species, wherever possible.	14	04,05,08,09,10, 11,12,13,14,15, 16, 18,20&21	But Enchytraeids to family(08,12,16,18);Family generally for Tubificids and Enchytraeids(09);Enchytraeids to genus(14);Enchytraeids and naids to family(20).

1C.) How important do you consider oligochaete identification? Add comments

LabCode	Comments
LB0802	If they were to form a large proportion of the samples we identify we would probably consider identification more important, particularly with estuarine samples, but as most samples we encounter are fully marine, and we come across very few oligochaetes it is not currently considered important
LB0803	Oligochaete ID is as important as any other infaunal taxon wrt the type of studies we are involved in. However, the majority of the literature suggests you need sexually mature adults for correct ID and this involves mounting and clearin each specimen. We have analysed data where oligochaetes have been ID'd to species, then lumped them at class level an reanalysed the data set. For the majority of our studies this has not affected the results wrt the objectives.
LB0804	When they are abundant, as important as any other taxa.
LB0805	Important. Don't have any sites dominated by them and appear to have a limited species list (so far)
LB0806	Low priority because of the limited use made of it in interpretation of most data sets.
LB0807	For our samples the time taken to identify oligochaetes to species and the few animals, the time is not justified.
LB0808	No more or no less important than other groups. However, as with other groups, when faced with high numbers of individuals a pragmatic approach is usually adopted and a representative sub-sample of animals (@100) would be identified to the lowest taxonomic level possible and the resulting proportions of species identified applied to the whole sample.
LB0809	Oligochaetes are not often major components in our samples, and when they are, it is mostly*Tubificoides benedii* . However, the more components of a sample that can be identified to species the more information obtained.Therefore, I would like to be able to identifiy more of the Oligochaetes to the species level.
LB0810	Extremely. They are the dominant fauna at several sites, and estimates of species diversity can be seriously skewed by not including diversity within the oligochaeta. They show species partitioning on salinity and sediment/habitat characteristics which m...
LB0811	In the estuarine situation it is considered very important as it forms the main community structure. In transitional/coastal it is less important but they are not the main taxa in this area
LB0812	-
LB0813	Important, but more information on ecological requirements of individual species/groups needed in order to faciliate interpretation of community structure.
LB0814	Important in estuarine waters where they predominate. Ideally they should be treated as most other macrofauna and identified to species to provide best asssessment of composition and diversity of fauna.
LB0815	Depends on the origin of the sample, from an estuary with mud, depositing, and particularly when upper estuary, it is vital as Oligochaeta may be the only group represented.
LB0816	Just as important as identifying everything else.
LB0818	Having now acquired the experience and recently obtained the 1994 workshop keys/tables/descriptions, I consider oligochaete identification to be of significant importance in certain systems (Estuaries, rivers etc.)
LB0819	Not important, and more to the point, not practical to do routinely. Family level I.d. is quite sufficient as far as I'm concerned. There are also health and safety implications with processing specimens in chemicals such as Amman's Lactophenol
LB0820	Commercially clients have never insisted on higher levels of id.
LB0821	-

1D.) Place the following identification aids in rank order of importance for oligochaete identification at your laboratory (1: most important; 5: least important). Exclude any aids not used; add comments

	LB0802	LB0803	LB0804	LB0805	LB0806	LB0807	LB0808
Keys/tables	3	5	1	1	1	1	2
Publications/descriptions	2	5	5	4	3	4	5
Reference material	1	2	3	2	4	2	4
Experience/memory	5	3	2	2	2	3	1
Habitat information	4	1	4	5	5	-	3

	LB0809	LB0810	LB0811	LB0812	LB0813	LB0814	LB0815
Keys/tables	2	2	1	2	1	1	2
Publications/descriptions	1	3	3	3	5	3	2
Reference material	1	1	5	4	2	2	1
Experience/memory	1	4	4	1	3	4	2
Habitat information	-	5	2	5	4	5	2

	LB0816	LB0818	LB0819	LB0820	LB0821		AVERAGE
Keys/tables	1	1	1	1	1		1.6
Publications/descriptions	3	3	3	3	2		3.3
Reference material	4	4	5	2	5		2.8
Experience/memory	2	2	2	4	3		2.6
Habitat information	-	3	4	5	4		3.8

1E.) Do you find your oligochaete literature adequate? Add comments

LabCode	Comments
LB0802	No, although I think we have most literature that is available but still feel that it is inadequate and sparse compared with polychaete family keys
LB0803	No, A lot of the literature we have is from the USA which proves confusing wrt name changes etc
LB0804	We have Brinkhurst and Unico's table of features. Also have access to Brinkhurst (freshwater) and Brinkhurst & Jamieson (1971). We are lacking many descriptions.
LB0805	no. Still very subjective
LB0806	No. Something like the Lin.Soc. book would be ideal but this is very limited. Beyond this we use the workshop [notes]. Need better keys, full descriptions and diagrams of all common species collected together in one publication.
LB0807	At the level we are interested in- yes
LB0808	No. The Tubificid table provided by Unico is generally good. However, other groups are poorly covered and on the whole the information we have is generally poor.
LB0809	Yes, for the level to which the Oligochaeta are normally identified.
LB0810	The Unicomarine tabular key is useful, certainly more so than trawling all the literature, but I had some problems with the Tub pseudogaster complex, and some of the commonly encountered (freshwater) species that extend to brackish water are not well cov...
LB0811	It surfices for the amount we do but certainly after the RT I felt it would have been better to have more publications - especially with drawings of chaete etc.
LB0812	Yes, combination of Brinkhurst 1982, publications, workshop notes and own notes an sketches. A comprehensive key & descriptions, replacing Brinkhurst 1982 is overdue.
LB0813	Its ok, the Unicomarine Workshop notes are best, but a lot of oligo features are a bit subjective.
LB0814	NO! Although I have a good selection of literature, both old and new, it is difficult to find good descriptions and figures of some taxa - for example Monopylephorus rubroniveus/parvus, Clitellio arenarius, Aktedrilus spp.
LB0815	No, we used what we had, but some of the species that were previously had not been seen, and we had no reference material, the literature was inadequate.
LB0816	Yes for the majority of work we do, but not that comprehensive.
LB0818	Having acquired the 1994 workshop keys/tables/descriptions I am incearsingly more confident about the identification o oligochaetes, however, new descriptions species are appearing all the time, the table v.3 gives excellent indications.
LB0819	No. for the most part (and necessarily I assume) it concentrates on internal anatomy which is impractical to deal with on a routine basis (and doesn't work - unless you happen to be the internationally recognized expert writing the paper of course)
LB0820	No -not at all.
LB0821	No - It would be better to have more pictures of the whole animal and external features rather than the internal features

1F.) Did you attend the 1994 Oligochaete workshop hosted by Unicomarine Ltd.? Add comments

LabCode	Comments
LB0802	None of the current benthic ecologists attended the workshop although the literature supplied for the workshop was used for this ring test
LB0803	One of our ex-employees did.
LB0804	No
LB0805	yes
LB0806	Yes. Only covered a limited range of species, we are not able to supply a large amount or wide range of oligs ours … a supply of reference material. Very dependent on being able to clear material (COSHH problem) also access to high …
LB0807	Yes
LB0808	Yes
LB0809	No.
LB0810	No -but I have the literature from it - Essential: makes you aware of the large variation in some of the penis sheaths of some species, which if you only have chaetae and penis sheaths visible can be troublesome.
LB0811	Yes - and the folder and papers from that meeting was my main source for the RT.
LB0812	Yes, it was very useful.
LB0813	yep, it was good
LB0814	Yes. I found it boosted my confidence considerably - the most useful side was actually seeing what certain species actualy looked like in the flesh rather than the poor line drawings from publications.
LB0815	One member of staff did attend, we have the paperwork, which we still use.
LB0816	No
LB0818	No, I hate to say it but perhaps it was about time we had an update (possible theme for next w/shop)
LB0819	No, but I've got the literature from it, which unfortunately I don't find useful.
LB0820	No I didn't get the chance
LB0821	No

1G.) How would you rank your experience with oligochaete identification?

	No. Labs.	LabCodes
1. Very experienced	0	-
2.	0	-
3. Reasonably experienced	9	05,07,08,10,12, 14,16,18&19
4.	10	02,03,04,06,09, 11,13,15,20&21
5. No experience	0	-

1H.) What methods do you normally use for oligochaete identification?

	No. Labs.	LabCodes	Comments
Stereo-examination of gross morphology	17	02,03,04,05,06, 08,09,10,11,12, 14,15,16,18,19, 20& 21	T.benedii & Grania spp.
Temporary mounts for chaetal examination	19	02,03,04,05, 06,07,08,09,10, 11,12,13,14,15, 16,18,19,20&21	Most Tubificoides, most Naididae; Temporary cleared mounts(14).
Permanent cleared mounts for examination of internal anatomy	4	05,07,10&18	Depending on specimen, size, condition and features, also abundance(05);Never done due to time(08);Where difficult specimens are present(18);Used to clear specimens with Ammans Lactophenol and mount them but found that the process did not improve my ability to identify specimens and wasted an awful lot of time(19).

Tables 2A - 2H. Data from section 2 of the oligochaeta questionnaire.

2A.) Did you find the habitat notes supplied with the ring test useful? Give comments

LabCode	Comments
LB0802	yes very useful as they helped to rule out species when identification using traditional keys was difficult
LB0803	A small amount. They were used to eliminate which species it definitely could not be during the ID process.
LB0804	Yes, as reinforcement of possible species designation.
LB0805	Yes. Unico '94 notes mainly marine, head to Brinkhurst for the freshwater ones!
LB0809	Yes, in that I was able to check whether or not my identification matched the known habitat for a given species.
LB0811	Yes - but I mainly used them as a check where I had doublers. Occasionally they were useful when the oli descriptions had habitat info with them.
LB0814	Only used to confirm a few ids. - eg for low salinity taxa such as Tubifex
LB0815	Yes, could not have have even guessed at the id. without this information.
LB0816	No
LB0821	Yes. Salinity and habitat was useful

2B.) Did you clear any of the ring test specimens? Give numbers, examples and reasons

LabCode	Comments
LB0802	No
LB0803	Yes, one, but we didn't note down which one it was.
LB0804	None.
LB0805	#8 A lot of broken chaetae, looking for some feature to aid id!
LB0809	No. All identifications were based on gross morphology and chaetal shape and arrangement. Given that there was only one specimen and my lack of experience and knowledge regarding internal morphology (e.g. male reproductive organs) it was not worthwhile for me to examine any of the specimens in this way.
LB0811	No.
LB0814	Yes - number 15 to get better view of penes.
LB0815	No
LB0816	No
LB0821	No

2C.) Did you use reference material to assist your identifications? Give examples

LabCode	Comments
LB0802	We would have if we had reference material. But as we hardly ever come across oligochaetes in our samples we have very few. Most if not all are Grania species and they did not feature in the ring test - I hope!
LB0803	Yes we used our own lab reference collection material although we do not have many marine oligochaetes within this.
LB0804	Some Psammoryctides from a previous ... survey.
LB0805	No. Ref. material limited and mainly obvious taxa
LB0809	Yes, although reference material for the Oligochaeta is available for only a few species. Ring test specimens were compared with reference specimens for Nais elinguis and Paranais litoralis. Reference materail was also used for Tharyx sp.
LB0811	No.
LB0814	Yes - checked our ref. material of T.swirencoides, T.scoticus , T.brownae
LB0815	Yes, we have a small collection of worms from ..., all of which have been verified -*Tubificoides benedii, T.heterochaetus, Heterochaeta costatus* , etc.
LB0816	No, as our reference specimens are far from complete.
LB0821	Don't have any reference material as of yet.

2D.) How long did the ring test take to complete?

LabCode	Time (hours)	Comments
LB0802	15	
LB0803	12	
LB0804	7	
LB0805	7	
LB0809	27	Including literature compilation
LB0811	20	
LB0814	7	
LB0815	6	
LB0816	21	
LB0821	-	

2E.) How many people were involved in the ring test identifications?

LabCode	No. People
LB0802	5
LB0803	3
LB0804	3
LB0805	1
LB0809	1
LB0811	1
LB0814	1
LB0815	1
LB0816	3
LB0821	1

2F.) How difficult did you find the ring test?

	No. Labs.	LabCodes
1. Very easy	0	-
2.	0	-
3.	2	14&15
4.	5	05,09,11,16&21
5. Very hard	3	02,03&04

2G.) How many of the 25 RT specimens do you think you identified correctly to species?

LabCode	Predicted **No. correct**	Actual **No. correct**	Oligochaetes Only **No. correct (22max)**
LB0802	3	*4*	*3*
LB0803	10	*14*	*11*
LB0804	12	*20*	*18*
LB0805	16	*16*	*14*
LB0809	12	*19*	*16*
LB0811	15	*16*	*13*
LB0814	20	*20*	*18*
LB0815	15	*23*	*20*
LB0816	15	*23*	*20*
LB0821	15	*18*	*18*

2H.) Please use the space below if you have any further comments regarding the ring test.

LabCode	Comments
LB0802	The majority of the participants feel that oligochaetes are inherently difficult and that we don't come across them often enough to warrant spending vast amounts of time searching through the literature. This is undoubtedly reflected in our identifications. However, there is every chance that we will be taking on estuarine samples in the near future so some expertise will be necessary.
LB0803	As we do not routinely identify oligochaetes to species and we have a poor selection of reference material we found this ring test difficult.We are hoping to use the ring test as a training exercise more than anything else. We would be interested to hear of key 'tips' for IDing these to species as we have been advised by other taxonomists that sexually mature adults are required for accurate identification to species as specimens need to be cleared in lactophenol to enable the penis sheath to be examined. This is very time consuming for one specimen, never mind for multiple individuals depending on the number you encounter in you samples.The majority of the specimens included in the ring test were not thought to be sexually mature so following the key with respect to using features of the penis sheath could not be done. However, we are not discounting that our lack of expertise may have led us to believe they were immature specimens!
LB0804	-
LB0805	-
LB0809	I found the Ring Test difficult, but a good opportunity to amass literature on the Oligochaeta and to try and improve my taxonomic knowledge of this group. However, what would be most beneficial would be to include the Oligochaeta in a taxonomic workshop, such as that held last autumn in Portaferry.
LB0811	Although initially terrified by the prospect of the RT I actually enjoyed it. I might not get many right but at least when I checked the habitat data against the specimens I had doublers of and they agreed that was a revelation!
LB0814	Several species appear to be repeated in this ring test - although this may be an error on my part. I had hoped to see more new taxa (only Psammoryctides and T.heterochaetus? Were new to me) and have the opportunity to save some digital images of their penes etc. I reckon a series of good digital images of diagnostic features of real oligochaetes would get round the present problems of poor illustrations in taxonomic papers.
LB0815	Hopefully your feedback will be as full as usual, as this will help greatly when undertaking further Oli id. Can we keep some of the specimens????????? We need to improve our reference collection.
LB0816	Although we usually find oligochaetes in our samples, they consist of a small no. of species. We don't find the diversity of oligochaetes that were included in this ring-test.
LB0821	-

Appendix I.

The National Marine Biological Analytical Quality Control Scheme

Ring Test Bulletin – RTB#19

Unicomarine Ltd.
May 2002

RING TEST DETAILS

Ring Test #19
Type/Contents – Targeted Oligochaeta and similar fauna
Circulated – 17/01/2002
Completion Date – 29/03/2002
Number of Participating Laboratories - 18
Number of Results Received – 10

Summary of differences

Specimen	Genus	Species	Total differences for (10) laboratories	
			Genus	Species
RT1901	Tubificoides	benedii	1	1
RT1902	Tubifex	tubifex	3	4
RT1903	Paranais	litoralis	2	2
RT1904	Tubificoides	benedii	0	0
RT1905	Nais	elinguis	1	4
RT1906	Psammoryctides	barbatus	4	4
RT1907	Heterochaeta	costata	1	1
RT1908	Tubificoides	swirencoides	1	4
RT1909	Tubificoides	heterochaetus	3	5
RT1910	Paranais	litoralis	2	3
RT1911	Tubificoides	amplivasatus	1	2
RT1912	Tubifex	tubifex	6	6
RT1913	Tubificoides	insularis	0	0
RT1914	Tubificoides	amplivasatus	1	3
RT1915	Psammoryctides	barbatus	4	4
RT1916	Paranais	litoralis	1	1
RT1917	Heterochaeta	costata	1	1
RT1918	Tubificoides	swirencoides	1	7
RT1919	Tubificoides	cf. galiciensis	0	7
RT1920	Tharyx	sp. A	2	4
RT1921	Mediomastus	fragilis	2	2
RT1922	Capitella	capitata	2	2
RT1923	Nais	elinguis	2	3
RT1924	Tubificoides	cf. galiciensis	0	6
RT1925	Heterochaeta	costata	1	1
		Total differences	42	77
		Average differences /lab.	4.2	7.7

Detailed Breakdown of Identifications

RT1901 – *Tubificoides benedii*
Sediment: Mud. Salinity: High. Depth: Mid Shore. Geography: Blackwater Estuary. Condition: Good, Large.
One generic and one specific difference; Lab 02 identified as Tubificidae.

RT1902 – *Tubifex tubifex*
Sediment: Mud. Salinity: Low. Depth: Mid Shore. Geography: Thames Estuary. Condition: Good, Small.
Three generic and four specific differences; Lab 02 identified as *Paranais sp.*, Lab 03 identified as *Tubificoides insularis* (both lack pectinate chaetae), Lab 05 identified as *Tubificoides aculeatus?* (which is an abyssal species), and Lab 11 identified as *Tubifex nerthus* (which has ventral anterior chaetae with increasingly reduced lower teeth).

RT1903 – *Paranais litoralis*
Sediment: Mud. Salinity: Medium. Depth: Mid Shore. Geography: Blackwater Estuary. Condition: Average.
Two generic and two specific differences; Lab 02 identified as *Tubificoides sp.* and Lab 15 identified as *Tubificoides pseudogaster* (both have dorsal chaetae present from the first chaetiger).

RT1904 – *Tubificoides benedii*
Sediment: Mud. Salinity: High. Depth: Mid Shore. Geography: Suffolk. Condition: Average-Poor.
No differences recorded.

RT1905 – *Nais elinquis*
Sediment: Mixed. Salinity: Low. Depth: Low Water Mark. Geography: Thames Estuary. Condition: Good, Faint Eyes.
One generic and four specific differences; Labs 02, 11 and 14 identified as *Nais variabilis* (which lacks dorsal chaetae with long parallel teeth), and Lab 03 identified as *Tubificoides sp.* (which has dorsal chaetae on the first chaetiger).

RT1906 – *Psammoryctides barbatus*
Sediment: Mixed. Salinity: Low. Depth: Mid Shore. Geography: Thames Estuary. Condition: Very Good, Large.
Four generic and four specific differences; Labs 02, 03, 09 and 11 identified as *Tubifex tubifex* (which lacks palmate chaetae).

RT1907 – *Heterochaeta costata*
Sediment: Muddy Sand. Salinity: Medium. Depth: Low Water Mark. Geography: North Lincolnshire. Condition: Very Good, Large.
One generic and one specific difference; Lab 02 did not identify this specimen.

RT1908 – *Tubificoides swirencoides*
Sediment: Mixed. Salinity: High. Depth: Shallow Subtidal. Geography: Strangford Lough. Condition: Very Poor, Incomplete.
One generic and four specific differences; Lab 02 identified as *Tubifex tubifex* (which lacks papillations and has pectinate chaetae), Lab 03 identified as *Tubificoides sp.*, Lab 05 identified as *Tubificoides amplivasatus* (which lacks posterior papillations), and Lab 09 identified as *Tubificoides scoticus* (which lacks anterior bifid chaetae with closely applied 'clothes peg' teeth).

RT1909 – *Tubificoides heterochaetus*
Sediment: Mud. Salinity: Medium. Depth: Shallow Subtidal. Geography: Thames Estuary. Condition: Average.
Three generic and five specific differences; Lab 04 identified as *Tubificoides pseudogaster,* Lab 09 identified as *Limnodrilus hoffmeisteri* (both of which lack simple pointed dorsal chaetae), Lab 03 identified as Tubificinae sp., Lab 05 identified as *Tubificoides spp?* (spp. indicates more than one species present, the vial should have contained just one specimen), and Lab 02 did not identify this specimen.

RT1910 – *Paranais litoralis*
Sediment: Mixed Gravel. Salinity: Medium. Depth: Mid Shore. Geography: Severn Estuary. Condition: Good, Asexual Evidence.
Two generic and three specific differences; Lab 02 identified as *Paranais sp.*, Lab 05 identified as *Chaetogaster spp.* (which has no dorsal chaetae; spp. indicates more than one species present, the vial should have contained just one specimen), and Lab 11 identified as *Tubificoides pseudogaster* (which has dorsal chaetae present from the first chaetiger).

RT1911 – *Tubificoides amplivasatus*
Sediment: Mixed. Salinity: High. Depth: Shallow Subtidal. Geography: Milford Haven. Condition: Average.
One generic and two specific differences; Lab 02 identified as Naididae (which have characteristic chaetae and body-forms), and Lab 21 identified as *Tubificoides insularis* (which has papillations).

RT1912 – *Tubifex tubifex*
Sediment: Mixed. Salinity: Low. Depth: Mid Shore. Geography: Thames Estuary. Condition: Poor.
Six generic and six specific differences; Lab 02 identified as Naididae (which have characteristic chaetae and body-forms), Lab 03 identified as *Tubificoides amplivasatus*, Lab 04 identified as *Tubificoides indet.*, Lab 05 identified as *Monopylephorus irroratus* (which has twisted hair chaetae and lacks pectinate chaetae), Lab 11 identified as *Tubificoides aculeatus* (which is an abyssal species), and Lab 21 identified as *Eiseniella tetraedra* (which has lacks hair and pectinate chaetae).

RT1913 – *Tubificoides insularis*
Sediment: Mixed. Salinity: High. Depth: Shallow Subtidal. Geography: Stour Estuary. Condition: Average. Notes: Co-habitant with specimen RT1914.
No differences recorded.

RT1914 – *Tubificoides amplivasatus*
Sediment: Mixed. Salinity: High. Depth: Shallow Subtidal. Geography: Stour Estuary. Condition: Good. Notes: Co-habitant with specimen RT1913.
One generic and three specific differences; Lab 02 identified as Tubificidae, Lab 03 identified as *Tubificoides sp.*, and Lab 16 identified as *Tubificoides scoticus* (which lacks posterior banding and has broad lance shaped anterior dorsal chaetae).

RT1915 – *Psammoryctides barbatus*
Sediment: Mixed. Salinity: Low. Depth: Shallow Subtidal. Geography: Thames Estuary. Condition: Good.
Four generic and four specific differences; Labs 02, 03, 09 and 11 identified as *Tubifex tubifex* (which lacks palmate chaetae).

RT1916 – *Paranais litoralis*
Sediment: Mud. Salinity: Medium. Depth: Mid Shore. Geography: Thames Estuary. Condition: Poor. Notes: Exact specimen for each laboratory as circulated in RT17.
One generic and one specific difference; Lab 02 identified as *Psammoryctides barbatus* (which has hair and palmate chaetae – possible vial mix up).

RT1917 – *Heterochaeta costata*
Sediment: Mud. Salinity: Medium. Depth: Mid Shore. Geography: Essex. Condition: Good.
Notes: Exact specimen for each laboratory as circulated in RT17.
One generic and one specific difference; Lab 03 identified as *Tubificoides amplivasatus* (which has hair chaetae and lacks pectinate chaetae).

RT1918 – *Tubificoides swirencoides*
Sediment: Mud. Salinity: High. Depth: Shallow Subtidal. Geography: Tees Estuary. Condition: Good.
One generic and seven specific differences; Labs 04, 05, 15 and 21 identified as *Tubificoides cf. galiciensis* (which has bifid chaetae accompanying the posterior hair chaetae), Lab 02 identified as *Clitellio arenarius?* (which has no hair chaetae), Lab 03 identified as *Tubificoides sp.*, and Lab 11 identified as *Tubificoides scoticus* (which lacks anterior bifid chaetae with closely applied ‘clothes peg’ teeth).

RT1919 – *Tubificoides cf. galiciensis*
Sediment: Mud. Salinity: High. Depth: Shallow Subtidal. Geography: Tees Estuary. Condition: Good.
Seven specific differences; Labs 03, 04, 11 and 14 identified as *Tubificoides swirencoides* (which has simple pointed chaetae accompanying the posterior hair chaetae), Lab 02 identified as *Tubificoides benedii* (which lacks hair chaetae), Lab 09 identified as *Tubificoides insularis* (which has anterior papillations), and Lab 16. Identifies as *Tubificoides scoticus* (which lacks bifid chaetae accompanying the posterior hair chaetae).

RT1920 – *Tharyx sp. A*
Sediment: Mud. Salinity: High. Depth: Mid Shore. Geography: North Wales. Condition: Good.
Two generic and four specific differences; Labs 04 and 14 identified as *Tharyx killariensis* (which has a longer thinner body and is subtidal), Lab 05 identified as *Chaetozone setosa agg.*, and Lab 21 identified as *Chaetozone sp. B* (both of which have posterior simple pointed acicular chaetae in both rami).

RT1921 – *Mediomastus fragilis*
Sediment: Mixed. Salinity: Full. Depth: Subtidal. Geography: Orkney. Condition: Good.
Two generic and two specific differences; Labs 02 identified as *Capitomastus minimus* (which has three anterior segments with capillary chaetae and no achaetus segment), and Lab 21 did not identify this specimen.

RT1922 – *Capitella capitata*
Sediment: Mud. Salinity: Medium. Depth: Mid Shore. Geography: Suffolk. Condition: Average.
Two generic and two specific differences; Lab 02 identified as *Tubificoides amplivasatus* (which lacks hooded hooks and has dorsal hair chaetae throughout its body), and Lab 21 did not identify this specimen.

RT1923 – *Nais elinguis*
Sediment: Mixed. Salinity: Low. Depth: Shallow Subtidal. Geography: Thames Estuary. Condition: Good, No Eyes.
Two generic and three specific differences; Labs 02 and 05 identified as *Paranais litoralis* (which lacks dorsal hair chaetae), and Lab 14 identified as *Nais variabilis* (which lacks dorsal chaetae with long parallel teeth).

RT1924 – *Tubificoides cf. galiciensis*
Sediment: Mud. Salinity: High. Depth: Shallow Subtidal. Geography: Tees Estuary. Condition: Good.
Six specific differences; Labs 05, 09 and 14 identified as *Tubificoides swirencoides*, Lab 11 identified as *Tubificoides amplivasatus* (both have simple pointed chaetae accompanying the posterior hair chaetae), Lab 21 identified as *Tubificoides benedii* (which lacks dorsal hair chaetae), and Lab 03 identified as *Tubificoides sp.*.

RT1925 – *Heterochaeta costata*
Sediment: Mud. Salinity: Medium. Depth: Mid Shore. Geography: Blackwater Estuary. Condition: Good.
One generic and one specific difference; Lab 02 identified as *Tubificoides pseudogaster agg.* (which lacks palmate chaetae).

PLEASE RETURN ALL RING TEST SPECIMENS BY 24TH MAY 2002. THESE ARE REFERENCE COLLECTION SPECIMENS AND MUST BE RETURNED TO OUR MUSEUM. YOUR LABORATORY WILL BE INELEGIBLE FOR FUTURE RING TESTS IF SPECIMENS ARE NOT RETURNED.

Appendix II. NMBAQCS RT19 'Oligochaeta' Questionnaire.

LabCode: _____

Please take a few moments to complete this questionnaire so that your ring test results can be qualified correctly.
If you did not participate in the ring test, please complete Section 1 only.

SECTION 1 - GENERAL

1A.) How often do you encounter oligochaetes in your macrobenthic samples?
Mark the appropriate box:

- ☐ 1. Always
- ☐ 2. Often
- ☐ 3. Occasionally
- ☐ 4. Rarely
- ☐ 5. Never

1B.) At what taxonomic level do you normally identify oligochaetes? Give qualifying comments (e.g. species but Enchytraeids to family)
Mark the appropriate box:

- ☐ Class
- ☐ Family
- ☐ Species, wherever possible.

1C.) How important do you consider oligochaete identification? Add comments

1D.) Place the following identification aids in rank order of importance for oligochaete identification at your laboratory
(1: most important; 5: least important). Exclude any aids not used; add comments

…….. Keys/tables
…….. Publications/descriptions
…….. Reference material
…….. Experience/memory
…….. Habitat information

1E.) Do you find your oligochaete literature adequate? Add comments

1F.) Did you attend the 1994 Oligochaete workshop hosted by Unicomarine Ltd.? Add comments

1G.) How would you rank your experience with oligochaete identification?
Mark the appropriate box:

- ☐ 1. Very experienced
- ☐ 2.
- ☐ 3. Reasonably experienced
- ☐ 4.
- ☐ 5. No experience

1H.) What methods do you normally use for oligochaete identification?
Give examples of taxa and proportions of specimens identified by each method
Mark the appropriate boxes:

- ☐ Stereo-examination of gross morphology
- ☐ Temporary mounts for chaetal examination
- ☐ Permanent cleared mounts for examination of internal anatomy

SECTION 2 - RT19 (to be completed by RT19 participants only)

2A.) Did you find the habitat notes supplied with the ring test useful? Give comments

2B.) Did you clear any of the ring test specimens? Give numbers, examples and reasons

2C.) Did you use reference material to assist your identifications? Give examples

2D.) How long did the ring test take to complete?

…….. Hours (total working hours, i.e. 2 persons for 2 hrs = 4 hrs)

2E.) How many people were involved in the ring test identifications?

2F.) How difficult did you find the ring test?
Mark the appropriate box:

- ☐ 1. Very easy
- ☐ 2.
- ☐ 3.
- ☐ 4.
- ☐ 5. Very hard

2G.) How many of the 25 RT specimens do you think you identified correctly to species?

…… out of 25.

2H.) Please use the space below if you have any further comments regarding the ring test, or suggestions for future target ring tests

Thank you for completing this questionnaire. Please either email or post your completed form to:
davidhall@unicomarine.com; David Hall, Unicomarine Ltd., Works Road, Letchworth, Hertfordshire SG6 1LW.

Appendix III. Unicomarine Ltd. Extraction/Recording/Biomass SOP for Macrobenthic Samples
NMMP Version 1.1 Oligochaeta

			Extracted*		Preservation		Recorded/Identification				Biomass			Key literature (not comprehensive)
Class	Family	Genus	All	In part	Alcohol	Dried	Enumeration	Present/absent	Tax. level**	Juv. separated	Weighed	Fragments incl.	Tubes/shells incl.	
Oligochaeta			☑		☑		☑		Varied	☑☒	☑	☑	n/a	Brinkhurst, 1971 & 1982; 1994 Oligochaete workhop notes; In-house tables & notes.
	Naididae		☑		☑		☑		Varied		☑	☑	n/a	
		Amphichaeta	☑		☑		☑		Species		☑	☑	n/a	
		Chaetogaster	☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971
		Nais	☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971, 1982
		Paranais	☑		☑		☑		Species		☑	☑	n/a	
		Stylaria	☑		☑		☑		Species		☑	☑	n/a	
		Uncinais	☑		☑		☑		Species		☑	☑	n/a	
	Tubificidae		☑		☑		☑		Varied (Family, except where stated below)		☑	☑	n/a	Brinkhurst 1971, 1982; In-house notes.
		Monopylephorus	☑		☑		☑		Family (except M.irroratus to species)		☑	☑	n/a	
		Limnodriloides	☑		☑		☑		Genus		☑	☑	n/a	
		Clitellio	☑		☑		☑		Species		☑	☑	n/a	
		Heterochaeta	☑		☑		☑		Species		☑	☑	n/a	
		Limnodrilus	☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971
		Tubifex	☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
		Tubificoides	☑		☑		☑		Species (except T.brownae, T.crenacoleus, T.diazi and T.pseudogaster, all as T.pseudogaster agg.)		☑	☑	n/a	
		Psammoryctides	☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Enchytraeidae		☑		☑		☑		Family (except Grania spp. to genus)		☑	☑	n/a	Brinkhurst 1982
		Grania	☑		☑		☑		Genus		☑	☑	n/a	
	Branchiobdellidae		☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971
	Aeolosomatidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Haplotaxidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Lumbriculidae		☑		☑		☑		Family		☑	☑	n/a	Brinkhurst 1971
	Dorydrilidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Glossoscolecidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Lumbricidae		☑		☑		☑		Family (except Eiseniella tetraedra to species)		☑	☑	n/a	Brinkhurst 1982

*=some taxa will be counted in situ/subsampled if present in high numbers

**=minimum level required (good condition given)