The National Marine Biological

Analytical Quality Control Scheme

Fish Component Report from the Contractor

Scheme Operation - Year 18

2011/12

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FISH COMPONENT REPORT FROM THE CONTRACTOR

SCHEME OPERATION –   YEAR 18 –   2011/12

Scheme Year 18 Exercise Reports (hyperlinked in this report)

Reverse Ring Test Report –   F_RRT03

Fish Ring Test Bulletin –   F_RT05

Previous Scheme Exercise Reports (hyperlinked in this report)

Reverse Ring Test Report –   F_RRT01

Reverse Ring Test Report –   F_RRT02

Ring Test Bulletin –   RT28 (F_RT01)

Ring Test Bulletin –   RT31 (F_RT02)

Ring Test Bulletin –   RT33 (F_RT03)

Fish Ring Test Bulletin –   F_RT04

1. Introduction

The eighteenth year of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme (2011/12) followed the format of the seventeenth year. A series of exercises involved the distribution of test materials to participating laboratories and the centralised examination of returned data and samples. The labelling and distribution procedures employed previously have been maintained and specific details can be found in the Scheme's annual reports for 1994/95 and 1995/96 (Unicomarine, 1995 & 1996).

The Fish Component of the scheme commenced in its twelfth year (2005/06). Twenty-nine laboratories / fish teams participated in the Fish Component of the Year 18 NMBAQC Scheme. Twenty-four participants were government laboratories / fish teams; five were private consultancies. Although some fish are sampled under the Clean Seas Environment Monitoring Programme (CSEMP) the number of target species is relatively few. However the requirement to monitor transitional water fish communities for the Water Framework Directive (WFD) provides the major impetus for fish component exercises.

1.1 Summary of Performance

This report presents the findings of the Fish component for the eighteenth year of operation of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme.

This component consisted of two official modules, each with a single exercise:

- Re-identification of a set of fifteen fish specimens supplied by each of the participating laboratories (Fish Reverse Ring Test module).
- Identification of one set of fifteen fish specimens circulated by the scheme contractor (Fish Ring Test module)

The analytical procedures of both modules were the same as for the seventeenth year of the Scheme. The results for each of the Scheme exercises are presented and discussed.

Fish Reverse Ring Test (F_RRT): The identification of a set of fifteen fish species selected and supplied by the participating laboratories was generally accurate (29/349 errors). The majority of specimens were collected by fish teams during their 2011 autumn monitoring surveys. One recurring error that was highlighted by this exercise concerned the identification of grey Mullets, with over one third of the submissions of this taxon incorrectly identified. Other recurring errors were noted for Gobies (several species). However, there were differences in the approach to this exercise by the individual laboratories; some laboratories used this as a test for confirming voucher specimens whilst others sought a means of having uncertain or 'unknowns' identified making it difficult to directly compare results.

A **Fish Ring Test (F_RT)** of fifteen fish specimens was distributed by Thomson Unicomarine Ltd. This fish ring test (F_RT05) produced good agreement between the identifications made by the participating laboratories and those made by Thomson Unicomarine Ltd. On average each laboratory recorded 2.9 generic errors and 4.1 specific errors. Three specimens were responsible for 53% of all generic and 46% of specific errors recorded.

1.1.1   Statement of Performance

Each participating laboratory / fish team has a 'Statement of Performance', which includes a summary of results for each of the Schemes modules and details the resulting pass/fail flags where appropriate. These statements were first circulated with the 1998/1999 annual report, for the purpose of providing evidence of Scheme participation and for ease of comparing year on year progress.

2.   Summary of Fish Component

2.1   Introduction

There were two modules in the fish component for Scheme year eighteen; Fish Reverse Ring Test identification (F_RRT) module and Fish Ring Test identification (F_RT) module.

Both fish modules are described in more detail below. A brief outline of the information to be obtained from each module is given, together with a description of the preparation of the necessary materials and brief details of the processing instructions given to each of the participating laboratories.

2.1.1   Logistics

The labelling and distribution procedures employed previously have been maintained and specific details can be found in the Scheme's annual reports for 1994/95 and 1995/96 (Unicomarine, 1995 & 1996). Email was the primary means of communication for all participating laboratories. This has considerably reduced the amount of paper required for the administration of the Scheme.

2.1.2   Data returns

Return of data to Thomson Unicomarine Ltd. followed the same process as in previous years. Spreadsheet based forms (tailored to the receiving laboratory) were distributed via email, with additional hard copies where appropriate. All returned data have been converted to Excel 2003 format for storage and analysis. In this and previous Scheme years slow or missing returns for exercises lead to delays in processing the data and resulted in difficulties with

reporting and rapid feedback of results to laboratories. Reminders were distributed shortly before each exercise deadline.

2.1.3   Confidentiality

To preserve the confidentiality of participating laboratories, each are identified by a four-digit Laboratory Code. In September 2011 each participant was given a confidential, randomly assigned Scheme year eighteen LabCode. Codes are prefixed with the Scheme year to reduce the possibility of obsolete codes being used inadvertently by laboratories, *e.g.* Laboratory number four in Scheme year eighteen will be recorded as LB1804.

In the present report all references to Laboratory Codes are the post-August 2011 codes (Scheme year eighteen), unless otherwise stated. To further reduce potential errors and simplify administration, LabCodes were assigned in a single series for all laboratories participating in the benthic invertebrates, fish and particle size analysis components of the NMBAQC Scheme (due to Thomson Unicomarine administering these three components).

2.2   Fish Reverse Ring Test (**F_RRT**) Module

2.2.1   Description

This training module enables the identification of fish specimens to be externally verified and encourages laboratories / fish teams to build extensive, verified reference collections to improve identification consistency. The value of reference material / images in assisting the process of identification cannot be over-emphasised; the creation and use of reference collections are viewed as best practice. The module follows the format of the Benthic Invertebrate Component's Laboratory Reference (LR) module which was introduced in Scheme year three (1996/97). These modules assess the ability of participating laboratories to identify material from their own area, or with which they are familiar, or to have difficult specimens examined externally. This was the third official Fish Reverse Ring Test exercise (F_RRT03). The participants were required to submit a reference collection of fifteen specimens for re-examination by Thomson Unicomarine Ltd. Laboratories are also permitted to use this exercise to verify identifications of difficult or problematic taxa about which they are unsure.

2.2.1.1 Selection of fauna

The different geographical distributions of species meant that a request for a uniform set of species from all laboratories was unlikely to be successful. Accordingly a list of instructions was distributed to participating laboratories. Each laboratory / fish team was permitted to include one unidentified or problematic taxon. Specimens wherever possible were to be representatives from WFD monitoring surveys.

2.2.1.2 Analysis

A prepared results sheet was distributed with the exercise's instructions and attached labels for the laboratories to identify each of the specimens. Polystyrene produce boxes and ice-strips were also supplied, if requested, to enable the best transportation protocol for frozen fish. Full instructions for the preparation and postage of specimens were provided. Participating laboratories / fish teams were permitted approximately **thirteen weeks** to prepare and submit their reference specimens. All specimens were re-identified and the identification made by Thomson Unicomarine Ltd. compared with that made by the participating laboratories. All specimens were returned to the laboratories after analysis, if requested.

2.2.2   Results

2.2.2.1 General comments

In total twenty-five laboratories / fish teams subscribed to F_RRT03, with all laboratories returning specimens for verification. Four laboratories submitted data and specimens after the submission deadline (LB1802, LB1809, LB1853 and LB1854). Six laboratories submitted less than the specified number of taxa (LB1843, LB1844, LB1846, LB1847, LB1848 and LB1862). In total three hundred and forty-nine fish taxon bags were submitted for verification.

2.2.2.2 Returns from participating laboratories

Table 1 (Fish Reverse Ring Test Report, F_RRT03) presents a summary of the data sets and specimens received for the F_RRT03 exercise. The identification of the specimens received from the participating laboratories was checked using a variety of identification literature and in-house reference material. Detailed results have been reported to each of the participating laboratories / fish teams via a single exercise report containing the individual report sheets for all participants. Due to this module's emphasis upon training and the diversity of submissions, comparisons of results are not applicable and as such no summary statistics are provided in this report.

Each participant received a Fish Reverse Ring Test Report (Fish Reverse Ring Test Report, F_RRT03), outlining the AQC identifications and providing brief notes for identification discrepancies. Participating laboratories were given the option to request the return of all or some of their specimens for re-examination.

Specific details of each participant's results can be found in the Fish Reverse Ring Test Report (Fish Reverse Ring Test Report, F_RRT03) which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org).

2.2.3   Discussion

In the majority of instances identifications made by Thomson Unicomarine Ltd. were in agreement with those made by the participating laboratories, just twenty-nine errors (from a potential three hundred and forty-nine). In view of the different species that were sent by laboratories for identification it is difficult to make detailed inter-lab comparisons with such a small data set and the potentially differing approaches taken to this exercise. However over a third of the sixteen specimens of grey Mullets sent by participating laboratories were identified incorrectly (LB1804, LB1846, LB1848, LB1859 and LB1861 (3 specimens)). Another recurring error was noted for Gobies (*Pomatoschistus minutus, P. pictus* and *P. microps*). Similar errors were noted in F_RRT02. Such trends will be monitored in future reverse fish ring tests and potentially difficult taxa could be specifically targeted in future fish ring tests (F_RT exercises) to quantify and resolve problems via the circulation of standardised specimens.

The results for this exercise should be viewed giving consideration to the different approaches by participant laboratories. Some laboratories appear to be sending well known species while others elect to obtain a 'second opinion' on more difficult species or atypical individuals. Thus the scores are not comparable and it is not considered appropriate to assign any rank to the laboratories. Each participant should deliberate upon the aims of this component in terms of data quality assessment.

2.3   Fish Ring Test Specimens (F_RT) Module

2.3.1   Description

This training module of the Scheme examined inter-laboratory variation in the participants ability to identify fish taxa and attempted to determine whether any errors were the result of inadequate keys, lack of reference material (*e.g.* growth series), or the incorrect use of satisfactory keys.

One set of fifteen fish specimens (F_RT05) was distributed in 2012. Details of substratum, salinity, depth and geographical location were provided for all ring test specimens to assist identification.

2.3.1.1 Preparation of the Samples

The specimens distributed were obtained from a range of surveys from around the UK and Europe. Specimens were also donated by other organisations. Every attempt was made to provide animals in good condition and of similar size for each laboratory/fish monitoring team. Each specimen sent was uniquely identifiable by means of a coded label and all material has been retained for subsequent checking. Where relevant, every effort was made to ensure all specimens of a given species were of the same sex.

All specimens were taken from replicate trawls or grabs within a single survey and in most cases they were replicates from a single sampling station.

2.3.1.2 Analysis required

The participating laboratories were required to identify each of the F_RT specimens to species and provide the Species Directory code (Howson & Picton, 1997) for the specimen (where available). If a laboratory would not routinely have identified the specimen to the level of species then this should be detailed in the 'confidence level' field. Laboratories can also add brief notes and information on the keys or other literature used to determine their identifications. The specimens were retained by the participant laboratories for incorporation into their in-house reference collections or training material. **Twelve weeks** were allowed for the analysis of the fish RT exercise (F_RT05).

2.3.2 Results

2.3.2.1 General comments

The implementation of this part of the Scheme was the same as in previous years. The F_RT circulation was accompanied by details of each specimen's habitat details (depth, salinity, substratum, and geographical location). The F_RT circulations are designed as a learning exercise to discover where particular difficulties lie within specific common taxa. A number of laboratories use these modules of the Scheme for training purposes and have selected them preferentially over other modules. CSEMP laboratories are required to participate in this component though it is not used when assigning 'pass' or 'fail' flags.

For F_RT05 fifteen fish specimens were circulated to eleven participating laboratories. As with previous Scheme years, participating laboratories were permitted to supply multiple data entries for each exercise to maximise results and enhance the training aspect of this module. Other aspects of the circulation, in particular the method of scoring results, were the same as for previous circulations. Participating laboratories were permitted to retain F_RT05 fish specimens as part of their in-house reference collections. All eleven laboratories returned data for this exercise; seventeen individual data sets in total via multiple data submissions.

2.3.2.2 Returns from participating laboratories

Each laboratory returned a list of their identifications of the taxa. The identifications made by the participating laboratories were then compared with the AQC identifications to determine the number of differences. A simple character-for-character comparison of the text of the two names (the AQC identification and the laboratory identification) was the starting point for this

determination and provided a pointer to all those instances where (for whatever reason) the names differed. Each of these instances was examined to determine the reason for the difference.

As previously found, a major cause of an identification being different from the AQC identification was through differences in spelling of what was clearly intended to be the same species. There were several examples of these differences, e.g. *Hyperlopus lanceolatus* for *Hyperoplus lanceolatus*, *Zues faber* for *Zeus faber* and *Trispoterus mintutus* for *Trisopterus minutus*. However, unlike previous F_RT exercises no errors were noted due to the use of synonyms indicating participants are using up-to-date identification guides.

NB. For the purposes of calculating the total number of differences in identification made by each laboratory a difference was ignored if it was clearly a spelling error.

Tables 1 and 2 (Ring Test Bulletin – F_RT05) present the identifications made by each of the participating laboratories for the fifteen specimens in circulation F_RT05, arranged by specimen and laboratory respectively. For clarity the name is given only in those instances where the generic or specific name given by the laboratory differed from the AQC identification. Where it was considered that the name referred to the same species as the AQC identification but differed for one of the reasons indicated above, then the name is presented in brackets "[name]". Errors of spelling or the use of a different synonym are not bracketed in this way if the species to which the laboratory was referring was not the same as the AQC identification. A dash, "-", in the Tables indicates that the name of the genus (and / or species) given by the laboratory was considered to be the same as the AQC identification. A pair of zeros, "0 0", in the Tables indicates that the subscribing laboratory did not return data.

2.3.2.2.1 Scoring of RT results

The method of scoring was to increase a laboratory's score by one for each difference between their identification and the AQC identification, *i.e.* for each instance where text other than a dash or a bracketed name appears in the appropriate column in the tables (Tables 1 and 2 in F_RT05). Two separate scores were maintained; for differences at the level of genus and species. These are not independent values, if the generic level identification was incorrect then the specific identification would normally also be incorrect, though the reverse is not necessarily the case.

2.3.2.3 Ring Test distribution results

Results were forwarded to the participating laboratories as soon as practicable. Each participant also received a ring test bulletin (F_RT05), outlining the reasons for each individual identification discrepancy. These bulletins contained images of the test material. Participating

laboratories were instructed to retain their ring test specimens, for addition to in-house reference collections or for future in-house training.

2.3.2.3.1 F_RT05

F_RT05 contained fifteen fish specimens. The results from the circulation are presented in Tables 1 and 2 (F_RT05) in the same manner as for previous circulations. The agreement at the generic level was good; with forty-nine errors (from a potential two hundred and fifty-five) were recorded from the seventeen data sets received via the eleven participating laboratories. Agreement at the specific level was also generally good; sixty-nine errors were recorded. The majority of participating laboratories correctly identified each of the specimens. Only a few of the taxa were responsible for the majority of differences and these are described briefly below.

The bulk of the errors could be attributed to three specimens. *Ammodytes tobianus* (12.5-14cm, poor specimen), *Oncorhynchus mykiss* (16-19cm, good specimen) and *Hyperoplus lanceolatus* (14.5-17.5cm, good specimen) accounted for a total of 53% of all generic errors and 46% of all the specific errors recorded. Five of the fifteen circulated specimens were correctly identified by all participating laboratories (*Hyperoplus lanceolatus*, *Lipophrys pholis*, *Zeus faber*, *Lophius piscatorius* and *Perca fluviatilis*). Further details and analysis of results can be found in the Fish Ring Test Bulletin (Fish Ring Test Bulletin – F_RT05) which was circulated to each laboratory that supplied results for this exercise and was posted on the Scheme's website (www.nmbaqcs.org).

2.3.2.4 *Differences between participating laboratories*

Figure 1 (F_RT05) presents the number of differences recorded at the level of genus and species for each of the participating laboratories. The laboratories are ordered by increasing number of differences at the level of species. The division of laboratories into three bands (Low, Medium and High) on the basis of the number of differences at the level of species is also shown.

2.3.3 *Discussion*

This is the fifth fish ring test circulated through the NMBAQC Scheme and the results were comparable with those from the four previous exercises RT28 (F_RT01), RT31 (F_RT02), RT33 (F_RT03) and F_RT04, with a high level of agreement between participating laboratories for the majority of distributed species. The F_RT component is considered to provide a valuable training mechanism and be an indicator of problem groups and possible areas for further 'targeted' exercises or inclusion at taxonomic workshops. Multiple data entries from some laboratories and the inclusion of images in the ring test bulletins (RTB) have further emphasised the learning aspect of these exercises.

F_RT05 indicated that the majority of laboratories are using the same literature to identify most specimens; Wheeler 1969 and Maitland & Herdson 2009. However, several of the participating laboratories did not provide information as to the literature used for identification. None of the participants identified all of the specimens correctly. Several participants mis-identified species that are perceived to be common and readily identifiable (*Pleuronectes platessa*, *Trispoterus minutus* and *Microchirus variegatus*). The most common error was for the lesser sandeel (*Ammodytes tobianus*). Deterioration of ring test material may also have contributed to some mis-identifications, for example fin damage due to repeated examination could produce inaccurate fin ray counts. It must be noted that the vast majority of participants in this exercise would not routinely encounter fixed and preserved fish specimens and these results do not necessarily compromise identifications in routine fish monitoring surveys. Further details and analysis of results can be found in the fish ring test bulletin (Fish Ring Test Bulletin – F_RT05) which was circulated to all participants and is available on the Scheme's website (www.nmbaqcs.org).

3.   Conclusions and Recommendations

A number of observations may be made from the results of the exercise described above. The following is a summary of the major points of importance.

1. The third Fish Reverse Ring Test (F_RRT03) was successfully implemented and the format can be brought forward for another exercise in the next Scheme year. Participants are encouraged to continue to provide feedback to enable the protocols to be refined.

2. An improved learning structure to the Scheme through detailed individual exercise reports has been successfully implemented and was continued in this Scheme year. After each F_RT exercise a bulletin is circulated, reviewing the literature used and detailing the correct identification of the taxa circulated. Participants are encouraged to review their exercise reports and provide feedback concerning content and format wherever appropriate.

3. The majority of participating laboratories submitted data before the deadline, however late submissions contributed in delaying the production of the final report. Laboratories should endeavour to submit their results within the requested time; this would greatly facilitate the analysis of results and effective feedback.

4. Previous Fish Ring Tests (RT28 (F_RT01), RT31 (F_RT02), RT33 (F_RT03), F_RT04) and Reverse Fish Ring Test (F_RRT01, F_RRT02) have highlighted instances of error due to the incorrect translation of a common name. A significant number of both F_RRT03 and F_RT05 data also contained several spelling errors. Fish teams are to incorporate scientific names in field data records and/or ensure that common to scientific name translations are correct prior to database submission.

5. Fish teams are encouraged to collate fish identification literature to improve their identification skills and follow the most recent taxonomy. <u>Unpublished keys from Scheme workshops could be posted on the Scheme's website. The Scheme has produced a UK Standard Taxonomic Literature database. Laboratories are encouraged to review the content and give details of additions wherever possible.</u>

6. The maintenance of a comprehensive reference collection has numerous benefits for improving identification ability, training new staff, maintaining consistency of identification between surveys and access to growth series material. The inclusion of growth series material is extremely useful for certain faunal groups. Ideally all surveys should have an associated reference collection to enable ease of cross-checking or adopting future taxonomic developments. <u>It is strongly recommended that laboratories implement and expand in-house reference collections of fish; these collections could include images and physical specimens.</u>

7. An improved learning structure to the Scheme through detailed individual exercise reports has been successfully implemented and was continued in this Scheme year. <u>Participants are encouraged to review their exercise reports and provide feedback concerning content and format wherever appropriate.</u>

8. Recurring errors have been highlighted in the identification of both grey Mullets and Gobies in all reverse ring test exercises. <u>These groups should be targeted at workshops or in future ring test exercises.</u>

9. Future Fish Ring Test (F_RT) circulations will target taxa identified in the Fish Reverse Ring Tests (F_RRT) as potentially problematic. <u>Participants are encouraged to inform Thomson Unicomarine of difficult taxa that they would like to be 'Ring Tested'. Participants are also invited to submit specimens for use in such exercises (approximately 20 specimens of equal size and condition would be required for inclusion).</u>

10. The RT and Reverse RT modules offer training and baseline data for fish; a quality control module (similar to the benthic invertebrate component's Own Sample module) should be devised to provide quantifiable data assurance.

11. This year's Fish Ring Test (F_RT05) produced seventeen data sets from eleven participating laboratories due to the submission of multiple data sets. <u>The option of multiple data submissions per participant laboratory will be continued into future F_RT exercises. Participants should not submit multiple sets of data if these data represent a replicated consensus; multiple data submissions are to allow sub-teams and individual analysts to receive specific results and feedback.</u>

4. References

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