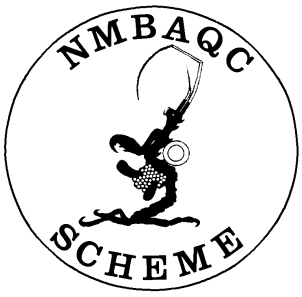

The National Marine Biological
Analytical Quality Control Scheme

Fish Component Report from the Contractor
Scheme Operation – Year 19
2012/13

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FISH COMPONENT REPORT FROM THE CONTRACTOR
SCHEME OPERATION – YEAR 19 – 2012/13

Scheme Year 19 Exercise Reports (hyperlinked in this report)

Reverse Ring Test Report – F_RRT04

Fish Ring Test Bulletin – F_RT06

Previous Scheme Exercise Reports (hyperlinked in this report)

Reverse Ring Test Report – F_RRT01

Reverse Ring Test Report – F_RRT02

Reverse Ring Test Report – F_RRT03

Ring Test Bulletin – F_RT01

Ring Test Bulletin – F_RT02

Ring Test Bulletin – F_RT03

Ring Test Bulletin – F_RT04

Ring Test Bulletin – F_RT05

1. Introduction

Year nineteen of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme (2012/13) followed the format of the eighteenth year. A series of exercises involved the distribution of test materials to participating laboratories and the centralised examination of returned data and samples.

The Fish Component of the scheme commenced in its twelfth year (2005/06). Twenty five laboratories / fish teams participated in the Fish Component of the Year 19 NMBAQC Scheme. Twenty one participants were government laboratories / fish teams; four 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 nineteenth 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 eighteenth 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 relatively accurate (F_RRT04) (17 errors for 325 specimens submitted). The majority of specimens were collected by fish teams during their 2012 autumn monitoring surveys. One recurring error that was highlighted by this exercise concerned the identification of the Grey Mullets with four individuals incorrectly identified. Other recurring errors included Wrasses, Dragonets and 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.

Fish Ring Test (F_RT): Fifteen fish specimens was distributed by Thomson Unicomarine Ltd. This fish ring test (F_RT06) produced good agreement between the identifications made by the participating laboratories and those made by Thomson Unicomarine Ltd. On average each laboratory recorded 1.05 generic differences and 1.90 specific differences.

1.1.1 Statement of Performance

To each participating laboratory / fish team, a Statement of Performance will be issued 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

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).

2.1.2 Data returns

Return of data to Thomson Unicomarine Ltd. followed the same process as in previous years. Spreadsheet based forms 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 2012 each participant was given a confidential, randomly assigned Scheme year nineteen 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 nineteen will be recorded as LB1904.

In the present report all references to Laboratory Codes are the post-August 2012 codes (Scheme year nineteen), 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

The Fish Reverse Ring Test is a training module which 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 Components 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 fourth official Fish Reverse Ring Test exercise (F_RRT04). 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 were 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 **nine 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. Specimens were returned to the laboratories after analysis, if requested.

2.2.2 Results

2.2.2.1 General comments

In total twenty-four laboratories / fish teams subscribed to F_RRT04, with twenty-two laboratories returning specimens for verification. Three laboratories submitted data and specimens after the submission deadline (LB1937, LB1941 and LB1942). Three laboratories submitted less than the specified number of taxa (LB1938, 1949 and 1953). In total three hundred and twenty five fish samples were submitted for verification.

2.2.2.2 Returns from participating laboratories

Table 1 (Fish Reverse Ring Test Report, F_RRT04) presents a summary of the data sets and specimens received for the F_RRT04 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_RRT04), outlining the AQC identifications and providing brief notes for identification discrepancies.

Specific details of each participant's results can be found in the Fish Reverse Ring Test Report (Fish Reverse Ring Test Report, F_RRT04) 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

2.2.3.1 General Discussion

In the majority of instances, identifications made by Thomson Unicomarine Ltd. were in agreement with those made by the participating laboratories with seventeen errors occurring from a potential three hundred and twenty five. The Grey Mullets (*Liza aurata*; *Chelon labrosus* and *Liza ramada*), caused the most identification errors, with four of the twenty specimens sent by participating laboratories identified incorrectly (LB1938, LB1940 and LB1952 (2 specimens). Gobies were the next taxonomic group that were incorrectly identified (*Pomatoschistus microps, P. minutus* and *Gobius niger*). Similar errors were noted in the previous report F_RRT03. There were also discrepancies for Corkwing Wrasse (*Symphodus melops*) and Common Dragonets (*Callionymus lyra*). 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.

2.2.3.2 Dragonet identification queries

(The following text: O'Reilly 2013, pers. comm., 30 May)

The identification of Dragonets has raised some queries. Representatives of the three British Dragonet species were submitted. Two specimens initially identified as Reticulated Dragonets (*Callionymus reticulatus*) were attributed to Common Dragonets (*Callionymus lyra*) during the exercise based on the presence of 4 preopercular spines. One of these specimens (8cm in length) showed distinctive black coloration of the first dorsal fin, as illustrated for the Reticulated Dragonet in Kay & Dipper (2009), but had 4 distinct preopercular spines. The specimen was collected in a SEPA survey in 2012 in Loch Eil, near Fort William in Western Scotland. This water body, a transitional water sea loch, also harbours Common Dragonets and Spotted Dragonets, so some care is required when identifying smaller specimens. Most fish guides (Wheeler 1969, Lythgoe & Lythgoe 1971, Maitland & Herdson 2009, Kay & Dipper 2009) indicate that the Reticulated Dragonet has only 3 preopercular spines although Fricke (1986) indicates the antrorse spine (facing forward) may be small, rudimentary, or absent in this species. Fricke's figures indicate that female Spotted Dragonets may also have a darkly coloured first dorsal fin which suggests that the Loch Eil specimen could be the latter. However the female first dorsal fin depicted by Lythgoe & Lythgoe (1971) for a Spotted Dragonet is spotted rather than dark. The apparent absence of any spots on the membrane of the second dorsal fin of the Loch Eil specimen (albeit now a preserved specimen) is more indicative of a female Reticulated Dragonet.

However, subsequent sampling by SEPA in the Gareloch and the adjacent Outer Clyde Estuary in May 2013 revealed seven female Common Dragonets (size range 12-14cm) with distinct short, black, first dorsal fins. The second dorsal fin had a single horizontal brown band. Such darkly pigmented first dorsal fins are not illustrated for the Common Dragonet in any if the fish guides.

It is evident that for small sized Dragonets (15cm or under) careful observation of the preopercular spines and colouration patterns of both the body and the dorsal fins of live or fresh specimens is required to help elucidate the species present. According to the fish guides (and the present observations) a dark first dorsal fin could occur in Common, Reticulated, or Spotted Dragonets! Some further clarification of the variation of fin colouration patterns, especially in juveniles or females, is required. It seems likely that due to confusion in this matter the occurrence of Spotted and Reticulated Dragonets could be under recorded in British waters.

2.3 Fish Ring Test (F_RT) Module

2.3.1 Description

The Fish Ring Test is a training module of the Scheme, which examines 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_RT06) was distributed in 2013.

2.3.1.1 *Preparation of the Samples*

The specimens distributed were obtained from a range of surveys from around the UK. 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 level and provide the respective Species Directory code (Howson & Picton, 1997) where available. If a laboratory would not routinely have identified the specimen to species level 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. **Twelve weeks** were allowed for the analysis of the fish RT exercise (F_RT06).

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 for assigning pass or fail flags.

For F_RT06 fifteen fish specimens were circulated to eighteen 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. Thirteen of the fifteen specimens were either discarded or retained by the participant laboratories for incorporation into their in-house reference collections or training material. The two preserved specimens (specimen 06; *Limanda limanda* and specimen 14; *Arnoglossus laterna*) were requested to be returned to Thomson Unicomarine by 1st October 2013. Eighteen laboratories out of nineteen returned data for this exercise, with twenty one 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. In a first instance, the correct spelling of the name was checked and then other differences were evaluated.

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. *Osmerus eperlanu* for *Osmerus eperlanus, Spratus spratus* for *Sprattus sprattus* and *Chelidonichthys lucernus* for *Chelidonichthys lucerna.* Errors calculated were just for identification errors, not synonyms or spelling errors. Synonyms and spelling errors were however highlighted in Tables 1 and 2 (Ring Test Bulletin - F_RT06) to those participants who need to check names against the FishBase (www.fishbase.org) or WoRMS websites (www.marinespecies.org).

Tables 1 and 2 (Ring Test Bulletin – F_RT06) present the identifications made by each of the participating laboratories for the fifteen specimens in circulation, 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]. Spelling errors or the use of a 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.

2.3.2.3 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_RT06). Two separate scores were maintained for differences at the genus or species level. A genus can be incorrect, but the species correct which is a form of synonymy as illustrated with the example of *Blicca bjoerkna* which has changed from *Abramis bjoerkna*.

2.3.2.3 Ring Test distribution results

Each participant was notified of the test bulletin (F_RT06) being published on the NMBAQC website outlining the reasons for each individual identification discrepancy. This bulletin contained images of the test material. Participating laboratories were instructed to return 2 preserved specimens (06 and 14).

2.3.2.3.1 F_RT06

F_RT06 contained fifteen fish specimens. The results from the circulation are presented in Tables 1 and 2 (F_RT06) in the same manner as for previous circulations. The agreement at the generic level was good; twenty-two errors (from a potential three hundred and fifteen) were recorded from the twenty-one data sets received via the eighteen participating laboratories. Agreement at the specific level was also good; forty 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 majority of the generic differences were recorded from *Blicca bjoerkna* and *Arnoglossus laterna* whereas the majority of specific differences recorded were from *Ammodytes marinus*, with thirteen laboratories recording as *Ammodytes tobianus*.

Four of the fifteen circulated specimens were correctly identified by all participating laboratories (*Sprattus sprattus*, *Osmerus eperlanus*, *Rutilus rutilus and Dicentrarchus labrax).* Specimen FRT603 was also recorded as being correctly identified by all participating laboratories despite not all specimens being re-checked due a mixture of *Scomber* species. Further details and analysis of results can be found in the Fish Ring Test Bulletin (Fish Ring Test Bulletin – F_RT06) 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_RT06) presents the number of differences recorded at genus and species level for each of the participating laboratories. The laboratories are ordered by increasing number of differences at species level. The division of laboratories into three bands (Low, Medium and High) on the basis of the number of differences at species level is also shown.

2.3.3 Discussion

2.3.3.1 General Discussion

This is the sixth fish ring test circulated through the NMBAQC Scheme and the results were comparable with those from the five previous exercises RT28 (F_RT01), RT31 (F_RT02), RT33 (F_RT03), F_RT04 and F_RT05 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 problematic 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_RT06 indicated that the majority of laboratories are using the same literature to identify most specimens; Wheeler 1969, Wheeler 1978 and Maitland & Herdson 2009. However, several of the participating laboratories did not provide information as to the literature used for identification.

Several participants mis-identified species that are perceived to be common and readily identifiable (*Limanda limanda* and *Lampetra fluviatilis*). The most common error was for the lesser sandeel (*Ammodytes marinus*). Deterioration of ring test material may also have contributed to some mis-identifications; reasons for this include fin damage due to repeated examination which could produce inaccurate fin ray counts. Some of the specimens arrived in a deteriorated condition after being in transit. 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_RT06) which was circulated to all participants and is available on the Scheme's website (www.nmbaqcs.org).

2.3.3.2 Mackerel identification queries

The identification of mackerels has raised some queries in F_RT06. A mixture of two *Scomber* species was sent out in the fish ring test (F_RT06) for specimen 03 which included *Scomber scombrus* and *Scomber colias / Scomber japonicus.* Only one of the two species was sent to each participating laboratory. *S. colias* and *S. japonicus* have been regarded by some as synonymous but recent molecular evidence suggests that they are grouped in distinct lineages within the *Scomber* cluster, indicating they are 2 separate species.

Identifications of species within the genus *Scomber* has produced some controversy over the years which has led to molecular research verifying the genetic differences between species. Each of the species are usually found to inhabit different habitats. Morphological differences of *Scomber* species, include a difference in head size, although this is reliable only in adults. Coloured markings on the sides and bellies may also be useful, but they can quickly fade after death. The most reliable character to separate *S. scombrus* from *S. colias* and *S. japonicus* is the first dorsal fin ray count; *S. scombrus* has 11 - 13 slender spines whereas *S. colias* and *S. japonicus* have 9 - 10. Recent molecular studies show, that *S. colias* and S*. japonicus* group in distinct lineages within the *Scomber* cluster which support the recognition of Atlantic *Scomber colias* and Pacific *Scomber japonicus* as distinct species.

3. Conclusions and Recommendations

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

1. The sixth Fish Reverse Ring Test (F_RRT04) 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_RRT exercise a bulletin is circulated (F_RRT04), reviewing the literature used and detailing the correct identification of the taxa circulated. Participants are encouraged to review the bulletin 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, F_RT05) and Reverse Fish Ring Test (F_RRT01, F_RRT02, FRRT03) have highlighted instances of error due to the incorrect translation of a common name. A significant number of both F_RRT04 and F_RT06 data also contained numerous 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 results in taxonomy. 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. The use of referring to websites such as FishBase and WoRMS is recommended to check the most recent names used.

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 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. It is strongly recommended that laboratories implement and expand in-house reference collections of fish; these collections could include images and physical specimens.

7. Recurring errors have been highlighted in the identification of Dragonets, Grey Mullets and Gobies in all reverse ring test exercises. These groups should be targeted at workshops or in future ring test exercises.

8. Future Fish Ring Test (F_RT) circulations will target taxa identified in the Fish Reverse Ring Tests (F_RRT) as potentially problematic. Participants are encouraged to inform Thomson

Unicomarine of difficult taxa that should be included in ring tests. Participants are also invited to submit specimens for use in such exercises approximately 30 specimens of equal size and condition would be required for inclusion).

9. 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.

10. This years Fish Ring Test (F_RT06) produced twenty one data sets from eighteen participating laboratories due to the submission of multiple data sets. 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.

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