NMBAQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

Fish Component Annual Report
Scheme Operation 2019/2020 (Year 26)

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FISH COMPONENT ANNUAL REPORT FROM APEM Ltd

SCHEME OPERATION – 2019/2020 (Year 26)

Linked Documents (hyperlinked in this report):

Fish Reverse Ring Test Bulletin — RRT11

Fish Ring Test Bulletin — FRT13

1. Introduction

The twenty-sixth year of the NE Atlantic Marine Biological Analytical Quality Control (NMBAQC) Scheme (2019/2020) closely followed the format of the twenty-fifth year, with a ring test (RT) and a reverse ring test (RRT) being organised. The Fish Component of the Scheme is currently in its fifteenth year (start 2005/06). It involved the distribution of test specimens and images to participating laboratories and the centralised examination of returned data for the first module (RT), and re-analysis of fish specimens submitted by participants (RRT). Additionally for the RT module, participants were supplied with a suite of images of each taxon and invited to complete an optional 'image based identification' answer sheet in the same format as the normal RT data sheet and submit results before the physical specimens were distributed. The labelling and distribution procedures employed previously have been maintained. Specific details can be found in previous Scheme annual reports.

Nineteen laboratories signed up for Scheme year 2019/2020 (with multiple participants from some organisations counted separately). Thirteen participants were government laboratories, three private consultancies, one university, one chartered laboratory and one research institute. 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 fish assemblages in transitional waters for the Water Framework Directive (WFD) provides a major impetus for the Fish Component modules. As in previous years, some laboratories elected to be involved in either one or both modules of the scheme.

1.1 Summary of Performance

This report presents the findings of the Fish component for year 2019/2020 (year 26) of the North East Atlantic Marine Biological Analytical Quality Control (NMBAQC) Scheme.

This component comprised two modules:

- Fish Reverse Ring Test module (FRRT) - re-identification by APEM Ltd. of a set of up to fifteen specimens supplied by participating laboratories;
- Fish Ring Test module (FRT) - identification of fifteen fish specimens with the option of submitting an additional image only answer sheet.

The analytical procedures of the various modules were the same as for 2018/2019 (year 25) of the Scheme. The results for each of the Scheme exercises are presented and discussed.

Comments are provided on the performance of participating laboratories in each of the exercises.

Fish Reverse Ring Test (FRRT): The identification of up to fifteen fish specimens selected and supplied by the thirteen participating laboratories (FRT11) was relatively accurate with five taxonomic errors for 190 specimens submitted. Twelve participants supplied collection dates for specimens, these were all collected between October and December 2019. Most participants used this as a test for confirming voucher specimens; four participants included a problematic specimen in their submission, misidentification of problematic specimens are not counted as taxonomic errors.

Fish Ring Tests (FRT): Samples of 15 specimens were distributed (FRT13). Whilst the FRT was not exclusively targeted, the aim was to include specimens that have been problematic in the past and small/juvenile specimens that might require more detailed examination.

For FRT13, the average numbers of differences per participating laboratory (for a total of 11 laboratories with 16 submissions) were 2.3 generic differences (15%) and 4.5 specific differences (28%). Three specimens (Clupeidae, Gadidae and Gobiidae) were responsible for half of the generic differences. Three specimens (two Gobiidae, one Mugilidae) were responsible for 43% of the specific differences.

1.1.1 Statement of Performance

Each participating laboratory was supplied with a 'Statement of Performance', which included a summary of results for each of the Scheme modules and details of the resulting flags, where appropriate. These statements were first circulated with the Year 5 annual report (1998/1999) 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 are two modules within the Fish component: Fish Reverse Ring Test (FRRT) and Fish Ring Test (FRT) modules.

Each of these modules is described in more detail below. A brief outline of the information 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. An effort was made this year to use more environmentally sustainable materials for the distribution and receipt of specimens. This included the replacement of polystyrene boxes with cardboard boxes and the use of reusable ice packs with natural wool insulation for the transportation of frozen material.

2.1.2 *Data Returns*

Return of data to APEM Ltd. followed the same process as in previous Scheme years. Spreadsheet-based forms (tailored to the receiving laboratory) were distributed to each laboratory via email, paper copies were also supplied. All returned data were converted to Excel 2010 format for storage and analysis. Reminders were distributed shortly before each exercise deadline.

2.1.3 *Confidentiality*

In September 2019, each participant was given a confidential, randomly assigned 2019/2020 (Scheme year 26) LabCode. Codes are prefixed with the component initials (*i.e.* F for Fish component), the Scheme Year and a unique number (between 01 and 25); for example, laboratory number one in Scheme Year 2019/2020 (Year 26) was recorded as F_2601.

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

2.2.1 *Description*

The Fish Reverse Ring Test module is a training module which encourages laboratories to build reference collections to improve identification consistency, and to seek additional opinions for difficult specimens. The value of reference material in assisting identification cannot be over-emphasized; the creation and use of reference collections is viewed as best practice. This module can help participating laboratories to assess their ability to identify material from their own samples. Laboratories are also able to use this exercise to obtain second opinion identifications for difficult or problematic taxa of which they are unsure. This was the eleventh Fish Reverse Ring Test exercise (FRRT11). The participants were able to submit up to 15 specimens for re-examination by APEM Ltd.

2.2.2 *Selection of fauna*

Participants were asked to submit, wherever possible, specimens from WFD monitoring surveys and could include one unidentified or problematic taxon. It is the intention of the exercise for participants to have fish specimens from their own surveys and geographical region re-examined, accordingly a diverse number of species and regions are included.

2.2.3 Results

2.2.3.1 General comments

Thirteen laboratories subscribed to the eleventh Fish Reverse Ring Test. Only one participant submitted data and specimens shortly after the deadline. One participant submitted less than the fifteen permitted specimens and four participants submitted a problematic specimen.

2.2.3.2 Analysis of material from participating laboratories

Fish Reverse Ring Test Bulletin FRRT11 presents a summary of the data sets and specimens received for analysis. The re-identification of the submitted specimens used a variety of identification literature and in-house reference material. Due to this module's emphasis upon training and due to the diversity of submissions, comparison of results is not applicable and, as such, no summary statistics are provided in this report.

A preliminary report with individual results was sent to each participant before the Fish Reverse Ring Test Bulletin (FRRT11) summarising the results of all participants was distributed. Table 1 of the bulletin summarises the species submitted by participants and Table 2 gives details on the taxonomic errors and discrepancies observed. Participants were given the option to request specimens returned following completion of the exercise. Participants were notified once the bulletin was available for download from the Scheme's website (www.nmbaqcs.org).

2.2.4 Discussion

In almost all cases identifications made by APEM Ltd. agreed with those made by the participants, only five taxonomic errors from one hundred and ninety specimens were recorded. The taxonomic errors were not limited to any single problematic group of species but from five different families. Six taxonomic discrepancies were recorded, three from spelling errors of species names and three from the uses of synonyms. The submission of authorities for species names was optional and as such errors submitted were not included as taxonomic discrepancies.

2.3 Fish Ring Test (**FRT**) Module

2.3.1 Description

The Fish Ring Test (FRT) module is a training module which examines variation in participants' ability to identify different species and attempts to determine whether

differences are the result of literature deficiencies, lack of reference material (e.g. growth series) or misinterpretation of identification resources.

A set of 15 fish specimens with accompanying images were distributed in November 2019. An effort was made to include species that had been previously highlighted as problematic and juvenile or small specimens that might lack diagnostic features present at a larger size. Basic habitat and geographic details recorded when specimens were collected were provided to assist identification.

2.3.2 Preparation of the samples

The specimens distributed were obtained from a range of surveys from around the UK. All specimens were collected by APEM Ltd. Care was taken to provide specimens of similar size and condition for each laboratory. Each specimen was uniquely identifiable by means of a coded label and all material can been retained by participants for subsequent checking. Specimens were taken from samples within a single survey and, in most cases, they were from a single sample or trawl.

2.3.2.1 Analysis Required

The participating laboratories were asked to identify each of the FRT specimens to species level and they were also asked to complete a 'confidence level' field to indicate whether they would ordinarily have left the specimen at a higher taxonomic level. Participants could also add brief notes and information detailing the literature used to determine their identifications. The implementation of this part of the Scheme was the same as in previous years with the exception that images were distributed three weeks prior to specimens to allow participants to enter an optional image only answer sheet. Seven laboratories chose to submit the optional 'image only' set of preliminary results. Participants were permitted to supply multiple returns (*i.e*. different sets of results from different analysts) to enhance the training value of the module, two laboratories chose to utilise this option. The protocols followed, particularly the method of counting differences, were the same as for previous circulations. Approximately nine weeks were allowed for the analysis of specimens.

2.3.3 Results

2.3.3.1 General Comments

Several laboratories use the ring tests for training purposes and select them preferentially over other modules. The results are not used to assign 'Pass' or 'Fail' flags. In total, eleven laboratories subscribed to FRT13 with a total of sixteen individual data sets. Eight data sets

for identification made from images alone were submitted. Only one participant submitted data shortly after the deadline.

2.3.3.2 Returns from Participating Laboratories

Identifications made by the participating laboratories were compared with those made by APEM Ltd. to determine the numbers of differences. Where identifications deviated from the APEM Ltd. identification due to the use of synonyms, or incorrect spellings of the name, the difference was ignored for the purpose of calculating the total number of differences.

Tables 2 and 3 of Fish Ring Test Bulletin FRT13 show identifications made by each of the participating laboratories for the fifteen specimens, arranged with laboratories as rows and specimens in columns in Table 2, specimens as rows and laboratories as columns in Table 3. For clarity, the participant's identification is given only where the name given by the laboratory differed from the APEM Ltd. identification. Where it was considered that the name referred to the same species as the APEM Ltd. identification, but differed for one of the reasons indicated above, the name is presented in brackets: "[name]". A dash, "-", in the tables indicates that the name of the genus (and / or species) given by the laboratory was the same as the APEM Ltd. identification.

2.3.3.3 Counting Result differences

For each laboratory, a count was made of each difference between their identification and the APEM Ltd. identification (*i.e*. for each instance where text other than a dash or a bracketed name appears in the appropriate column in Tables 2 and 3). Separate counts were maintained for differences at genus and species level.

2.3.3.4 Ring Test Results

The intention of this training module is to discover where difficulties lie in the identification of certain taxa. Results for Scheme Year 2019/2020 were presented in Fish Ring Test Bulletin FRT13 along with the reasons for each identification discrepancy. This bulletin contains images of the test material and of all available taxa that were named as alternative identifications by participants. Participating laboratories were advised to retain ring test specimens after receiving their results, in order that they could review their identifications, if necessary. Participants are encouraged to question APEM Ltd. identifications if they still believed their original identifications to be correct. On completion of the exercise specimens can be incorporated into participants in-house reference collections.

2.3.3.5 Taxonomic differences observed

The results discussed below are given in Table 2 of Fish Ring Test Bulletin FRT13, which displays the data arranged with columns for species to enable quick reference to the range of answers received and in Table 3, which presents the results arranged with columns for participants.

The agreement at generic level was generally good; 36 differences (15% of all genus identifications received from participants) were recorded in the 16 data sets received from 11 participating laboratories. There was much less agreement at species level, with 66 differences recorded (28% of all species identifications received from participants).

Three of the specimens circulated were responsible for half of the generic differences. These were *Clupea harengus, Merlangius merlangus* and *Crystallogobius linearis*. Three of the specimens circulated were responsible for 43% of the specific differences. These were *Chelon labrosus*, *Pomatoschistus lozanoi* and *C. linearis*.

Following re-examination of specimen 4 (*Ammodytes tobianus*), it was deemed possible some laboratories received a specimen where the diagnostic features needed to separate the species from *A. marinus* were indeterminant. Therefore, for this ring test specimen, both *A. marinus* and *A. tobianus* were accepted as correct for this exercise. Concerns over the identifications of specimens 8, 11 and 13 (*Sprattus sprattus*, *C. labrosus* and *Trisopterus minutus*) were also raised by one participant. Further information was supplied to support the AQC identification for these taxa.

Only one of the 15 specimens circulated was correctly identified by all participants (*Limanda limanda*).

Further details and analysis of results, including difference recorded from image only submissions can be found in the Fish Ring Test Bulletin FRT13. The bulletin was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org).

2.3.3.6 Differences between Participating Laboratories

Differences recorded at genus and species level for each of the participating laboratories are summarised in Table 1 and Figure 1 in the Fish Ring Test Bulletin FRT13. The laboratories are ordered by increasing number of differences at species level followed by genus level. The division of laboratories into three bands (Low, Mid and High) based on the number of differences at species level is also shown.

Only one participant correctly identified all specimens and only two participants submitted one difference at species level.

2.3.4 Discussion

The results were in broadly comparable with those from previous exercises. However, the number of differences were higher than generally seen in the past (average generic differences were only higher in FRT03 and FRT05; specific differences only higher in FRT05). The higher number of differences are indicative of the more challenging specimens distributed from problematic groups or of juvenile sizes, and they should not be used as a direct comparison with previous fish ring tests results.

In FRT13, the number of differences observed were mostly related to four problematic groups (juvenile clupeids, sand eels, the sand goby complex and juvenile grey mullet). There were also relatively high numbers of differences for *Merlangius merlangus*, *Trisopterus minutus* and *Crystallogobius linearis*. Many differences were due to the use of identification features from literature that were not consistent for juvenile specimens. The quality of some specimens might have also contributed to identification differences, most participants would identify species in the field and might not be familiar with preserved specimens. Some participants also commented that some of the specimens were smaller than they would encounter or consider recording/identifying to species. Due to these factors the results from the exercise are not intended to make a comparison with participants identifications in routine fish monitoring surveys.

The main literature used to identify most specimens was consistent with past exercises (Henderson 2015, Maitland & Herdson 2009 and Wheeler 1969/1978). There was also a significant increase in the use of scientific papers, workshop keys and early life history books (Munk & Neilsen 2005 and Russell 1969).

Production of the Fish Ring Test Bulletin FRT13 was delayed past the initial deadline. The delay in publication was mostly due to an increased effort to cover all differences and comments raised by participants and to provide detailed summaries of features used for problematic species in a revised reporting format.

The FRT exercise is intended to be a valuable training tool and can be an indicator of problem groups. It can highlight possible taxa for further ‘targeted’ ring test exercises or for inclusion at taxonomic workshops. The allowance of multiple submissions per laboratory and the inclusion of images in the Ring Test Bulletins help to enhance the training value of this component. All participating laboratories have been made aware of the problems identified

by these ring tests via Fish Ring Test Bulletin FRT13, which also included literature citations and detailed discussion of the problem taxa highlighted by the exercise.

3. Conclusions and Recommendations

Several 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 latest Fish Reverse Ring Test (FRRT11) and Fish Ring Test (FRT13) were successfully implemented and their format can be continued in the next scheme year. **Participants are encouraged to provide feedback to enable protocols and implementation to be improved where possible.**

2. Most participating laboratories submitted data / specimens in accordance with the Scheme's timetable. There were only two late submissions, although they did not delay initial analysis and distribution of interim reports. **Participants should endeavour to supply data / specimens according to the exercise deadlines to ensure timely summary reporting.**

3. Some identification differences might be the results of inadequate literature. Participants are encouraged to collate fish identification literature for problematic groups or juvenile specimens and follow the most recent taxonomy. **Participants are encouraged to review the bibliography of taxonomic literature available on the NMBAQC website (Section 3 in Worsfold *et al.* 2018) and give details of additions where possible. Reference to online databases for the validity of scientific names (FishBase, WoRMS and Eschmeyer's Catalog of Fishes) is also recommended.**

4. The maintenance of a comprehensive reference collection has numerous benefits for improving identification ability, maintaining consistency of identification between surveys and access to growth series material. The FRRT exercise can be used as a means of verifying reference specimens. Laboratories are strongly recommended to **implement and expand in-house reference collections of fish; these should include images alongside physical specimens**. The inclusion of juvenile material is useful for

certain groups, *e.g.* clupeids. Ideally **all surveys should include a photographic reference of all species encountered as a minimum.**

5. Laboratories participating in the ring test exercises should attempt to identify all specimens to species and **complete the 'confidence level' section of their ring test datasheets** to enable additional information to be gathered regarding the difficulty of ring test specimens.

6. Since the beginning of the scheme, continual improvement to the learning structure of the Scheme reports has been crucial. For the FRRT and FRT detailed results have been forwarded as **individual exercise reports** to each participating laboratory as soon after the exercise deadlines as practicable. The results and subsequent differences raised in both exercises should **benefit all scheme participants**. A bulletin was circulated after each exercise, reviewing the literature used, detailing the accepted identification of the taxa received or circulated, and including images of relevant specimens. Participants are encouraged to review all exercise reports and **provide feedback concerning content and format** wherever appropriate.

7. Despite being raised as a problematic group in the past gobies and grey mullet continued to be groups with a high number of differences recorded. Future Fish Ring Test exercises are expected to target taxa that were highlighted as potentially problematic in FRT13 and FRRT11. **Participants are encouraged to provide feedback on problem taxa that could be included in future exercise and are invited to submit specimens for use in future exercises** (approximately 20 specimens of similar size and condition).

8. The distribution and analysis of an 'Image only' FRT provided lots of feedback and helped raise potential difficulties that would need to be overcome for the use of image only circulations in future exercises. Notably, clear images of all potential diagnostic features (requiring manipulation of the specimen) would need to be supplied; fin ray counts or similar would also need to be supplied. The use of 'image only' specimens also remains a potentially useful option for the inclusion of

conservation species or scarce species that would otherwise be impractical to circulate. **Participants are encouraged to provide feedback on the use of 'image only' specimens in future exercises.**

9. The Fish Ring Test (FRT13) included the distribution of some specimens that were smaller than usually encountered by some participants, this was probably a factor in more identification differences recorded. **Participants are encouraged to provide feedback on the circulation of juvenile specimens in future exercises.**

10. Two of the eleven laboratories submitted multiple data sets for the Fish Ring Test. **Participants are encouraged to submit multiple data sets for sub-teams and individual analyst where possible to improve the training aspect of the exercise.**

11. Specific protocol documents are yet to be produced for the Fish Component of the Scheme. To avoid possible confusion **protocol documents for the exercise will be produced and made available on the scheme website.**

12. APEM Ltd. always strives to ensure smooth running and **transparency of the Scheme**. APEM Ltd. log and make available all correspondence to the Fish Component Contract Manager (Jim Ellis, CEFAS). Participants can be assured that their anonymity will be protected if this correspondence is required to be shared with the Committee.

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