

**SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE
LABORATORY FOR ANISAKIS APRIL 2013 - MARCH 2014**

1. Introduction.....	1
2. Provision of a completed website for the Anisakis NRL.....	1
2.1 Overview	1
2.2 Website structure	1
3. Production of Standard Operating Procedures (SOPs) for the NRL.....	2
3.1 Adoption of standard operating procedures	2
3.2 Establishment of the OCL network, provision technical advice and training.	3
3.3 Establishment of reference material collection.....	3
4. Participation in EURL proficiency test scheme	3
4.1 Completion of EURLP proficiency test.....	3
5. Attendance at the European Reference Laboratory for Parasites (EURP) annual meeting.....	3
5.1 Participation in EURLP annual meeting	3
5.2 Implementation of the first year of EURLP recommendations.....	4
5.3 Meetings, workshops and task forces	4

1. Introduction

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) Weymouth is designated as the UK National Reference Laboratory (NRL) for Anisakis. This report summarises the activities carried out by the NRL for the financial year 2013-14 according to the requirements of Regulation (EC) No. 882/2004 and as defined in the Service Level Agreement between the Food Standards Agency and Cefas. The description of activities included herein comprises co-ordination of UK Official Control Laboratories (OCL), provision of advice to the Competent Authority (CA) and collaboration with the European Union Reference Laboratory (EURL) through participation in comparative testing.

For the period of this report Dr Matt Longshaw was NRL Director from 1 April until 31 December. Dr Stephen Feist took over the role from 1 January 2014. Statistical support previously provided by Allan Reese who recently retired is now provided by Dr Nick Taylor who has joined the project team.

2. Provision of a completed website for the Anisakis NRL

2.1 Overview

This has been completed and will continue to be updated and refreshed as more information becomes available and usage increases. In addition to the primary purpose as a national information source on the responsibilities and function of the NRL it is intended that the website will form an important site for general information on anisakid nematodes and their importance at potential zoonotic agents.

2.2 Website structure

- NRL Home
 - Background
 - Regulations
 - Links to regulations relating to Anisakis
 - Methods
 - List of methods from EURL
 - Proficiency testing
 - References
 - List of references
 - Contact NRL



UK National Reference Laboratory (NRL) for Anisakis

Providing information and guidance on public health related to Anisakis



3. Production of Standard Operating Procedures (SOPs) for the NRL

3.1 Adoption of standard operating procedures

The EURLP has provided us with their current SOP 'Identification of Anisakidae larvae at the species level by multiplex PCR'. Polymerase Chain Reaction or PCR allows the detection and amplification of nucleotides specific for a particular species using primers. This multiplex PCR uses eight primers that allow detection of ten species of Anisakidae larvae. (*A. pegreffii*, *A. simplex* S.I, *A. physeteris* (including *A. brevispiculata* and *A. paggiae*), *A. typical*, *Contraecaecum osculatum*, *C. rudolphii*, *Pseudoterranova* sp and *Hysterotilacium aduncum*). The primers are specific for the Internal Transcribed Spacer region (ITS) which varies between species and so the different species can be separated on the basis of the size of this amplified product.

Using parasites from the proficiency test, this SOP will be adapted to suit our current methods of extraction, PCR and gel electrophoresis but retaining critical parameters (ie primer ratio and concentration, thermocycler parameters) in order to achieve the same result. The fragments obtained by PCR amplification with oligonucleotides specific for each species of Anisakidae larvae will also be sequenced as a confirmatory measure at least during the initial testing of the method. A brief literature review was also completed to ensure that any relevant background information was assimilated. During the next period it is planned to acquire field collected material for reference and quality assurance purposes (see 3.3).

3.2 Establishment of the OCL network, provision technical advice and training.

As part of the continuing process for identifying those laboratories who are or wish to take part in Anisakis sample testing as part of the NRL proficiency test, 29 laboratories were contacted in England, Wales, Scotland and Northern Ireland via email and were asked to respond to an enclosed questionnaire. Of these 29 labs only 8 responded with only 1 confirming that they wished to take part once the correct testing systems were in place. One OCL that responded to the questionnaire requested advice on molecular diagnosis for *Anisakis*. Communication on this is ongoing.

The NRL director contacted AHVLA colleagues responsible for duties under the NRL for parasites. In particular for awareness of our role but also to ensure that the NRL Anisakis is consulted for information regarding NRL Anisakis activities prior to EURLP meeting for which UK participation is provided by the AHVLA.

3.3 Establishment of reference material collection

As part of our duties for Defra Aquatic Animal Health we maintain a Registry of Aquatic Pathology which contains a large number of parasite specimen, both within tissue sections and as gross specimens. We are building on this reference collection for the NRL Anisakis with a view to build a 'tissue bank' of samples for training purposes, quality assurance and potential research projects. Cefas is well placed to collect suitable parasite samples from various fisheries cruises as well as local sources and planning for this is advanced.

4. Participation in EURL proficiency test scheme

4.1. Completion of EURLP proficiency test

Initial contact was made with Dr Eduard Pozio Director of the EURL for parasites (www.iss.it/crlp/index.php) to notify them of our function and requesting that we participate in the Anisakis proficiency test (PT). Subsequently we contacted Dr. Marco Lalle at the EURL and arranged our participation in the annual PT. Fillet samples were received 19/3/14 and screened using 'protocol 2. UV on squeezed and frozen fish fillet'. Results were returned to the EURL within the required deadline and we are currently awaiting confirmation of receipt. Isolated parasites from this material were stored in G2 buffer for molecular speciation and assessment of the molecular methods/primers currently in place.

5. Attendance at the European Reference Laboratory for Parasites (EURP) annual meeting

5.1 Participation in EURLP annual meeting

Dr Matt Longshaw confirmed that we had received verbal instruction from the FSA that our attendance at the EURLP annual meeting was not required.

5.2 Implementation of the first year of EURLP recommendations

None received.

5.3 Meetings, workshops and task forces

The NRL director attended the International Council for the Exploration of the Seas (ICES) Working Group for Pathology and Diseases of Marine Organisms (WGPDMO) meeting at ICES headquarters in Copenhagen 24th February to 1 March 2014. One of the key functions of this meeting is to collate information on changes in disease prevalence or trends in marine organisms. This includes data on Anisakid nematodes. As indicated above, Cefas currently collects fish health data from sentinel species (the common dab) and on an opportunistic basis commercial fish. We report the occurrence of *Anisakis* infections to this working group. In addition, at this meeting a specific term of reference on 'Parasites and other infectious agents in marine finfish and shellfish species posing a hazard to human health' was discussed. Although UK data does not suggest any significant changes in prevalence in anisakid prevalence in fish, increased prevalence has been noted in some regions such as the Baltic. Previously identified anisakid infection in wild caught salmon (causing 'red vent syndrome') are still being recorded by the Environment Agency.

Participation at the meeting provided the opportunity to establish independent scientific support from an expert on *Anisakis* from Poland (Dr M. Podolska, National Marine Fisheries Institute, Gdynia, Poland) who has kindly agreed to provide advice on anisakid biology and diagnostics on request.



Dr Stephen Feist
NRL Director

Date: 26/03/14