



Centre for Environment  
Fisheries & Aquaculture  
Science



# Annual report for the FAO Reference Centre

## Bivalve Mollusc Sanitation 2022 — 2023

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Title of FAO Reference Centre

**FAO Reference Centre for Bivalve Mollusc Sanitation**

Name of the Institution and contact details

**Centre for Environment, Fisheries and Aquaculture Science (Cefas).**

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Reporting period

**January 2022 - March 2023**

Date of reporting

**June 2023**



Food and Agriculture Organization  
of the United Nations

# 1. Introduction

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) is designated as the FAO Reference Centre for Bivalve Mollusc Sanitation. The Reference Centre's mandate is to contribute to the implementation of FAO's scientific, technical, and economic programme priorities and to provide specific, independent technical/scientific advice aimed at strengthening capacities in countries and regions. This designation is made under an agreement between FAO and the United Kingdom's Department of Environment, Food and Rural Affairs (Defra). This fourth annual report describes the technical and scientific activities delivered via the Reference Centre to support the FAO Department of Fisheries and Aquaculture, and the associated costs for the period January 2022 – March 2023. Financial support for operation of the Reference Centre was afforded by Defra and the United Kingdom's Food Standards Agency (FSA). Details of finances are provided in Annex I.

## 2. Areas of Collaboration

The work programme of the FAO Reference Centre for Bivalve Mollusc Sanitation for the period covered in this report was submitted in December 2021 [<https://www.cefas.co.uk/faobivalves/>].

Main areas of collaboration for 2022-23 were:

- To provide support for the development and maintenance of FAO resources and eLearning material on bivalve mollusc sanitation.
- To provide guidance on relevant laboratory protocols, accreditation, and use of methods for bivalve mollusc testing to FAO Member Countries.
- To prepare and deliver a workshop in Senegal on bivalve mollusc sanitation.
- To plan, organize and distribute a second PT scheme (after the pilot scheme in 2021) for Member Countries.
- To undertake additional activities, within scope, supporting FAO mandate in Member Countries.
- On request, support for FAO Member Countries on topics related to contamination of bivalve molluscs with harmful algal biotoxins.

## 2.1 Provision of support for the development and maintenance of FAO eLearning material and resources on bivalve mollusc sanitation.

The FAO/WHO Technical Guidance for the Development of the Growing Area Aspects of Bivalve Mollusc Sanitation Programmes<sup>1</sup> was first published in 2018, shortly before the original designation of the FAO Reference Centre in 2019. Since then the Reference Centre has been assisting the FAO eLearning academy to develop open access online training based upon the technical guidance. Modules 1 and 2 (covering growing area risks, assessments and review) have been published so far and have proven valuable resources for a range of stakeholders as well as useful pre-workshop familiarisation material for attendees at training events. In the period covered by this report, work has continued on the third and final module in the series which focuses on growing area classification, monitoring and management, and will be published in two parts. In 2021 following requests received from stakeholders to enhance provision on microbiological method implementation, it was agreed to include additional detailed eLearning on key microbiological methods required to generate the data which are used to inform growing area management – technical details which were not included in the original guidance but are included in the Codex Alimentarius Standard for Live and Raw Bivalve Molluscs (CXS 292-2008). This additional material was generated for application and quality assurance of methods for faecal indicator organisms. This has been submitted to the eLearning team for further development. All other technical developmental work has been completed and will be subject to final technical review by the Reference Centre team prior to publication. Module three covers 'Classification, Monitoring and Growing Area Management' and will be split into two parts, comprising of 8 lessons in total, ranging from between 20 – 70 minutes duration. This module covers growing area monitoring (chapter 4), classification (chapter 5) and growing area management (chapter 6) of the Technical Guidance for the Development of the Growing Area Aspects of Bivalve Mollusc Sanitation Programmes. The desired learning outcome for module 3 are set out in Figure 2.

Figure 1. Development of additional eLearning material to cover microbiological methods for bivalve molluscs



<sup>1</sup> FAO and WHO (2018) Technical guidance for the development of the growing area aspects of Bivalve Mollusc Sanitation Programmes. Food Safety and Quality Series No. 5 Rome, 292 pp Licence: CC BY-NC-SA 3.0 IGO.

The Reference Centre agreed additional staged funding to enable the translation of eLearning into French and Spanish languages; to date French versions of modules 1 and 2 have been completed



[\[Cours: Contrôle sanitaire des mollusques bivalves: profil de risques des zones de production conchylicole \(fao.org\)\]](#)



[\[Cours: Contrôle sanitaire des mollusques bivalves: évaluation et examen des zones de production conchylicole \(fao.org\)\]](#)

## 2.2 Provision of advice and guidance on protocols, accreditation, and use of methods and approaches for bivalve mollusc production.

### 2.2.1 Laboratory protocols and guidance available from the Reference Centre (accredited ISO/IEC 17025 and non-accredited)

During 2022-23 the Reference Centre has continued to make a comprehensive series of laboratory protocols for analysis of bivalve molluscs and associated guidance documents available on its website



[Protocols and Technical Guidance - Cefas \(Centre for Environment, Fisheries and Aquaculture Science\)](#). These are listed in Figure 2.

Figure 2. Protocols and technical guidance made available via the Reference Centre website



**EXCELLENCE PAGE**

### Protocols and Technical Guidance

**FIND OUT MORE —**



1. Determination of faecal coliform bacteria in seawater using most probable number technique (based on the US FDA BAM chapter 4)
2. Enumeration of *Escherichia coli* in bivalve shellfish using the most probable number technique (based on ISO 16649-3) \*
3. Detection of potentially pathogenic *Vibrio* spp. in bivalve shellfish
4. Detection of *Salmonella* spp. in bivalve molluscs (based on ISO 6579-1) \*
5. Quantitative detection of norovirus and hepatitis A virus in bivalve molluscan shellfish (using ISO 15216-1) \*
6. Enumeration of FRNA phage in bivalve shellfish
7. Guidance on the derivation of MPN results for official control testing of bivalve molluscs
8. Guidance for determining uncertainty of measurement for the enumeration of *E. coli* in bivalve molluscs by ISO 16649-3
9. Guidance on the use of proprietary *Salmonella* detection kits for detection and identification of *Salmonella* spp. in bivalve molluscs
10. Guidance providing supplementary data on the performance of vvHA real-time PCR assays for the detection of *Vibrio vulnificus*
11. Guidance for the determination of limits of detection and quantification for determination of viruses in bivalve shellfish
12. Example datasets for determination of limits of detection and quantification for determination of viruses in bivalve shellfish
13. Calculation spreadsheet for quantification of viruses in bivalve shellfish using ISO 15216-1
14. Guidance for best practice for norovirus testing in shellfish

\*indicate method accredited to ISO IEC 17025

## 2.2.2 Method standardisation, and provision of ad hoc scientific and technical advice

Personnel at the Reference Centre led or contributed to activities supporting method development, validation, and standardisation with relevance to bivalve molluscs, and provided technical and scientific advice to national bodies in 2022-23. In brief this included:

- Provision of leadership and representation for the ISO/TC34/SC9/W27 (Vibriosis), developing methods (quantitative and qualitative) for determination of human pathogenic *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*) in seafoods including bivalve molluscs.
- Provision of representation on CEN/TC463/WG1 (standardisation of molecular methods for food microbiology)
- Provision of representation on ISO/TC34/SC9/WG3 (validation of food microbiology methods)
- Advice to the Department of Forestry, Fisheries and the Environment (Competent Authority), South Africa, on appropriate conditions and confirmatory testing for depuration of bivalve molluscs.
- Advice and practical training for a representative of the National Public Health Laboratory, Malaysia on *E. coli* and virus testing, and organisation of proficiency testing schemes.
- Advice and practical support (provision of materials) to the Institut National des Sciences et Technologies de la Mer, Tunisia on methods for testing of norovirus and hepatitis A virus in bivalve molluscs, particularly with regard to control materials.
- Advice to bivalve mollusc industry representatives in Senegal on interpretation of *E. coli* results.
- Advice to the Jamaican Ministry of Fisheries on biotoxin monitoring and testing methods for bivalve molluscs and other marine species.
- Advice to the CSIR-National Institute of Oceanography, Goa, India on analysis of saxitoxins in bivalve molluscs.
- Support to the United Kingdom Food Standards Agency and Defra on issues associated with trade of live bivalve molluscs with the European Union.

### 2.3 Prepare and deliver regional training workshops on bivalve mollusc sanitation

The report on the Latin America and the Caribbean virtual regional workshop on Bivalve Mollusc Sanitation (held in November 2021) was published in April 2022;



[Latin America and the Caribbean virtual regional workshop on Bivalve Mollusc Sanitation, November 2021 - Cefas \(Centre for Environment, Fisheries and Aquaculture Science\).](#)

Figures 3 & 4. Cefas training support for Department of Fisheries, Bangladesh



The Reference Centre supported a training and familiarisation visit to the Department of Fisheries in Bangladesh, partly funded by the UK Ocean Country Partnership Programme, held in Dhaka from 21st-22nd September 2022, with the aim of increasing expertise within Bangladesh in service of development of commercial bivalve mollusc production in the country. This workshop was attended by 25 delegates from the Bangladeshi government and official control laboratories and combined classroom learning and exercises and practical training in microbiological methods.



[Bangladesh Familiarisation and Training Workshop, September 2022 - Cefas \(Centre for Environment, Fisheries and Aquaculture Science\).](#)

A series of recommendations were agreed with the Department of Fisheries to aid feasibility studies on the development of bivalve mollusc aquaculture in Bangladesh, as a national fisheries and aquaculture diversification objective.

Figures 5 & 6. Cefas Training workshop with FAO Regional Offices for Africa, West Africa and Senegal



To support the PPG/STDF/672 (MTF/SEN/079/STF) Senegalese project as identified as a priority in the Work Programme for 2022-23, the Reference Centre collaborated with the FAO Regional Offices for Africa, West Africa and Senegal to deliver a Training Workshop on Risk Profiling and Sanitation of Bivalve Molluscs for stakeholders within Senegal. The Workshop was staged at the Royal Horizon Baobab Hotel, Somone, Senegal from 21st-23rd February 2023. Approximately 30 delegates from different elements of the Senegalese bivalve mollusc sector (industry representatives, local and national government officials) joined experts from the FAO and the Reference Centre for the three-day training programme.

The programme included both presentations from the experts, and training exercises (on identification and ranking of hazards, planning growing area assessments and interpretation of classification data), carried out by delegates working together in groups. These interactive exercises helped to raise awareness of the public health considerations required for further development of the industry and increased understanding amongst delegates of management controls. In addition, experts from the Reference Centre led the delegates in a physical survey of the shoreline in the Somone lagoon adjacent to the workshop venue to assess potential hazards to the bivalve production in the lagoon.

A full report on the workshop and presentations in French and English can be accessed via the website



[Senegal training workshop on Risk Profiling and Sanitation of Bivalve Molluscs, February 2023 - Cefas \(Centre for Environment, Fisheries and Aquaculture Science\).](#)

The expected benefit and impact of the workshop was to increase expertise within the bivalve mollusc sector in Senegal to facilitate the growth of the sector in a safe and sustainable way. The workshop also builds links between the Senegalese stakeholders, the FAO and the Reference Centre to enable development of further projects that can support the overall aims of the FAO in Senegal.

## 2.4 Proficiency testing (PT) for Member Countries

Following a successful pilot proficiency testing scheme (PT 88) for official laboratories of countries in Africa and the Asia and Pacific region carried out during 2021, a second PT scheme (PT 93) was organised during 2022. The Reference Centre invited official laboratories of all countries that had previously attended training workshops for Africa (2019), Asia and Pacific (2021) and Latin America and the Caribbean regions (2021) to participate in this distribution. The distribution took place in September 2022, and comprised easily transportable, fully characterised laboratory-constructed materials (Lenticules™) which once reconstituted mimic the bacteriological flora of either bivalve mollusc flesh and intravavular fluid (*E. coli*) or bivalve mollusc growing area waters (faecal coliforms). As with the pilot scheme, the aim of this PT was to assist in the establishment of approved laboratory methods for the determination of faecal indicators in national laboratories where the capability does not yet exist, or where participation in PT will assist laboratories with demonstration of the quality of test results and provide valuable material for staff training.

Twenty-one laboratories from 11 different FAO member countries (Argentina, Brazil, Chile, Kenya, Angola, South Africa, Mauritius, Bangladesh, Malaysia, Indonesia, the Philippines) were included in the distribution. Fourteen participating laboratories returned results of which 12 analysed Lenticules™ mimicking both *E. coli* in shellfish and faecal coliforms in water, 1 analysed for *E. coli* in shellfish only and 1 analysed for faecal coliforms in water only.

A separate PT report containing information on participants, samples and performance was produced, distributed to participants and published on the Reference Centre website.

 [PT Reports - Cefas \(Centre for Environment, Fisheries and Aquaculture Science\)](#)

Laboratories used a variety of methods of analysis. For *E. coli* in shellfish the most commonly used method was the EU reference method ISO 16649-3, whereas for faecal coliforms in seawater the most commonly used method was derived from the Food and Drug Administration (FDA) Bacteriological Analytical Manual – BAM Determination of faecal coliform bacteria in seawater by the most probable number (MPN) technique.

For *E. coli* in shellfish, 4 out of 13 laboratories reported all replicate results for all samples within satisfactory ranges. For faecal coliforms, no statistical evaluation of quantitative results was carried out, however 6 out of 13 laboratories correctly identified the presence or absence of faecal coliforms in all replicates for all samples. Overall, these results, indicate some difficulties with implementation of the methods required for microbiological analyses supporting monitoring and classification of bivalve growing areas (faecal indicators). Furthermore, they confirm the wide variety of different methodological approaches used (Table 1). More detailed eLearning on methods has been previously identified as a potentially useful addition to online technical training (see 2.1) however, it is clear that some laboratories would benefit from further support on the use of standardised approaches. Following this latest distribution, where appropriate, individual laboratories will be offered troubleshooting advice.

Microbiological analyses underpin bivalve sanitation programmes, with important public health decisions made on the basis of results, thus reliability of laboratory test methods and results is critical. It is therefore proposed that the next series of physical training workshops targets selected Official Laboratories and focuses on microbiological methods for faecal indicators and the quality of test results – this work item has been included in the Reference Centre work programme for 2023/24.

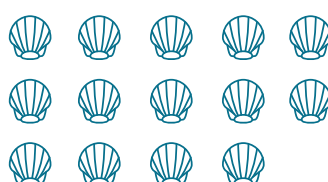
### Proficiency testing for *E.coli*

21



laboratories **received samples**  
from 11 FAO member countries

14



laboratories **reported results**



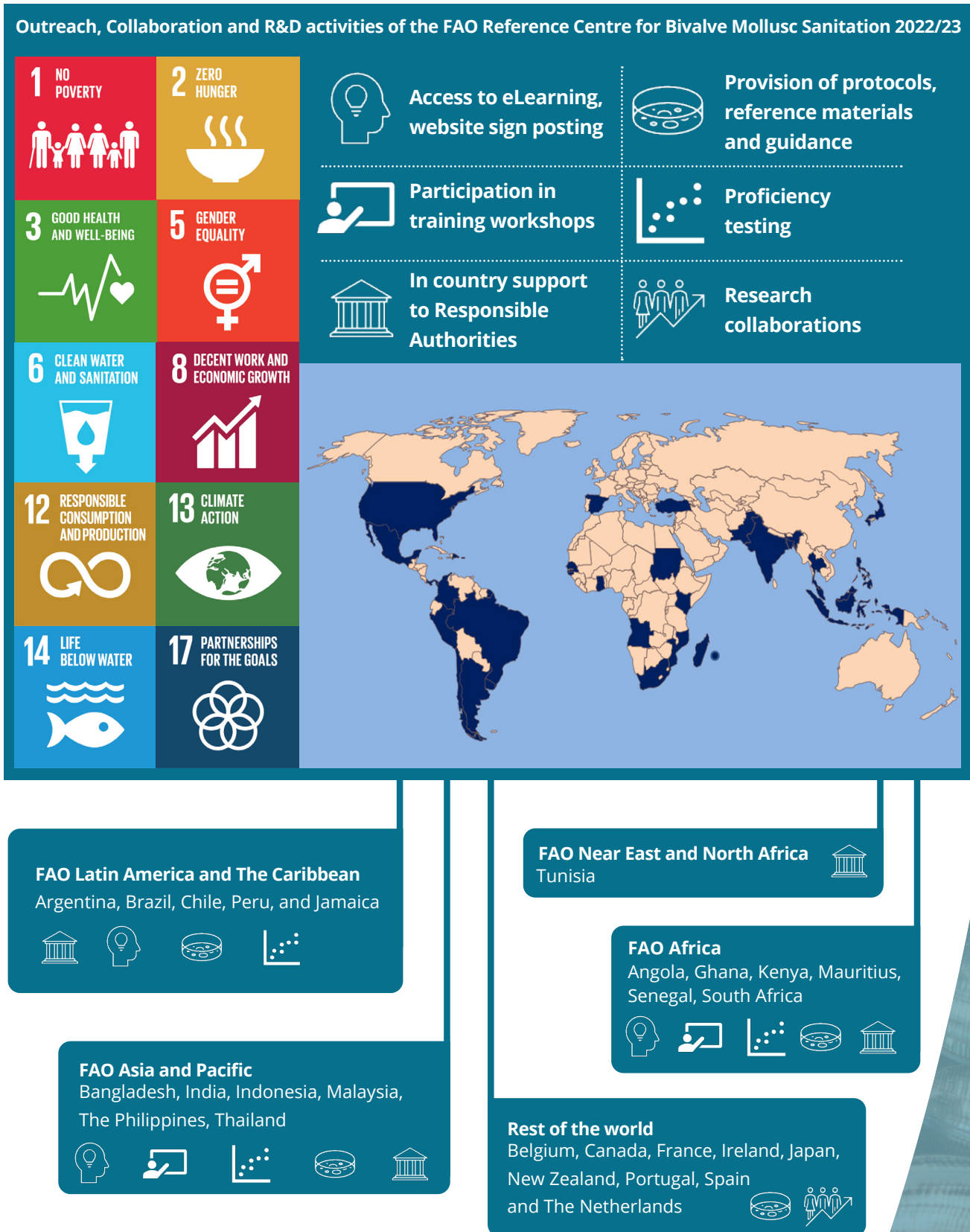
**Table 1 – Methods used by laboratories in the second pilot PT distribution in 2022**

<b>Method reference – determination of <i>E. coli</i> in bivalve molluscs</b>	<b>No. of labs</b>
ISO 16649 - 3 (MPN - 5 tubes, 3 dilutions) (FAO Reference Centre Generic protocol)	6
Bacteriological Analytical Manual, Chapter 4: Enumeration of <i>Escherichia coli</i> and the Coliform Bacteria	3
MPN – Media used MacConkey Broth Pour Plate Method-Media used Tryptone Bile X-Glucuronide (TBX) Medium	1
NCH 3056:2007	1
ISO 7218:2007	1
Simplate	1
<b>Method reference – determination of faecal coliforms in water</b>	<b>No. of labs</b>
Determination of faecal coliform bacteria in seawater by the most probable number (MPN) technique (Based on Bacteriological Analytical Manual – BAM) (FAO Reference Centre Generic Protocol)	4
Standard Methods for the examination of water and wastewater 23th Edition 2017.	3
ISO 9308-1: 2014	2
Membrane filtration APHA 9222B	1
Membrane filtration; SANS 5221, 4.5:2018	1
MPN-Media used MacConkey Broth.	1
COLILERT-18	1

### 3.0 Outreach and collaboration activities – summary

The reference centre was active in over 25 countries in 2022-23 (Figure 7). Activities ranged from provision of eLearning, delivery of training workshops, proficiency testing, provision of control materials, distribution of protocols and technical guidance, support to FAO Member Country Competent/Responsible Authorities, and research collaborations. The Reference Centre website continues as the main repository of information and portal for the dissemination of information and guidance.

Figure 7. Outreach, Collaboration and R&D activities of the FAO Reference Centre for Bivalve Mollusc Sanitation



## 4.0 Publications in 2022-23

1. Aguirre-Sánchez JR, **Martínez-Urtaza J** *et al.* Phylogenetic group and virulence profile classification in *Escherichia coli* from distinct isolation sources in Mexico. *Infect Genet Evol.* 2022 Dec;106:105380. doi: 10.1016/j.meegid.2022.105380. Epub 2022 Oct 22. PMID: 36283634.
2. Archer EJ, **Baker-Austin C**, Osborn TJ, Jones NR, **Martínez-Urtaza J**, Trinanés J, Oliver JD, González FJC, Lake IR. Climate warming and increasing *Vibrio vulnificus* infections in North America. *Sci Rep.* 2023 Mar 23;13(1):3893. doi: 10.1038/s41598-023-28247-2. PMID: 36959189; PMCID: PMC10036314.
3. Batista FM, Hatfield R, Powell A, **Baker-Austin C**, **Lowther J**, **Turner AD**. Methodological advances in the detection of biotoxins and pathogens affecting production and consumption of bivalve molluscs in a changing environment. *Curr Opin Biotechnol.* 2023 Apr;80:102896. doi: 10.1016/j.copbio.2023.102896. Epub 2023 Feb 9. PMID: 36773575.
4. Bowley J, **Baker-Austin C**, Michell S, Lewis C. Pathogens transported by plastic debris: does this vector pose a risk to aquatic organisms? *Emerg Top Life Sci.* 2022 Dec 1;6(4):349-358. doi: 10.1042/ETLS20220022. PMID: 36205551.
5. González-López I, **Martínez-Urtaza J** *et al.* Prevalence and genomic diversity of *Salmonella enterica* recovered from river water in a major agricultural region in Northwestern Mexico. *Microorganisms.* 2022 Jun 14;10(6):1214. doi: 10.3390/microorganisms10061214. PMID: 35744732; PMCID: PMC9228531.
6. Goya AB, **Maskrey BH**, **Turner AD** *et al.* Marine biotoxins in whole and processed scallops from the Argentine Sea. *Mar Drugs.* 2022 Oct 10;20(10):634. doi: 10.3390/md20100634. PMID: 36286458; PMCID: PMC9604692.
7. Lau DY, Aguirre Sánchez JR, **Baker-Austin C**, **Martinez-Urtaza J**. What Whole Genome Sequencing has told us about pathogenic Vibrios. *Adv Exp Med Biol.* 2023;1404:337-352. doi: 10.1007/978-3-031-22997-8\_16. PMID: 36792883.
8. Light E, **Baker-Austin C** *et al.* Establishing a marine monitoring programme to assess antibiotic resistance: A case study from the Gulf Cooperation Council (GCC) region. *Environ Adv.* 2022 Oct;9:None. doi: 10.1016/j.envadv.2022.100268. PMID: 36466197; PMCID: PMC9710716.
9. Núñez-Vázquez EJ, **Turner AD** *et al.* Paralytic Shellfish Toxins of *Pyrodinium bahamense* (Dinophyceae) in the Southeastern Gulf of Mexico. *Toxins (Basel).* 2022 Nov 3;14(11):760. doi: 10.3390/toxins14110760. PMID: 36356010; PMCID: PMC9694361.
10. Stentiford GD, **Turner AD**, **Baker-Austin C**, **Lowther JA**, **Maskrey BH**, **Hartnell RE** *et al.* A seafood risk tool for assessing and mitigating chemical and pathogen hazards in the aquaculture supply chain. *Nat Food.* 2022 Feb;3(2):169-178. doi: 10.1038/s43016-022-00465-3. Epub 2022 Feb 24. PMID: 37117966.
11. **Turner AD**, **Maskrey BH** *et al.* A feasibility study into the production of a mussel matrix reference material for the cyanobacterial toxins microcystins and nodularins. *Toxins (Basel).* 2022 Dec 30;15(1):27. doi: 10.3390/toxins15010027 PMID: 36668847; PMCID: PMC9867187.

12. **Turner AD** *et al.* Interlaboratory evaluation of multiple LC-MS/MS Methods and a commercial ELISA method for determination of tetrodotoxin in oysters and mussels. *J AOAC Int.* 2023 Mar 1;106(2):356-369. doi: 10.1093/jaoacint/qsad006. PMID: 36617186; PMCID: PMC9978588.
13. Vezzulli L, **Martinez-Urtaza J**, Stern R. Continuous Plankton Recorder in the omics era: from marine microbiome to global ocean observations. *Curr Opin Biotechnol.* 2022 Feb;73:61-66. doi: 10.1016/j.copbio.2021.07.016. Epub 2021 Jul 24. PMID: 34314925.
14. Yang C, **Martinez-Urtaza J** *et al.* Outbreak dynamics of foodborne pathogen *Vibrio parahaemolyticus* over a seventeen year period implies hidden reservoirs. *Nat Microbiol.* 2022 Aug;7(8):1221-1229. doi: 10.1038/s41564-022-01182-0. Epub 2022 Aug 2. PMID: 35918422.
15. **Younger A**, Kershaw S, Campos CJA. Performance of storm overflows impacting on shellfish waters in England. *Land.* 2022 Sep; 11(9):1576. doi: 10.3390/land11091576.

## 5.0 Acknowledgments

The FAO Reference Centre would like to thank the following individuals for their active support in the delivery of the work programme in 2022-23:

- Dr Esther Garrido Gamarro, FAO Food Safety and Quality, Fisheries Division – programme leadership.
- Ms Dalene Goosen at the FAO eLearning academy for their exceptional dedication and work to deliver the eLearning modules.
- Mr Blaise Ouattara, Mr Mamadou Ndiaye, Mr Sansan Kambire and Ms Secondine Sagna for great collaboration in the organisation of the Training Workshop on Risk Profiling and Sanitation of Bivalve Molluscs hosted in Senegal.
- Dr Iddya Karunsagar, University of Nitte, Mangalore, India for advice and collaboration during the year.
- Dr Jaime Martinez-Urtaza, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona (UAB) and an associate member of the FAO Reference Centre.
- Dr Andrew Turner, Principal Chemist and marine biotoxin scientist at Cefas for provision of ongoing technical advice related to marine biotoxins and bivalve molluscs during 2022-23.
- Ms Megan Pring and Mr Gregory Ngarachu – Cefas interns from University of West of England, Bristol for their outstanding contribution to the delivery of the work programme in 2022-23.
- Dr Michelle Stone, Cefas – leadership and logistics for the Bangladeshi familiarisation training in Dhaka.
- Mr Andy Powell, Cefas – Practical demonstrations and contributions to the Bangladeshi familiarisation training in Dhaka.

*Annex I. Financial support for the FAO Reference Centre for Bivalve Mollusc Sanitation received at Cefas (Financial year 2022/23)*

Funding Body	Amounts
UK Department for Environment, Food and Rural Affairs (Defra)	£80,000
UK Food Standards Agency (FSA)	£125,000
Total	£205,000

## World Class Science for the Marine and Freshwater Environment

We are the government's marine and freshwater science experts. We help keep our seas, oceans and rivers healthy and productive and our seafood safe and sustainable by providing data and advice to the UK Government and our overseas partners. We are passionate about what we do because our work helps tackle the serious global problems of climate change, marine litter, over-fishing and pollution in support of the UK's commitments to a better future (for example the UN Sustainable Development Goals and Defra's 25 year Environment Plan).

We work in partnership with our colleagues in Defra and across UK government, and with international governments, business, maritime and fishing industry, non-governmental organisations, research institutes, universities, civil society and schools to collate and share knowledge. Together we can understand and value our seas to secure a sustainable blue future for us all, and help create a greater place for living.



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