Food and Agriculture Organization of the United Nations

FAO Reference Centre - Annual report for the FAO Reference Centre for Bivalve Mollusc Sanitation 2023-24

Title of FAO Reference Centre	FAO Reference Centre for Bivalve Mollusc Sanitation
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Reporting period	April 2023 – March 2024
Date of reporting	June 2024

1. Introduction

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) is designated as the Food and Agriculture Organization of the United Nations (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 (UK) Department of Environment, Food and Rural Affairs (Defra). This fifth 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 April 2023 – March 2024. Financial support for operation of the Reference Centre was afforded by Defra and the UK Food Standards Agency (FSA).

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 April 2023 [https://www.cefas.co.uk/faobivalves/]. Main areas of collaboration for 2023-24 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 training workshops at the FAO Reference Centre at Cefas, Weymouth, UK for FAO Member Country representatives on methods for determination of faecal indicator organisms in bivalve molluscs.
- To plan, organize and distribute a Proficiency Testing (PT) scheme for faecal indicator bacteria for FAO Member Country Official Laboratories.
- 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.

2.1.1 eLearning on Bivalve mollusc sanitation

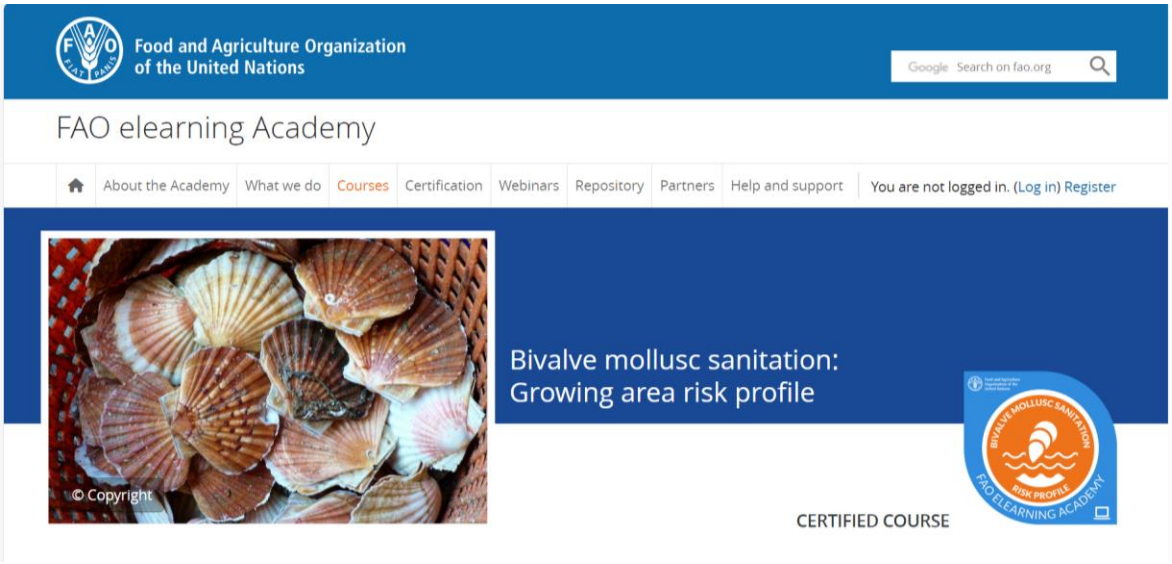
The FAO/WHO Technical Guidance for the Development of the Growing Area Aspects of Bivalve Mollusc Sanitation Programmes[1] was published as a second edition in 2021. The Reference Centre has been assisting the FAO eLearning academy to develop open access online training based upon the technical guidance. Modules 1, 2, 3 and 4 have now been published (Figure 1). This eLearning is a helpful resource for a range of practitioners with interests in

[1] 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.

bivalve mollusc production, as well as useful pre-workshop familiarisation material for attendees at Reference Centre training events. The objective of all 4 modules is to assist in the implementation of Codex Alimentarius guidance and standards in their various application and provide detailed information on how to establish and monitor bivalve mollusc growing areas. The focus of the eLearning is on bivalve molluscs intended for consumption raw and/or live, with a particular focus on microbiological hazards that may pose risks in primary production. In the period covered by this report, work has continued on the final module (4); technical development of this module was completed in March 2024.

Figure 1. Bivalve mollusc sanitation – eLearning modules

Module 1 – Bivalve mollusc sanitation: Growing area risk profile

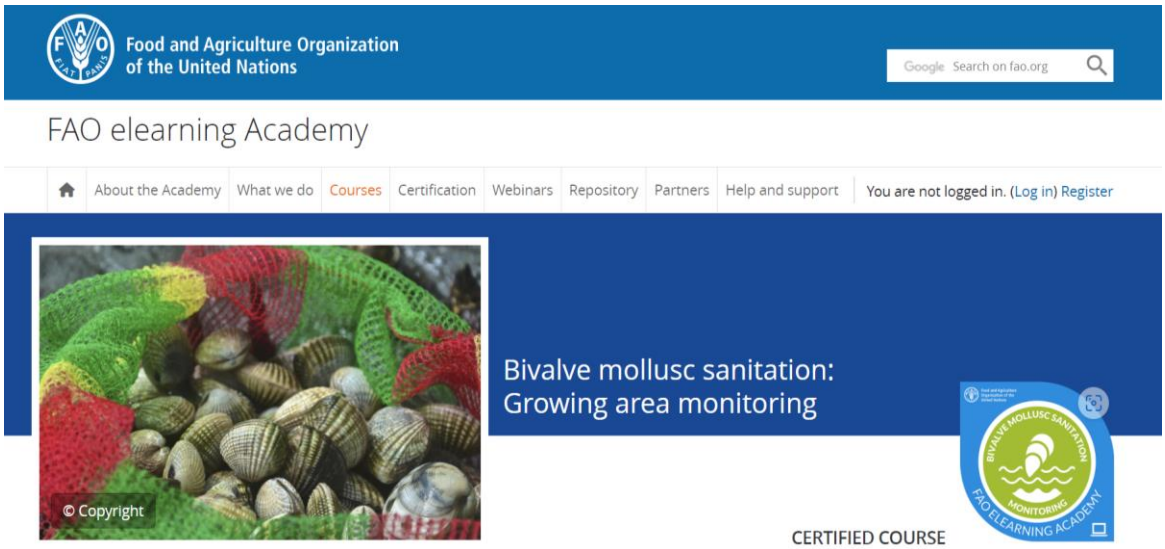


Course: Bivalve mollusc sanitation: Growing area risk profile | FAO elearning Academy

Module 2: Bivalve mollusc sanitation: Growing area assessment and review

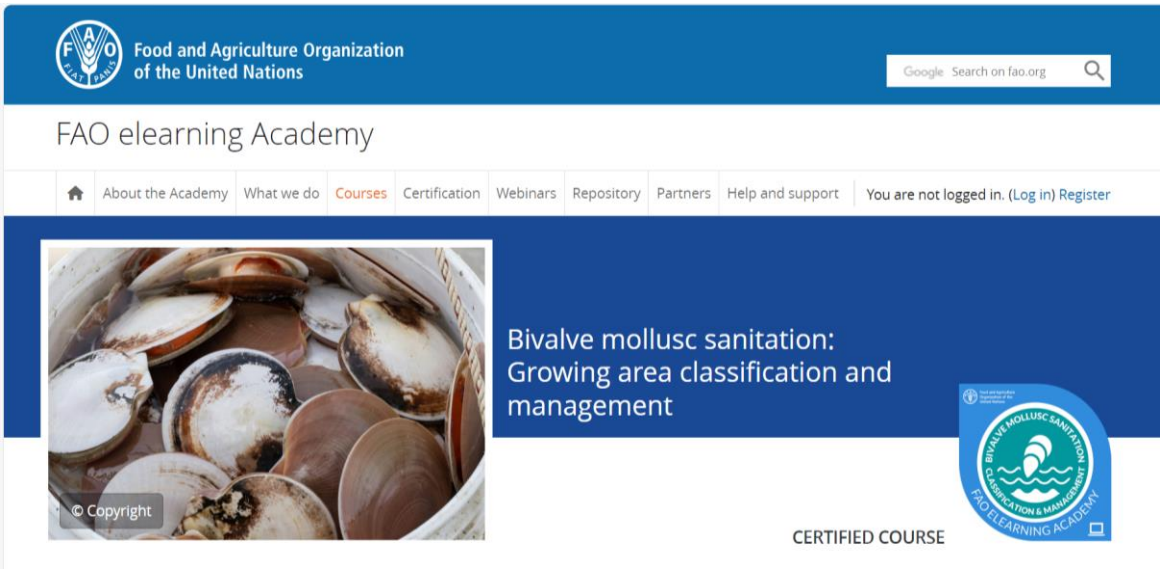
Course: Bivalve mollusc sanitation: Growing area assessment and review | FAO elearning Academy

Module 3 – Bivalve mollusc sanitation: Growing area monitoring



Course: Bivalve mollusc sanitation: Growing area monitoring | FAO elearning Academy

Module 4 – Bivalve mollusc sanitation: Growing area classification and management



Course: Bivalve mollusc sanitation: Growing area classification and management | FAO elearning Academy

2.1.2 eLearning course certification

During this reporting period each module has been included in the FAO eLearning digital badge certification scheme. The digital badge system enables learners to verify the acquisition of skills and competencies and provides evidence of the learning which can be shared with others, or form part of individual continuous professional development. Digital badging is underpinned by 4 key concepts of equity, inclusion, and transparency; lifelong learning; skills-based recruitment opportunities and verification of credentials. These concepts are closely aligned to United Nations (UN) Sustainable Development Goal 4 'Ensure inclusive and equitable quality education and lifelong learning opportunities for all'.

To further increase the accessibility of the eLearning modules, modules 1 and 2 are available in Spanish and French language versions. Modules 3 and 4 will be translated to Spanish and French in 2024.

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

During 2023-24 the Reference Centre has continued to make 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

1. Enumeration of *Escherichia coli* in bivalve shellfish using the most probable number technique (based on ISO 16649-3) *
2. Determination of faecal coliform bacteria in seawater using most probable number technique (based on the US FDA BAM chapter 4)
3. Detection of *Salmonella* spp. in bivalve molluscs (based on ISO 6579-1) *
4. Detection of potentially pathogenic *Vibrio* spp. in bivalve shellfish
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 bivalves
8. Guidance on the use of proprietary Salmonella detection kits for detection and identification of *Salmonella* spp. in bivalve molluscs, live echinoderms, gastropods and tunicates
9. Guidance providing supplementary data on the performance of vvHA real-time PCR assays for the detection of *Vibrio vulnificus*
10. Guidance for the determination of limits of detection and quantification for determination of viruses in bivalve shellfish
11. Guidance for best practice for norovirus testing in shellfish

**indicates 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 2023-24. In brief this included:

- Provision of leadership and representation for the ISO/TC34/SC9/W27 (international standardisation group on Vibrios), 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 (international standardisation of molecular methods for food microbiology)
- Provision of representation on ISO/TC34/SC9/WG3 (international standardisation of methods for validation of food microbiology methods)
- Advice and practical support (provision of proficiency testing 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.
- Provision of structured training programmes and ongoing support to the Jamaican Ministry of Fisheries on biotoxin monitoring approaches and associated quality control measures.
- Provision of training at the Reference Centre and ongoing support to the Microchem Laboratory, South Africa.
- Participation in the MTF SEN 079 STF: CEFAS training workshop on sampling, packaging, and transporting for stakeholders in Senegal, including provision of an opening presentation, expert feedback throughout the meeting and concluding remarks.
- Provision of advice to FAO officers on requirements for fisheries inspections at bivalve mollusc processing or dispatch centres in the Gambia.

2.3 Preparation and delivery of training workshops on methods for determination of faecal indicator organisms in bivalve molluscs

Between November 2023 and February 2024, the Reference Centre hosted three separate training workshops on bivalve mollusc sanitation, with particular focus on practical methods for determination of *E. coli* (as the most commonly used statutory faecal indicator organism) in bivalves using the most probable number (MPN) method (based on ISO 16649-3), at its laboratory in Weymouth, UK (Figure 3).

Over the course of the workshops, 16 delegates from appropriate government departments and testing laboratories in five FAO Member Countries (Bangladesh, Ghana, Kenya, Mozambique, Senegal) were provided with information in classroom sessions on public health risks related to bivalves, approaches to sampling and the principles of *E. coli* analysis. Delegates also spent time in the microbiology laboratories at Cefas receiving hands-on training in sample preparation and *E. coli* testing, participated in a practical shellfish sampling exercise on the shoreline, and were able to visit local oyster production areas and a depuration facility to learn more about shellfish production and post-harvest treatments in the UK. A small number of delegates also received specialist practical training in methods for detection of algal biotoxins in bivalves.

These training workshops were very well received by the delegates with 87% of participant feedback scores for different workshop elements (administration and accommodation, content,

presentations, practical sessions, and workshop location) “very good”, and the other 13% “good”; for workshop content, 100% of participant feedback scores were “very good”. A standalone report covering the workshops is currently in preparation and will be published on the Reference Centre website.

Figure 3 Training workshops on methods for faecal indicator organisms

2.4 Provision of proficiency testing (PT) for Member Countries

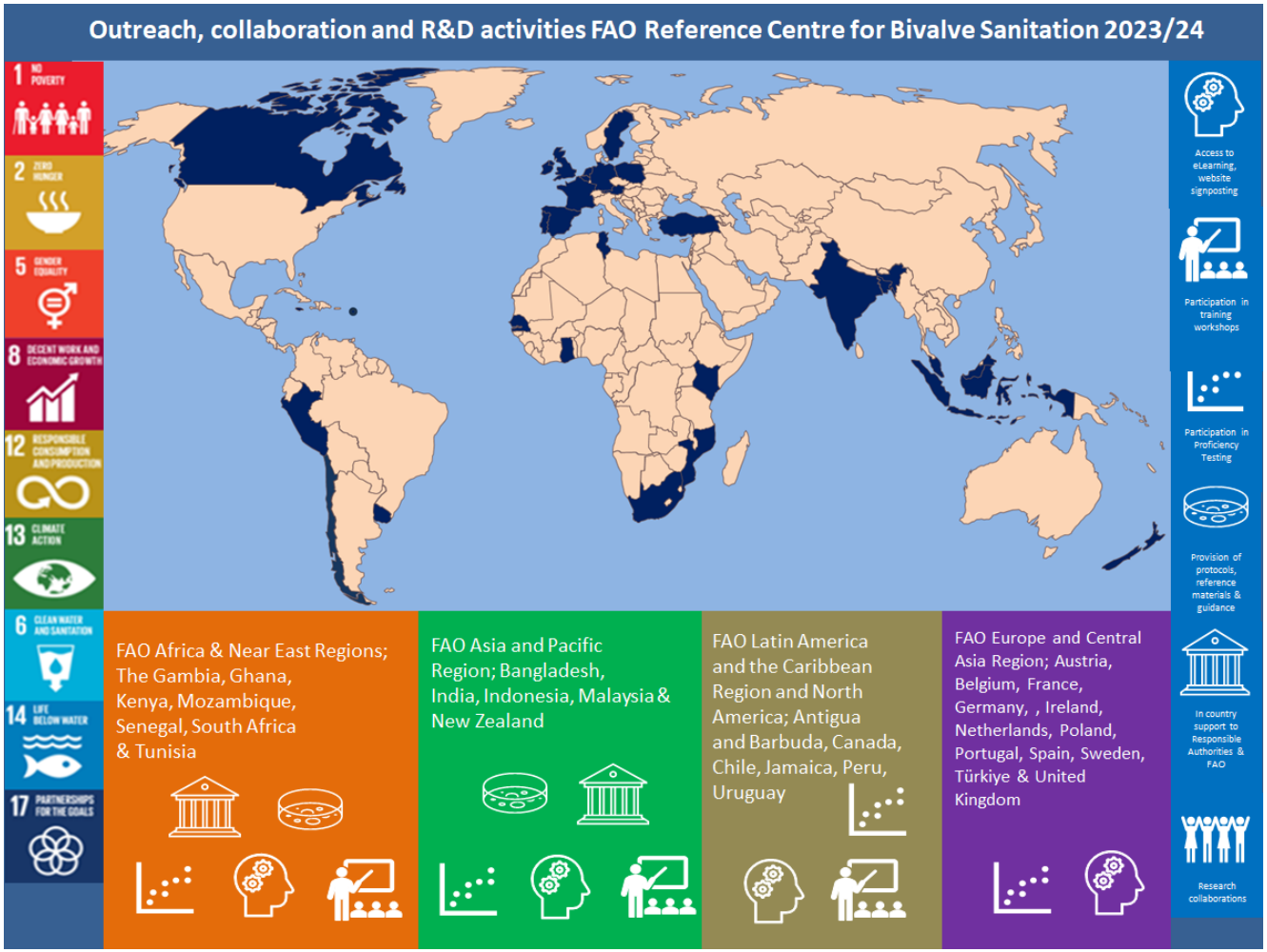
Following successful proficiency testing (PT) schemes for FAO member countries in 2021 and 2022, a third scheme was organised in early 2024. The Reference Centre invited official laboratories of all countries that had previously attended training workshops for Africa (2019), Asia and Pacific (2020) and Latin America and the Caribbean regions (2021) to participate. The distribution took place in March 2024, 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 previous schemes, 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.

Thirteen laboratories from 8 different FAO member countries (Antigua and Barbuda, Bangladesh, Chile, Indonesia, Malaysia, South Africa, Turkey, Uruguay) were included in the distribution. The deadline for reporting of results was May 2024. Results are currently being collated and analysed, and a report on the PT including anonymised results of all participants is in preparation and will be published on the Reference Centre website. This report will help participant laboratories to assess their performance with the relevant methods and identify areas for improvement.

3.0 Outreach and collaboration activities – summary

The Reference Centre was active in more than 20 countries worldwide in 2023-24 (Figure 7). Activities ranged from provision of eLearning, delivery of training workshops, proficiency testing, 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 4. Outreach, Collaboration and R&D activities of the FAO Reference Centre for Bivalve Mollusc Sanitation



4.0 Publications & international scientific presentations in 2023-24

1. Alex-Sanders N, **Scott G, Walker DI** et al. Development and validation of a duplex RT-qPCR assay for norovirus quantification in wastewater samples. J Virol Methods. 2023 Nov;321:114804. doi: 10.1016/j.jviromet.2023.114804.
2. **Baker-Austin, C., Martinez-Urtaza, J**. The evolution of molecular methods to study seafood-associated pathogens. Present Knowledge in Food Safety, 2023. 493-500. https://doi.org/10.1016/B978-0-12-819470-6.00004-4.
3. Bigoraj E, Kozyra I, Kaupke A, Osiński Z, **Lowther J**, Rzeżutka A. Prevalence and quantitative assessment of foodborne viruses on the imported mussels in Polish market. Food Control 2024 Mar; 157, 110145. doi:10.1016/j.foodcont.2023.110145.
4. Boxman ILA, **Lowther JA, Walker DI, Stapleton T** et al. An international inter-laboratory study to compare digital PCR with ISO standardized qPCR assays for the detection of norovirus GI and GII in oyster tissue. Food Microbiol. 2024 Jun;120:104478. doi: 10.1016/j.fm.2024.104478.
5. Campbell AM, Hauton C, **Baker-Austin C**, **van Aerle R**, **Martinez-Urtaza J**. An integrated eco-evolutionary framework to predict population-level responses of climate-sensitive pathogens. Curr Opin Biotechnol. 2023 Apr;80:102898. doi: 10.1016/j.copbio.2023.102898.
6. **Dhanji-Rapkova M**, **Martinez-Urtaza J**, **Baker-Austin C**, **Turner AD** et al. Sea temperature influences accumulation of tetrodotoxin in British bivalve shellfish. Sci Total Environ. 2023 Aug 10;885:163905. doi: 10.1016/j.scitotenv.2023.163905.
7. Funesto EGM, **Lewis AM, Turner AD**, et al. Immediate and delayed effects of a heatwave and *Prorocentrum lima* ((Ehrenberg) Stein 1878) bloom on the toxin accumulation, physiology, and survival of the oyster *Magallana gigas* (Thunberg, 1793). Sci Total Environ. 2023 Sep 20;892:164485. doi: 10.1016/j.scitotenv.2023.164485. Epub 2023 May 29.PMID: 37257593.
8. **Hartnell R**. International guidance on food safety for bivalve molluscs. Presentation at the World Seafood Congress/International Conference on Molluscan Shellfish Safety, Peniche, Portugal. 2023 Sep.
9. Leynse AK, Mudge EM, **Turner AD, Maskrey BH**, Robertson A. Gambierone and sodium channel specific bioactivity are associated with the extracellular metabolite pool of the marine dinoflagellate *Coolia palmyrensis*. Mar Drugs. 2023 Apr 15;21(4):244. doi: 10.3390/md21040244.
10. **Maskrey BH, Dean K, Morrell N, Younger A, Turner AD, Katsiadaki I**. Seasonal profile of common pharmaceuticals in edible bivalve molluscs. Mar Pollut Bull. 2024 Mar;200:116128. doi: 10.1016/j.marpolbul.2024.116128.
11. McQuillan JS, **Turner AD, Morrell N**, et al. Quantitative Polymerase Chain Reaction for the estimation of toxigenic microalgae abundance in shellfish production waters. Harmful Algae. 2023 Oct;128:102497. doi: 10.1016/j.hal.2023.102497.
12. **Treagus S, Lowther J**, Longdon B, Gaze W, **Baker-Austin C, Ryder D, Batista FM**. Metabarcoding of hepatitis E virus genotype 3 and norovirus GII from wastewater samples in England using nanopore sequencing. Food Environ Virol. 2023 Dec;15(4):292-306. doi: 10.1007/s12560-023-09569-w.

13. Vasey J, **Martinez-Urtaza J** et al. Comparative genomics uncovered differences between clinical and environmental populations of *Vibrio parahaemolyticus* in New Zealand. Microb Genom. 2023 Jun;9(6):mgen001037. doi: 10.1099/mgen.0.001037.
14. Whyte C, **Turner AD, Hatfield RG, Morrell N, Rowland-Pilgrim S**, et al. The Presence of *Pseudo-nitzschia australis* in North Atlantic Aquaculture Sites, Implications for Monitoring Amnesic Shellfish Toxins. Toxins (Basel). 2023 Sep 5;15(9):554. doi: 10.3390/toxins15090554.

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 2023-24:

- 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.
- 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 2023-24.
- Dr Ben Maskrey Principal Chemist and marine biotoxin scientist at Cefas for provision of ongoing technical advice related to marine biotoxins and bivalve molluscs during 2023-24.
- Mr Gregory Ngarachu and Mr Joel Dorning – Cefas interns from University of West of England, Bristol for their outstanding contribution to the delivery of the work programme in 2023-24.
- Dr Michelle Stone, Cefas – organisation of logistical elements of the training workshops.