

# **FAO Reference Centre**

Annual report for the FAO Reference Centre for Bivalve Mollusc Sanitation Calendar Year 2021

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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Centre for Environment Fisheries & Aquaculture Science



### 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.

According to the 'Terms and Conditions of the Designation' agreed between FAO and the United Kingdom's Department of Environment, Food and Rural Affairs (Defra), the duration of the existing FAO Reference Centre for Bivalve Mollusc Sanitation is 4 years.

This third 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 calendar year 2021. 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 annual work programme of the FAO Reference Centre for Bivalve Mollusc Sanitation was agreed in December 2019 [https://www.cefas.co.uk/faobivalves/].

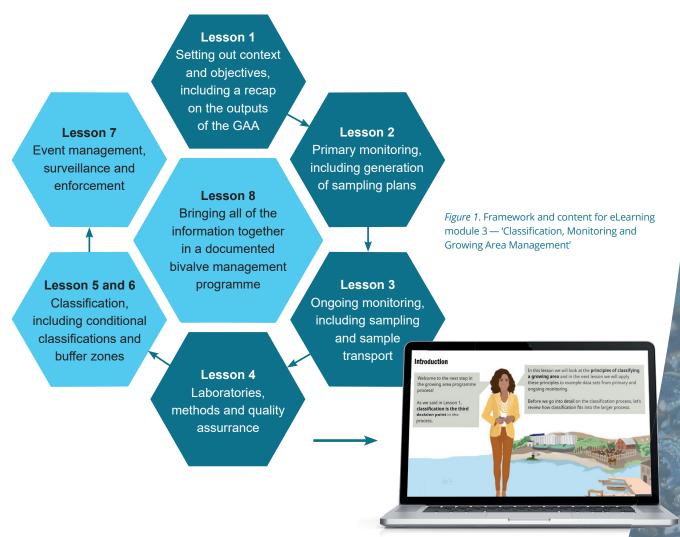
Main areas of collaboration for 2021 were:

- 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 regional workshop on bivalve mollusc sanitation.
- To undertake pilot proficiency testing (PT) 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.

In 2018, the FAO/WHO Food Safety and Quality Series, Technical Guidance for the Development of the Growing Area Aspects of Bivalve Mollusc Sanitation Programmes was published¹. In 2019 the first part of this technical guidance was successfully translated into an eLearning module via a collaboration between FAO Reference Centre for Bivalve Mollusc Sanitation, FAO Department of Fisheries and Aquaculture and the FAO eLearning Academy. In 2020 Module two, covering 'Growing Area Assessment and Review' was developed and published. In 2021 the development of Module three began (Figure 1). ELearning is intended for policy makers, development practitioners, sectoral specialists and researchers, bivalve farmers, trainers, and extension agents; it serves to compliment capacity and capability building activities of the Reference Centre.



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.

<sup>&</sup>lt;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.



Figure 2. eLearning outcomes — module 3 'Classification, Monitoring and Growing Area Management'

In 2021, lessons 2, 3 and 5 have been finalised with scheduled completion of the entire module in 2022. On completion, the three-module eLearning package will form an easily accessible, open access online resource detailing all the stages required to select, maintain, and manage growing areas to help ensure the production and harvest of microbiologically safe bivalves. FAO eLearning attracts over 600,000 active learners annually. The increased accessibility that this approach enables is substantial, helping meet capacity-building needs in Low- and Middle-Income Countries, (LMICs): it also reduces the necessity for in-country visits (reducing carbon emissions), the latter particularly relevant in 2021 enabling continuity of provision throughout movement restrictions imposed during the COVID-19 pandemic.



#### **Cefas Impact Prize for Grey Literature 2021**

In May 2021, Module two was awarded the Cefas Impact Prize for Grey Literature; an annual award recognising material that has a substantial impact on a process or decision with societal impact and relevant to Cefas' mission; and where Cefas scientists and/or advisers made a significant contribution to the work.

**Certification** — As part of the eLearning and in collaboration with the FAO academy, a digital badge system has been developed in 2021. This initiative will provide learners with recognised certification on completion of a formal test after each module. Once all three final certification tests are passed, the participant will receive a master badge verifying the acquisition of skills and competencies. Completing the digital badging exams provides the learner with evidence for Continuous Professional Development (CPD). The Cefas team were pleased to note that members of the UK Food Standards Agency (FSA) have registered as learners, demonstrating that the eLearning has benefits both nationally and internationally.

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

During 2021, generic protocols and guidance documents were reviewed, and made available via the website. Protocols describing microbiological methods for bivalve shellfish and growing area water support Member Countries in application of standard methods <a href="Protocols and Technical Guidance">Protocols and Technical Guidance</a> — Cefas (Centre for Environment, Fisheries and Aquaculture Science).

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

Figure 3. Protocols and technical guidance made available via the reference centre website

- Determination of faecal coliform bacteria in seawater using most probable number technique (based on the US FDA BAM chapter 4)
- 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



Protocols and
Technical Guidance

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#### 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 2021. In brief this included:

- Provision of leadership and representation for the ISO/TC34/SC9/W27 "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 (molecular methods for food microbiology).
- Review of the South African Model Ordinance for Growing Area Management of Shellfisheries.
- Input into the FAO Fisheries and Aquaculture Technical Annex of Technical Aspects of the Development of Growing Areas for Bivalve Molluscs' for distribution to relevant Member Countries.
- Provided content and editorial assistance to FAO in the re-publication of the technical guidance (version 2), now updated to reflect

- changes in EU Regulations, and changes to requirements in US standards for marine sanitation devices for larger vessels.
- Advice to South African Competent Authorities on elements of classification and requirements for monitoring flesh and phytoplankton in Shellfisheries, specifically in abalone.
- Advice to Competent Authorities in Ghana on unexplained die off in marine mammals, potentially associated with harmful algal blooms.
- Reference Centre personnel continued to support the Food Standards Agency and Defra on issues associated with trade of live bivalve molluscs with the European Union.
- Reference Centre personnel presented the work of the Centre to the FSA Chief Executive Officer and Chief Scientist.

The FAO Fisheries and Aquaculture Technical Annex of Technical Aspects of the Development of Growing Areas for Bivalve Molluscs' second edition was published in English and Spanish language versions in 2021.



#### 2.3 Prepare and deliver an International/regional workshop on bivalve mollusc sanitation

The report of the Asia Pacific virtual regional workshop on bivalve shellfish sanitation was published in April 2021 <u>Asia and Pacific virtual regional workshop on Bivalve Mollusc Sanitation</u>, December 2020 — Cefas (Centre for Environment, Fisheries and Aquaculture Science).



According to the designation cycle the reference centre supported FAO's scientific, technical, and economic programme priorities in the Latin America and Caribbean region via the co-delivery of a virtual regional workshop on Bivalve Mollusc Sanitation on the 2<sup>nd</sup> – 4<sup>th</sup> November 2021 with FAO, Fisheries division (Rome). The aim of the workshop was to explore targeted programmes of capability building and networks in Responsible Authorities and Official Laboratories. Over 60 National Competent Authority delegates from **Antigua and Barbuda**, **Argentina**, **Brazil**, **Chile**, **Colombia**, **Dominica**, **Panama**, **Mexico**, **St Vincent and the Grenadines**, **Suriname**, **Trinidad and Tobago**, and **Uruguay** attended the three-day workshop, together with representatives from FAO and the Reference Centre. The workshop comprised presentation of existing capability and capacity data presented by each Responsible Authority, dissemination of aspects of the technical guidance for growing areas aspects of bivalve mollusc production areas, an interactive question and answer session, and a bivalve mollusc 'quiz'. Due to a continued increase in interest in controls for marine biotoxins the Reference Centre invited Cefas' principal biotoxin scientist Dr Andrew Turner back again to present on methods for detection of biotoxins in bivalve molluscs.

A full report of the workshop, and presentations, including those describing bivalve production and national capabilities from Responsible Authorities, can be accessed via the website Latin America and the Caribbean virtual regional workshop on Bivalve Mollusc Sanitation, November 2021 — Cefas (Centre for Environment, Fisheries and Aquaculture Science).





Interactive material was further developed in 2021 to improve the experience of virtual attendees. In brief, two fictional case study scenarios, microbiological and biotoxin focused, allowed the delegates to envision what they might do in certain circumstances (Box 1, for example). In addition, two further interactive elements included a new hazard perception walk through (Box 2) and a data analysis scenario. Workshop working languages were English and Spanish, with Spanish translation services provided by the FAO translation team.

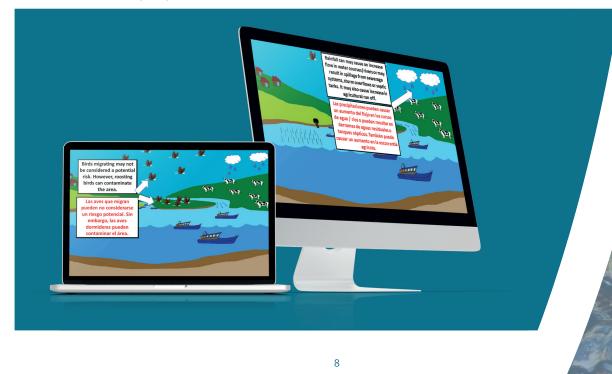
Box 1 — fictional scenario 1 virtual workshop bivalve sanitation — Latin America and The Caribbean

Delegates were provided with a fictional scenario - Scene setting - There is a significant rise in reported hepatitis A virus (HAV) infection in your region and the surrounding area, this happens about a month after a sailing regatta. Many of the reports are linked to the consumption of seafoods.

Delegates were provided with a Scenario PACK A with 7 pieces of information stepwise over the session. Information included laboratory reports, area maps, communicable disease data and export certificates. With guidance from FAO and Reference Centre facilitators they were asked to consider their response and role as a Responsible Authority.



Box 2 — Interactive hazard perception exercise — Latin America and The Caribbean



The expected benefit and impact of the workshop is to build networks to provide support to countries wishing to sustainably develop or expand their production of bivalve molluscs for domestic consumption or Interregional and international trade (Figure 4).

Figure 4. Input, output, and outcome expectations of series of regional FAO/ Cefas workshops on bivalve sanitation

#### **Inputs from Cefas and FAO**

Selection of appropriate delegate countries through a selection matrix

Production of the workshop agenda

Content of the workshop presentation

Planning of the workshop break out sessions

#### **Outcomes**

Formation of a network of Competent Authorities across the Latin America and The Caribbean region with responsibility for the development and operation of bivalve mollusc sanitation programmes

#### **Outputs of the workshop**

Presentations from the workshop

Minutes of the workshop

Answers to delegate chat questions from the workshop

Anonymous feedback from workshop participants

#### **Potential impacts**

Helping the delegate countries achieve enhanced production of safe bivalve molluscs

Development of an industry with environmental and societal benefits

Provision of safe and nutritious food products for domestic consumption

Increase in international and intercommunity trade opportunities, GDP increase

#### 2.4 Pilot proficiency testing (PT) for Member Countries

In 2021, the Reference Centre invited official laboratories of all countries, that had previously attended training workshops in Africa (2019) and Asia and Pacific (2020) to participate in a pilot PT distribution. PT can help in the development and implementation of new methods in laboratories. The distribution took place in March 2021, and comprised easily transportable, fully characterised laboratory constructed materials (Lenticules™) which once reconstituted mimic the bacteriological flora of either bivalve mollusc growing area waters or bivalve mollusc flesh and intravavular fluid. The aim of the 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-eight laboratories from 11 countries took part in the PT distribution. Results from



#### **EXCELLENCE PAGE**

Proficiency Testing and Quality Assurance

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National official control laboratories completing pilot PT distribution

the distribution in March were received from 19 laboratories (68%) (see below).



in 2021: Information valid as of May 2022

Chattogram, Bangladesh

Khulna, Bangladesh

Marine and Fisheries Research Institute, Kenya

3 Laboratories in Malaysia

The Philippines

Laboratoire National de Commerce, Senegal

South African Bureau of Standards, South Africa

6 Laboratories in Turkey



Dhaka, Bangladesh

The Gambia

Ghana

Fisheries Service, Kenya

3 Laboratories in Mozambique

Pakistan

Bureau of Fisheries and Aquatic Resources, Senegal

> Mérieux NutriSciences, South Africa

Analyses of data showed satisfactory performance in most laboratories, with higher uptake of samples and methods for *E. coli* in shellfish flesh than faecal coliforms in water. Methods utilised to test for *E. coli* and faecal coliforms are included in Table 1. Most laboratories used the EU reference method ISO 16649-3 for determination of E. coli, whereas the majority of those testing for faecal coliforms used a protocol 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.

Table 1. Methods used by laboratories in the pilot PT distribution in 2021

Method reference — <i>E. coli</i>	No.
ISO 16649 - 3 (MPN - 5 tubes, 3 dilutions)	13
NMKL 96 (RAPID E. coli agar based chromogenic media)	1
APHA (1992). Compendium of Methods for the Microbiological Foods. 2nd Edition, American Public Health Association, Washington, DC. (MPN - 4 dilutions, 3 tubes)	1
Indonesian National Standard, SNI 2332.1: 2015	1
MPN and Simplate	1

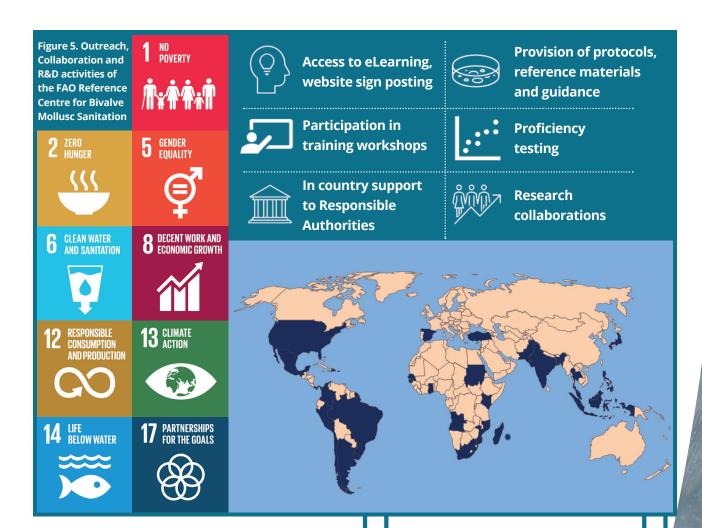
Method reference — faecal coliforms	No.
Determination of faecal coliform bacteria in seawater by the most probable number (MPN) technique (Based on Bacteriological Analytical Manual – BAM)	4
APHA (2001). Standard Methods for the Examination of Water and Wastewater. 20th Edition, American Public Health Association, Washington, DC. (MPN Method - 4 dilutions, 5 tubes)	1
COLILERT-18 Test Kit (MPN Method)	1
Membrane Filtration APHA 9222B	1
ISO 9308-1	1
SANS 5221	1

A separate PT report containing information on participants, samples and performance was produced and distributed to participants; laboratory performance will be discussed at the next series of workshops and individual laboratories will be offered troubleshooting advice. Invitations to participate in PT2023 have been sent to official laboratories and extended to attendees of the Latin America and Caribbean regional workshop.

## 3.0 Outreach and collaboration activities — summary

The reference centre was active in over 30 countries in 2021 (Figure 5). Activities ranged from provision of eLearning, training workshops, proficiency testing, distribution of protocols and technical guidance, support to FAO Member Country Responsible Authorities and research collaboration, the latter specifically with regard to the maintenance of the International Vibrio Strain Bank Vibrio Online Tools — Cefas (Centre for Environment, Fisheries and Aquaculture Science). The Reference Centre website continues as the main repository of information and portal for the dissemination of information and guidance. The next series of regional workshops will commence in 2022.





FAO Latin America and The Caribbean Antigua & Barbuda, Argentina, Brazil, Chile, Columbia, Dominican Rep., Grenada, Mexico, Panama, Peru, St. Vincent's & Grenadines, Suriname, Trinidad & Tobago and Uruguay





FAO Asia and Pacific Region - Bangladesh, India, Indonesia, Malaysia, Pakistan, The Philippines, Thailand, Turkey











FAO collaborations and R&D, Spain, United States and Japan



FAO Africa Region – Angola, Gambia, Ghana, Kenya, Madagascar, Mauritius, Mozambique, Senegal, South Africa











### 4.0 Publications in 2021

- Genomic signatures of adaptation to natural settings in non-typhoidal Salmonella enterica Serovars Saintpaul, Thompson and Weltevreden, J.R. Aguirre-Sanchez, J.R. Ibarra-Rodriguez, I.F. Vega-Lopez, J. Martínez-Urtaza, C. Chaidez-Quiroz, Infection, Genetics and Evolution, Volume 90, 2021, <a href="https://doi.org/10.1016/j.meegid.2021.104771">https://doi.org/10.1016/j.meegid.2021.104771</a>
- Oceanic hitchhikers-assessing pathogen risks from marine microplastic J Bowley, C
   Baker-Austin, A Porter, R Hartnell, C Lewis Trends in Microbiology, 2021.
- The foodborne transmission of Hepatitis E virus (exeter.ac.uk) Treagus, S; Wright, C; Baker-Austin,
   C; et al. The foodborne transmission of Hepatitis E virus 2021, Food and Environmental Virology.
- 4. Two Archaeal Metagenome-Assembled Genomes from El Tatio Provide New Insights into the Crenarchaeota Phylum, Santos, Andrés; Bruna, Pablo; Martinez-Urtaza, Jaime; Solís, Francisco; Valenzuela, Bernardita; Zamorano, Pedro; Barrientos, Leticia. 2021. Genes 12, no. 3: 391. <a href="https://doi.org/10.3390/genes12030391">https://doi.org/10.3390/genes12030391</a>
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- 6. Bacterial Communities in Fecal Samples of Myotis chiloensis from Southern, Chile Núñez-Montero, Kattia, Andrés Santos, Damián Quezada-Solís, **Jaime Martínez-Urtaza**, Fulgencio Lisón, and Leticia Barrientos. *International Journal of Morphology* 39, no. 1, 2021.
- 7. Distribution of Tetrodotoxin in Pacific Oysters (*Crassostrea gigas*) Dhanji-Rapkova, Monika; Turner, Andrew D.; Baker-Austin, Craig; Huggett, Jim F.; Ritchie, Jennifer M. 2021. *Mar. Drugs* 19, no. 2: 84. https://doi.org/10.3390/md19020084
- 8. Concentration and Quantification of SARS-CoV-2 RNA in Wastewater Using Polyethylene Glycol-Based Concentration and qRT-PCR. Farkas, Kata; Hillary, Luke S.; Thorpe, Jamie; Walker, David I.; **Lowther, James A.**; McDonald, James E.; Malham, Shelagh K.; Jones, Davey L. 2021. *Methods Protoc.* 4, no. 1: 17. <a href="https://doi.org/10.3390/mps4010017">https://doi.org/10.3390/mps4010017</a>
- 9. Alison O'Neill, Nadine Morrell, Andrew D. Turner, Benjamin H. Maskrey, Method performance verification for the combined detection and quantitation of the marine neurotoxins cyclic imines and brevetoxin shellfish metabolites in mussels (Mytilus edulis) and oysters (Crassostrea gigas) by UHPLC-MS/MS Journal of Chromatography B, Volume 1179, 2021, <a href="https://doi.org/10.1016/j.jchromb.2021.122864">https://doi.org/10.1016/j.jchromb.2021.122864</a>
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- 11. Abdulhussain AH, Cook KB, **Turner AD**, Lewis AM, Bibby TS and Mayor DJ (2021) The Influence of the Toxin-Producing Dinoflagellate, *Alexandrium catenella* (1119/27), on the Survival and Reproduction of the Marine Copepod, *Acartia tonsa*, During Prolonged Exposure. *Front. Mar. Sci.* 8:652225. doi: 10.3389/fmars.2021.652225.

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- **15. Adam M. Lewis, Karl J. Dean, David M. Hartnell**, Linda Percy, **Andrew D. Turner**, Jane M. Lewis, The value of toxin profiles in the chemotaxonomic analysis of paralytic shellfish toxins in determining the relationship between British *Alexandrium* spp. and experimentally contaminated *Mytilus* sp. Harmful Algae, Volume 111, 2022, 102131, ISSN 1568-9883, <a href="https://doi.org/10.1016/j.hal.2021.102131">https://doi.org/10.1016/j.hal.2021.102131</a>
- **16.** Romanello, Marina, **Martinez-Urtaza Jaime** *et al.* The 2021 report of the *Lancet* Countdown on health and climate change: code red for a healthy future The Lancet, Volume 398, Issue 10311, 1619 1662.
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- 19. J.R. Aguirre-Sanchez, J.R. Ibarra-Rodriguez, I.F. Vega-Lopez, J. Martínez-Urtaza, C. Chaidez-Quiroz, Genomic signatures of adaptation to natural settings in non-typhoidal Salmonella enterica Serovars Saintpaul, Thompson and Weltevreden, Infection, Genetics and Evolution, Volume 90, 2021, 104771, ISSN 1567-1348, <a href="https://doi.org/10.1016/j.meegid.2021.104771">https://doi.org/10.1016/j.meegid.2021.104771</a>
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- Dr Ben Maskrey, Senior Chemist and marine biotoxin scientist at Cefas for his participation in the Virtual Regional Workshop on Bivalve Mollusc Sanitation in November 2021.



Lem publica perunti strordiendem untiaecum ti, ac tam noneri patum con igit noves! Vivere etoreco mante, patis achucto haberum audam cononve robse, dienat, non re, ut fac teatus cone furo vius, mihilicae cut C. Mulibus, Catra? Quonvoltuus bonsimmodi tus tastorum dium in dieme iurare, ninihil hacturis in tem te, nonsultusuli sil tilicaetil culissusquam re, conderdin tem int vit atiae te condum pari in Itam, qua manum autesil hostum, ut iam ocaede pravoculis fuidin tabus condea interae lutemer iorbiti stratquon deorumu ntericaudet publiur. Les hi, utericteme cae tare vasdam confit. Nam ommodic epont? Nam nostrum peres conum num ma, tantinari inte nunte nenit.

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