

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

(Thematic areas<sup>1</sup>)

Title of FAO Reference Centre	FAO Reference Centre for Bivalve		
	Mollusc Sanitation		
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reporting officer (if different			
from			
above)			
Reporting period	January 2021 – December 2021		
Date of reporting	May 2022		

### 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 [<u>https://www.cefas.co.uk/faobivalves/</u>]. Main areas of collaboration for 2021 were:

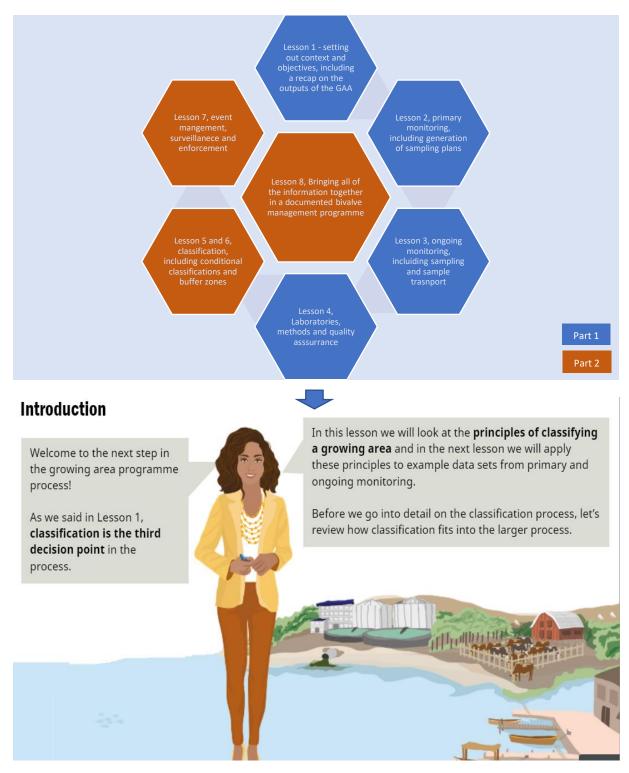
- To provide support for the development and maintenance of FAO resources and eLearning material on bivalve mollusc sanitation.
- To provide guidance on relevant laboratory protocols, accreditation, and use of methods for bivalve mollusc testing to FAO Member Countries.
- To prepare and deliver a regional 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 published<sup>2</sup>. 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.

<sup>&</sup>lt;sup>2</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 1. Framework and content for eLearning module 3 – 'Classification, Monitoring and Growing Area Management'



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

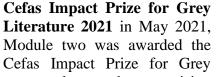
outcome for module 3 are set out in Figure 2.





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.





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

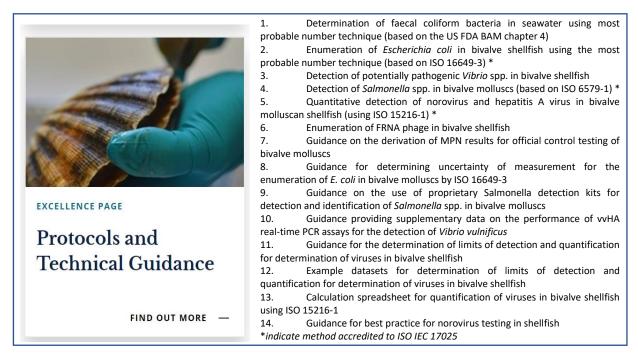
members of the UK Food Standards Agency (FSA) have registered as learners, demonstrating that the eLearning has benefits both nationally and internationally.

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 <u>Protocols</u> and Technical Guidance - 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



## 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</u> <u>Sanitation, December 2020 - Cefas (Centre for Environment, Fisheries and Aquaculture Science)</u>.

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 codelivery 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 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.

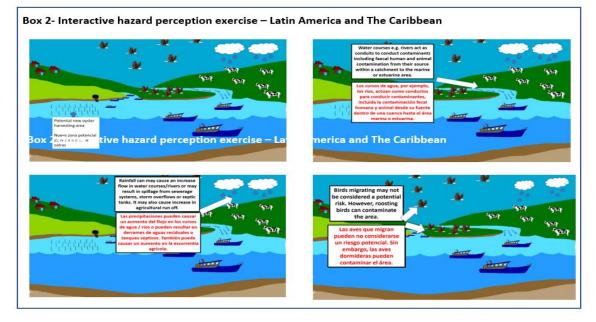
A full report of the workshop, and presentations, including those describing bivalve production and national capabilities from Responsible Authorities, can be accessed via the website Latin America and the Caribbean virtual regional workshop on Bivalve Mollusc Sanitation, November 2021 - Cefas (Centre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

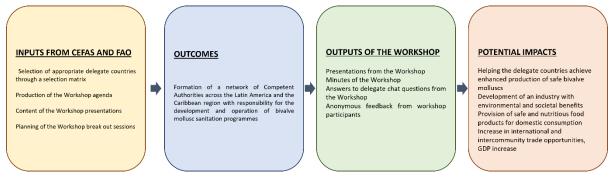


example). In addition, two further interactive elements included a new hazard perception walk through (Box 2) and a data analysis scenario. Workshop working languages were English and Spanish, with Spanish translation services provided by the FAO translation team.



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).





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



FIND OUT MORE -

Assurance

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<sup>™</sup>) 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 the distribution in March were received from 19 laboratories (68%) (Table 1).

Table 1. National	official control laborat	tories completing p	oilot PT di	stribution in 2021

Participating laboratory	Country	Results returned <sup>3</sup>
Quality Control Laboratory for Fisheries Products at INIPM, Angola	Angola	yes
Quality Control Laboratory, Department of Fisheries, Chattogram, Bangladesh		yes
Quality Control Laboratory, Department of Fisheries, Dhaka, Bangladesh		no
Quality Control Laboratory, Fish Inspection and Quality Control, Department Of Fisheries, Khulna,		yes
Bangladesh		
Department of Water Resources, The Gambia	The Gambia	no
National Food Safety Labs, Fish Health Unit, Ghana	Ghana	no
Loka Pemeriksaan Penyakit Ikan dan Lingkungan (Station for Investigation of Fish Health and Enviromental), Indonesia	Indonesia	yes
Kenya Marine and Fisheries Research Institute, Kenya	Kenya	yes
Kenya Fisheries Service, Kenya		no
Institut Pasteur de Madagascar, Madagascar	Madagascar	yes
Marine Micriobiology Laboratory, Mauritius	Mauritius	yes

<sup>&</sup>lt;sup>3</sup> Information valid as of May 2022

Pusat Biosekuriti Perikanan Kuantan, Malaysia	Malaysia	yes
Bacteriology Lab, Fisheries Research Institute, Malaysia		yes
Fisheries Biosecurity centre, Kaula Lumpa		yes
Laboratório de Inspecção do Pescado de Maputo, Mozambique	Mozambique	no
Laboratório de Inspecção do Pescado da Beira, Mozambique		no
Laboratório de Inspecção do Pescado de Quelimane, Mozambique		no
Livestock Experiment Station, Pakistan	Pakistan	no
Bureau of Fisheries and Aquatic Resources, The Philippines	The Philippines	yes
Bureau of Fisheries and Aquatic Resources, Senegal	Senegal	no
Laboratoire National de Commerce (LANAC), Senegal		yes
South African Bureau of Standards (SABS), South Africa	South Africa	yes
Mérieux NutriSciences, South Africa		no
Gida Kontrol Laboratuvar Mudurlugu, Turkey	Turkey	yes
Bursa Gıda ve Yem Kontrol Merkez Araştırma Enstitüsü Müdürlüğü, Turkey		yes
Ulusal Gıda Referans Laboratuvar Müdürlüğü, Turkey		yes
İstanbul Gıda Kontrol Laboratuvar Müdürlüğü, Turkey		yes
Kazım Dirik Mahallesi Sanayi Caddesi, Turkey		yes
Balıkesir Gıda Kontrol Laboratuvarı, Turkey		yes

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 2. 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 2 – Methods used by laboratories in the pilot PT distribution in 2021

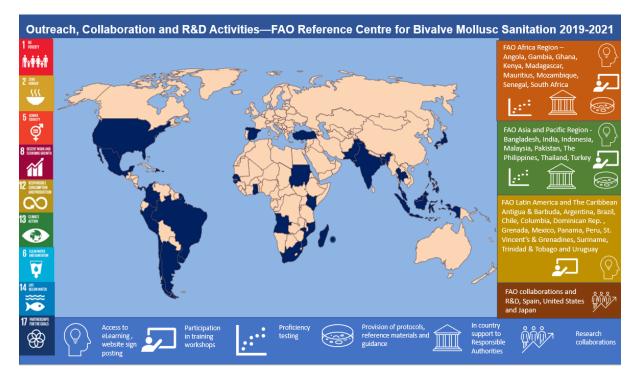
Method reference – <i>E. coli</i>	No.
ISO 16649 - 3 (MPN - 5 tubes, 3 dilutions)	13
NMKL 96 (RAPID E. coli agar based chromogenic media)	1
APHA (1992). Compendium of Methods for the Microbiological Foods. 2nd Edition, American Public Health Association, Washington, DC. (MPN - 4 dilutions, 3 tubes)	1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

Figure 5. Outreach, Collaboration and R&D activities of the FAO Reference Centre for Bivalve Mollusc Sanitation



### 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, https://doi.org/10.1016/j.meegid.2021.104771
- 2. <u>Oceanic hitchhikers–assessing pathogen risks from marine microplastic</u> J Bowley, C Baker-Austin, A Porter, R Hartnell, C Lewis Trends in Microbiology, 2021.
- 3. <u>The foodborne transmission of Hepatitis E virus (exeter.ac.uk)</u> **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. <u>https://doi.org/10.3390/genes12030391</u>

- 5. Vibrio spp. infections (vol 4, 1, 2018). **Baker-Austin, Craig**, et al. *NATURE REVIEWS DISEASE PRIMERS* 7.1,2021.
- 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.
- 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. <u>https://doi.org/10.3390/md19020084</u>
- 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. <u>https://doi.org/10.3390/mps4010017</u>
- 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, <u>https://doi.org/10.1016/j.jchromb.2021.122864</u>
- Joanna L. Kershaw, Silje-Kristin Jensen, Bernie McConnell, Shaun Fraser, Caroline Cummings, Jean-Pierre Lacaze, Guillaume Hermann, Eileen Bresnan, Karl J. Dean, Andrew D. Turner, Keith Davidson, Ailsa J. Hall, Toxins from harmful algae in fish from Scottish coastal waters, Harmful Algae, Volume 105, 2021, https://doi.org/10.1016/j.hal.2021.102068.
- 11. Abdulhussain AH, Cook KB, Turner AD, Lewis AM, Bibby TS and Mayor DJ (2021) Toxin-Producing Dinoflagellate, Alexandrium The Influence of the catenella (1119/27), on the Survival and Reproduction of the Marine Copepod, Acartia Exposure. Front. tonsa. During Prolonged Mar. Sci. 8:652225. doi: 10.3389/fmars.2021.652225.
- 12. Dillon, Michael, **Turner** et al. Current Trends and Challenges for Rapid SMART Diagnostics at Point-of-Site Testing for Marine Toxins. *Sensors* 21.7 (2021): 2499.
- Trinanes, J., and J. Martinez-Urtaza. Future scenarios of risk of Vibrio infections in a warming planet: a global mapping study (vol 5, pg e426, 2021). LANCET PLANETARY HEALTH 5.8 (2021): E504-E504.
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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, Volume111,2022,102131,ISSN1568-9883, https://doi.org/10.1016/j.hal.2021.102131.
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- 17. Trinanes J, Martinez-Urtaza J. Future scenarios of risk of Vibrio infections in a

warming planet: a global mapping study (vol 5, pg e426, 2021). LANCET PLANETARY HEALTH. 2021 Aug 1;5(8) :E504.

- **18**. Vezzulli, L., Oliveri, C., Borello, A. **Martinez-Urtaza J.** *et al.* Aquatic reservoir of *Vibrio cholerae* in an African Great Lake assessed by large scale plankton sampling and ultrasensitive molecular methods. *ISME COMMUN.* **1**, 20 (2021). https://doi.org/10.1038/s43705-021-00023-1.
- 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, https://doi.org/10.1016/j.meegid.2021.104771
- 20. Farkas, K.; Hillary, L.S.; Thorpe, J.; Walker, D.I.; Lowther, J.A.; McDonald, J.E.; Malham, S.K.; Jones, D.L. Correction: Farkas *et al.* Concentration and Quantification of SARS-CoV-2 RNA in Wastewater Using Polyethylene Glycol-Based Concentration and qRT-PCR. *Methods Protoc.* 2021, *4*, 17. *Methods Protoc.* 2021, *4*, 82. https://doi.org/10.3390/mps4040082.
- 21. Luz María García-García, Carlos J.A. Campos, Simon Kershaw, Andrew Younger, John Bacon, Scenarios of intermittent E. coli contamination from sewer overflows to shellfish growing waters: The Dart Estuary case study, Marine Pollution Bulletin, Volume 167, 2021,112332, ISSN 0025-326X,https://doi.org/10.1016/j.marpolbul.2021.112332
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#### 5.0 Acknowledgments

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**Annex I.** Financial support for the FAO Reference Centre for Bivalve Mollusc Sanitation received at Cefas (Financial year 2020/21)

Funding Body	Amounts
Department for Environment, Food and Rural Affairs (Defra)	£80,000
Food Standards Agency (FSA)	£125,000
Total	£205,000
Staff Pay	£187,391.66
Non-Pay (T&S / Consumables etc)	£17,608.34