Eastern IFCA

Inshore Fisheries and Conservation Authority

RESEARCH REPORT

EHO Biotoxin Sampling Report 2018

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Bacteriological and biotoxin sampling

Introduction

The Wash, which forms part of the Norfolk and Lincolnshire coastline provides some of the most productive fishing grounds for shellfish in UK waters. Cockles and mussels, characterised by a 2-valved (bivalves) shell joined by an elastic ligament, form the major component of landings with an average first sale value of over one million pounds per annum each (Jessop et al. 2013). To ensure shellfish from these production areas are safe for human consumption, and before they can be harvested and sold, they must be classified by the competent authority, the Food Standards Agency (FSA) as a requirement of food safety and public health in accordance with EC regulations 852/2004, 853/2004 and 854/2004 (The European Parliament and the Council of the European Union, 2004). The analytical approach is two-pronged, consisting of bacteriological analysis of shellfish meats for the purposes of bed classification and biotoxin analysis of both meat and water samples. Understanding the physiology of bivalves helps clarify why the consumption of such species can be problematic for human health. Bivalves are common in areas where nutrient levels are high, and waters sheltered, and these tend to be shallow coastal waters which are at higher risk of contamination from human sewage. Research has shown that bivalves are vectors of numerous human diseases, largely through their feeding mechanism, removing suspended particles of phytoplankton and detritus from the water column by pumping water over their gills. When pathogenic microorganisms contaminate the harvesting sites, they are filtered by the gills and become highly concentrated. The potential for contaminant capture and accumulation is enabled through this feeding mechanism, concentrating bacterial and viral pathogens found within food sources and the surrounding environment in liverlike digestive glands (Potasman et al. 2002).

Bacteriological Sampling – Bed Classification

Upon application for the establishment of a new shellfish production area it must first be classified. Bed classification involves an initial desktop and coastline study to determine potential pollutant sources in and around a proposed shellfish production area, including farmland, treatment plants and boats. Outlets near and into rivers and streams facilitate the transport of faecal coliforms onto shellfish beds where the degree and rate of deposition is affected by a range of physical and environmental factors such as bathymetry of the seabed, seasonality, rainfall and wind (Jessop et al. 2013).

Based on this information, a sanitary survey will be conducted with a view to evaluate the risk of microbiological contamination to shellfish within the proposed production area prior to a provisional classification being awarded. Once completed, provisional classification of the proposed production area can be made. Results of this preliminary survey provide the basis from which a sampling plan can be drawn up, enabling the identification of Representative Monitoring Points (RMP). RMP's are locations at which a pollution event is most likely to occur; therefore, periodic monitoring, each month in this case, should ensure detection of such an outbreak with more rapid response.

Under the current scheme, Local Action Groups and Local Action Plans provide an immediate and responsive mechanism for the investigation of E. coli sample results exceeding regulatory levels. Government targets aim to improve water quality in shellfish harvesting areas under the Water Framework Directive (European

Commission, 2013). Ultimately, water quality is one of the most important concerns for the shellfish industry and although there has been significant investment in the improvement of sewage systems, very few shellfish production areas are achieving an A-grade classification.

The testing procedure in the EIFCA district currently includes the analysis of *Cerastoderma edule* (cockle), *Mytilus edulis* (blue mussel), *Magallana gigas*[1] (Pacific oyster) and at times has included *Ostrea edulis* (native or flat oyster) and *Ensis directus* (razor clam). Shellfish and water samples are collected for analysis of microbiological contamination, marine biotoxins and chemical contamination by Eastern Inshore Fisheries and Conservation Authority (EIFCA), on behalf of three Local Authorities. EIFCA itself is currently responsible for collecting *C. edule* and *M. edulis* only. The Local Authorities are tasked with ensuring a sampling regime is active in order to address periodic monitoring of the shellfish production areas within The Wash.

Table 1. Classification criteria for harvesting areas (The Centre for Environment, Fisheries and Aquaculture Science (Cefas, 2019).

Class	**Microbiological standard**	**Treatment level**
A	80% of results contain ≤ 230 *E. coli*/100g shellfish flesh, no results exceeding 700 E.*coli/*100g shellfish flesh.	None required (direct human consumption).
B	90% of samples must be ≤ 4600 *E. coli*/100g shellfish flesh; all samples must be less than 46000 E. coli/100g shellfish flesh.	Depurate (using approved methodology in approved plant) OR relayed in an approved Class A relaying area OR EC approved heat treatment before being sold for human consumption.
C	All samples must not exceed ≤ 46000 *E. coli*/100g shellfish flesh.	Must be relayed (minimum of 2 months) in an approved Class B relaying area followed by treatment in an approved purification centre OR relaying for at least 2 months in an approved Class A relaying area OR after an EC approved heat treatment process.

[1] Formally *Crassostrea gigas.*

D	Do not conform to at least class C.	Prohibited.

Biotoxin sampling

Meats derived from *C.edule* and *M.edulis* sampling are used in the testing of Paralytic Shellfish Poisoning (PSP) caused by *Alexandrium spp*., Amnesic Shellfish Poisoning (ASP) associated with *pseudo nitzchia* and Diarrhetic Shellfish Poisoning (DSP) caused by *Dinophysis spp*. and *Prorocentrum lima*. Unusually high biotoxin concentrations can often be triggered by plankton blooms where an influx of phytoplankton to a system may bring with it toxic algal species. The presence of these may cause a temporary increase in the detection of toxic species associated with ASP, DSP and PSP. Although the occurrence of one is not necessarily preceded by the other, they can give an indication as to whether a toxic event may be imminent in the results (Jessop et al. 2013). Water samples are collected from designated shellfish growing areas and analysed using light microscopy. Phytoplankton monitoring in England and Wales is being carried out by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) on behalf of the Food Standards Agency. These samples are collected concurrently with the meat samples.

Table 2. Action levels of flesh, water toxic algae levels and methods of analysis (Food Standards Agency, 2019).

	Flesh	**Method of Analysis**	**Water**	**Method of Analysis**
ASP	>20 mg of domoic/epi-domoic acid/Kg flesh	High Performance Liquid Chromatography (HPLC)	Producing algae: Greater than or equal to 150,000 cells/Litre.	Utermöhl method (Light microscopy and electron microscopy)
DSP	Presence	Liquid Chromatography Mass Spectrometry (LC-MS)	Producing algae: Greater than or equal to 100 cells/Litre	
PSP	>800 micrograms STX/Kg flesh	High Performance Liquid Chromatography (HPLC)	Producing algae: Greater than 40 cells/litre	

During cultivation periods, shellfish may become contaminated by naturally occurring toxins derived from certain marine algae. One family of these chemicals is the fat-soluble (lipophilic) toxins. Historically, toxicity measurements have been undertaken using a qualitative, non-specific technique called the mouse bioassay (MBA) test. To reduce reliance on the MBA test and to improve risk management of contaminated shellfish, a chemical/analytical method was developed as a suitable alternative. In 2006, the UK was the first European Union country to introduce HPLC (High Performance Liquid Chromatography) methodology into a statutory monitoring programme. In 2011, the FSA approved the replacement of the MBA for the detection of lipophilic toxins, including toxins responsible for Diarrhetic Shellfish Poisoning (DSP) with Liquid chromatography mass spectrometry (LC-MS).

Toxins were extracted from shellfish flesh using a method published by the EU Reference Laboratory for Marine Biotoxins. A published liquid chromatographic (LC) method was then refined in order to separate 12 EU regulated lipophilic toxins including okadaic acid (OA) and dinophysistoxins (DTX), pectenotoxins (PTX), azaspiracids (AZA) and yessotoxins (YTX) within. Identification was then confirmed by (tandem) mass spectrometry (MS/MS) detection based on recording 'fingerprint' ions specific to each toxin compound. Following international protocols, the performance of the method was established and described after validation and application in EU-wide, inter-laboratory studies (Food Standards Agency, 2019).

The refined method provides an efficient, automated, multi-toxin approach which combines an effective extraction procedure with the specificity of LC-MS/MS analysis, allowing for specific identification and more precise quantification of the range of lipophilic toxins encountered in UK commercially significant shellfish species. It demonstrates many advantages over the former EU reference Mouse bioassay (MBA) method, which although is able to respond to the presence of marine lipophilic toxins, can present 'false' results (due to interference from co-extracted shellfish matrix compounds) and is not able to provide any quantitative information on toxin concentrations or specifically identify different toxins or toxin compounds. Furthermore, the introduction ensures increased confidence in monitoring results and addresses the scientific and ethical concerns identified with the mouse bioassay technique used in the monitoring programme.

Current Sampling Regime

The location of sampling sites in The Wash are detailed in Figure 1. Based on the current programme of monitoring, Table 3 outlines the current sample requirements from each site. Water quality readings are taken at the Stubborn Sand and Toft sites using a YSI data sonde.

Table 3. Current bacteriological sampling requirements for the Wash

Sample type	Sample Area	Zone	Species
EHO	Ouse Mouth	5	Cockle (*C.edule*)
	Nene Mouth	3	Cockle (*C.edule*)
	Black Buoy	2	Cockle (*C.edule*)
	North Lays	1	Cockle (*C.edule*)

	Toft	2	Mussel (*Mytilus sp.*)
	Welland Wall	6	Mussel (*Mytilus sp.*)
DSP	Toft	2	Mussel (*Mytilus sp.*)
Water	Stubborn Sand	4	n/a
	Toft	2	n/a

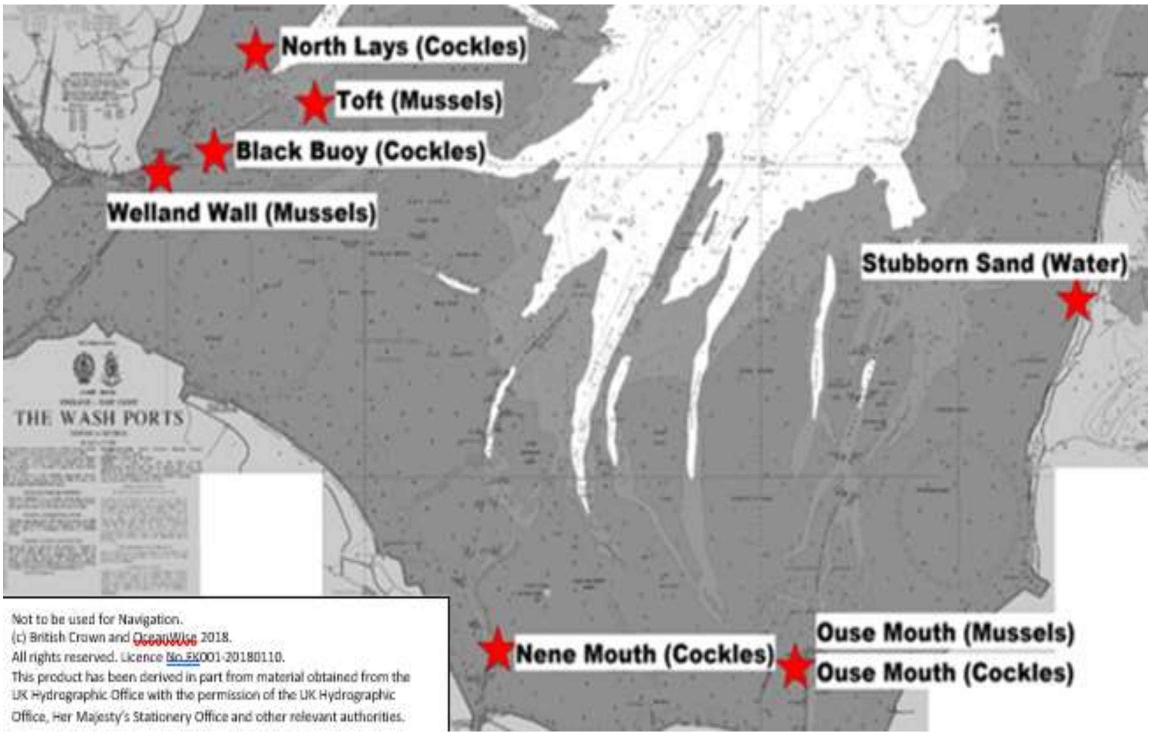

Figure 1. Bacteriological, Biotoxin and water sampling sites in The Wash for 2018-2019.

Results

Links below report a 5-year span of data collected during shellfish monitoring in The Wash for both *C. edule* and *M. edulis*, including microbiological results for individual harvesting beds in England and Wales (E. *coli* numbers in detail) and detailed maps of each zone.

Welland Wall (Mytilus sp., mussel)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=MUS&connection=SHS&PointID=B003M

North Lays (C. edule, cockle)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=COC&connection=SHS&PointID=B003W

Toft (Mytilus sp., mussel)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=MUS&connection=SHS&PointID=B003V

Black Buoy (C. edule, cockle)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=COC&connection=SHS&PointID=B04AO

Nene Mouth (C. edule, cockle)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=COC&connection=SHS&PointID=B04AL

Ouse Mouth (C. edule, cockle)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=COC&connection=SHS&PointID=B04AM

Hunstanton – Holmeside (Mytilus sp., mussel)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=MUS&connection=SHS&PointID=B004L

Stubborn Sand (C. edule, cockle)

https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/details/?species=COC&connection=SHS&PointID=B04AP

For biotoxin (ASP, DSP and PSP) and phytoplankton monitoring results see:

https://www.food.gov.uk/enforcement/monitoring/shellfish/ewbiotoxin

The full list of classifications given, effective from 3rd September 2018 for a period of one year for shellfish production areas in England and Wales can be found below (*Table 4*):

Table 4. Designated bivalve mollusc production areas in the eastern-IFCA district. Effective from the 3rd September 2018 (Food standards Agency, 2019).

Production area	Classification Zone	Bed Name	Species	Class	Explanatory note
The Wash - Boston	**Zone 1 South**	**North Lays**	***C. edule***	**B-LT**	

	Zone 2 East	**Black Buoy**	***C. edule***	**B-LT**	
	Zone 2 East	**Toft**	***Mytilus spp.***	**B-LT**	
		Welland Wall	***Mytilus spp.***	**Seasonal C**	**Transition period** 1st November-31st December **Class B season** 1st January-30th May (reverting to class C at all other times)
The Wash – King's Lynn	**Zone 5**	**Ouse Mouth**	***C. edule***	**B-LT**	
			Mytilus spp.	**B-LT**	
	Zone 5	**Nene Mouth**	***C. edule***	**B-LT**	
			Mytilus spp.	**B-LT**	
Brancaster		**Brancaster**	***C. edule***	**B-LT**	
			Mytilus spp.	**B-LT**	
			M. gigas	**B-LT**	
		Thornham[2]	***M. gigas***	**B-LT**	
Blakeney		**South Side**	***M. gigas***	**B-LT**	
		Wells – The Pool	***Mytilus spp.***	**B-LT**	
Butley		**Butley Oysterage**	***M. gigas***	**B-LT**	
Deben		**Girlings Hard**	***Mytilus spp.***	**B-LT**	
			O. edulis	**B-LT**	
			M. gigas	**B-LT**	
		Shottisham Creek	***Mytilus spp.***	**B-LT**	
			M. gigas	**B-LT**	
		Spinny Marsh	***M. gigas***	**B-LT**	
		Stonner Point	***M. gigas***	**B-LT**	

Discussion

Sampling requirements for water classification involve the collection of a minimum of ten monthly samples a year from each site. Inclement weather conditions during 2018 created a number of challenges in achieving the sampling regime but all requirements were achieved. Class B trigger thresholds of ≤ 4600 *E. coli*/100g shellfish flesh were exceeded at three (3) sampling sites in The Wash in April; the Nene Mouth sample (cockle) had an *E. coli* content of 7900/100g flesh, the Ouse Mouth sample (cockle) had an *E. coli* content of 13000/100g flesh and Black Buoy (cockle) had an *E. coli* content of 13000/100g flesh. Following this threshold being breached, these sites were

2. Thornham is now a declassified site as of January 2019.

prioritised for collection the following months. Analysis of samples showed that *E. coli* levels dropped back below the threshold the following month.

Classification results for designated shellfish production areas in The Wash (*table 4*) follow the same trend as results published in EIFCA's 2017 study. Results are effective from the 3rd of September 2018, for a period of one year. The sites in which Eastern IFCA collect samples are classified B, except for Welland Wall (Welland and Witham inner zone), which has been classified as a seasonal Class C. A Seasonal classification may be considered when sample results indicate a clear and consistent period when the shellfish are of a quality to be harvested compared to the rest of the year. For Class B beds in England and Wales, there is a long-term classification (LTC) system. This is in place to show greater stability in the classifications which are based on compliance over five years instead of the standard three years. All beds, with the exception of Welland Wall have continued to maintain B-LT (long term) status.

Shellfish gathered from a Class B site within the Eastern-IFCA district are required to undergo depuration using an approved methodology, relayed in an approved Class A relaying area or heat treated by approved methods before being sold for human consumption. Shellfish gathered from Welland Wall, a seasonal C-grade classification site within the Eastern-IFCA district between 31st May and 31st of October will require relaying (for a minimum of 2 months) to meet class A or B requirements or be heat treated. For the Class B season, the transition period from 1st November to 31st December for Welland Wall will depend on Class B season may commence prior to the stated class B season following 2 samples ≤4600 taken 1 week apart within the transition period. The site will revert to Class B between 1st January to 30th May. Shellfish will then be required to undergo depuration using an approved methodology, relayed in an approved Class A relaying area or heat treated by approved methods before being sold for human consumption.

Bed classification results for 2018-2019 are considered generally good. Many factors influence the levels of microbiological contamination in shellfish including seasonality, environmental conditions and quality and quantity of faecal contamination discharged into the local coastal area. Sources of contamination include human and animal pollution, occurring as either point source inputs (discharges, outfalls and cracked pipes) or as diffuse pollution predominantly from agricultural run-off. Increase in awareness of the impacts of these pollution sources has driven improvements in pollution management in recent years, however, identifying and preventing contamination from such sources presents a difficult ongoing challenge.

In 2018, the naturally occurring marine biotoxin Paralytic Shellfish Poison (PSP) was discovered in marine species within EIFCA's District outside of the EHO monitoring programme. PSP toxins are primarily associated with filter feeding bivalve molluscs who can accumulate the potent neurotoxins, all related to the parent compound Saxitoxin, which are produced naturally by certain species of microscopic algae. PSP toxins were identified in samples analysed by Cefas in relation to two dog deaths in Cley (Norfolk) and Felixstowe (Suffolk) in late December of 2017 and early January of 2018. The two dogs are thought to have eaten marine organisms washed up in winter storms including starfish and shore crabs. Very high concentrations of PSP toxins were found in the samples associated with the dog deaths; in starfish (15,000 to 22,000 µg PSP per Kg sample tissue tested) and in partially digested shore crab (2,500 to 3,500 000 µg PSP per Kg sample tissue tested). To put this into context, the

EU Regulatory maximum level for PSP toxins permitted in live bivalve molluscs is 800 µg PSP per kilogramme of tissue tested.

Eastern IFCA coordinated the public response to the incidents which was given the operating name of Operation Blake. This included the development of a testing regime to monitor levels of PSP in edible species and a media strategy to ensure that the public could be correctly informed in relation to the matter. The group published a number of press releases and advice to vets and pet owners on how to deal with suspected PSP incidents in pets. In March of 2019, funding was secured to continue the ongoing monitoring programme to inform the development of an action plan in relation to potential future incidents. The source of the funding is the European Maritime and Fisheries Fund.

The routine monitoring for the presence of toxin producing plankton in shellfish production and relaying areas, and biotoxins in bivalve molluscs, is a requirement of Regulation (EC) No 854/2004, which sets out official controls on products of animal origin intended for human consumption. The Food Standards Agency (FSA) is the body with overall responsibility for the official monitoring programme for marine biotoxins in live bivalve molluscs. Recent analysis of bivalve mollusc and water samples, collected through the Official Control monitoring programme, have been negative for PSP and the algae associated with PSP production in the UK (*Alexandrium* spp.) had remained below regulatory levels for commercially fished species in The Wash.

References

Centre for Environment, Fisheries and Aquaculture Sciences (2019). *Classification and microbiological monitoring.* (Online). Available at https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/shellfish-monitoring-results/ (accessed 14/01/2019).

Centre for Environment, Fisheries and Aquaculture Sciences (2018). Protocol for the Classification of Shellfish Harvesting Areas – England and Wales. Version 15.

Food Standards Agency (2019). *Shellfish Harvesting Classifications England and Wales: 2018-2019.* (Online). Available at: https://www.food.gov.uk/sites/default/files/media/document/classification-list-11-january-2019.pdf (Accessed 14/01/2019).

Food Standards Agency (2019). *Official control biotoxin monitoring (live bivalve molluscs) programme (England and Wales).* (Online). Available at: https://www.food.gov.uk/enforcement/monitoring/shellfish/ewbiotoxin

Jessop, R.W., Strigner, R., Thompson, S., Welby, P.R., (2013) Inshore Fisheries and Conservation Authority-Research Report-EHO biotoxin sampling. *Internal Publication.* 326-334.

Potasman, I., Alona, P., Majed, O., (2002) Infectious Outbreaks Associated with Bivalve Shellfish Consumption: A Worldwide Perspective. *Clinical Infectious Diseases*. Vol. 35 (8) 921–928. https://doi.org/10.1086/342330.

Regulation (EC) No. 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs (2004). (c.1-11). Brussels: The European Parliament and the Council of the European Union.

Regulation (EC) No. 853/2004 of the European Parliament and of the Council on the hygiene of foodstuffs laying down specific hygiene rules for the hygiene of foodstuffs (2004). (c. 1-4). Brussels: The European Parliament and the Council of the European Union.

Regulation (EC) No. 854/2004 of the European Parliament and of the Council on the hygiene of foodstuffs laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption (2004). (c.2). Brussels: The European Parliament and the Council of the European Union.