



RESEARCH REPORT

EHO bio toxin sampling report 2017

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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 (bivalves) form the major component of landings with an average first sale value of over one million pounds per annum (Jessop et al. 2013). To ensure these production areas are providing shellfish that 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. Bivalves are widely known as vectors of numerous human diseases and this is considered in the literature to be largely down to their feeding mechanism, removing suspended particles of phytoplankton and detritus from the water column by pumping water over their ctenidia. 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 shellfish production area. Potential sources of contaminants include 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 devised with a view to evaluate the risk of microbiological contamination to shellfish within the proposed production area and is conducted prior to a provisional classification being awarded. Upon completion of the sanitary survey, 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 (Jessop et al. 2013). 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 uses samples of *Cerastoderma edule* (common edible cockle), *Mytilus edulis* (blue mussel), *Crassostrea gigas* (Pacific oyster) and at times has included *Ostrea edulis* (native or flat oyster) and *Ensis directus* (razor clam). Samples are collected monthly by a

number of organisations, including the EIFCA, on behalf of Local Councils. EIFCA itself is currently responsible for collecting *C. edule* and *M. edulis* only. The Local Authority is tasked with ensuring a sampling regime is active in order to address periodic monitoring of the shellfish production areas within The Wash. 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 the Local Authority.

Table 1. Classification criteria for harvesting areas (The Centre for Environment, Fisheries and Aquaculture Science (Cefas 2017)).

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

Biotoxin sampling

C.edule, *M.edulis* and water samples are collected by EIFCA as part of this sampling regime on a monthly basis. Meats 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). 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. Water samples are collected from designated shellfish growing areas and analysed using light microscopy for various species of phytoplankton. 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, 2014).

	Flesh	Method of Analysis	Water	Method of Analysis
ASP	>10 milligrams of 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	>400 micrograms/Kg flesh	High Performance Liquid Chromatography (HPLC)	Producing algae: Greater than 40 cells/litre	

Previously, the method for detecting potentially harmful PSP and lipophilic toxins was based on a technique called the mouse bioassay (MBA) test. In 2006, the UK was the first European Union country to introduce HPLC (High Performance Liquid Chromatography) methodology into a statutory monitoring programme. This significantly reduced the reliance on the MBA test. 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) (Food Standards Agency, 2014). The introduction ensures increased confidence in monitoring results and addresses the scientific and ethical concerns identified with the mouse bioassay currently used in the monitoring programme.

Current Sampling Regime

Based on the current programme of monitoring, **Error! Reference source not found.** outlines the current sample requirements from each site in the Wash from which Officers collect organisms. **Error! Reference source not found.** depicts the locations of these sampling sites. During site visits, water quality readings are taken using a YSI data sonde. Such concurrent data collection provides water quality parameter details utilised by Cefas. Samples are delivered to Cefas using temperature controlled biotherm boxes.

Table 3. Current bacteriological sampling requirements for the Wash

Sample type	Sample Area	Zone	Species
EHO	Ouse Mouth	5	Cockle (<i>C.edule</i>)
	Nene mouth	3	Cockle (<i>C.edule</i>)
	Black Buoy	2	Cockle (<i>C.edule</i>)
	North lays	1	Cockle (<i>C.edule</i>)
	Toft	2	Mussel (<i>Mytilus sp.</i>)
	Welland Wall	6	Mussel (<i>Mytilus sp.</i>)
DSP	Toft	2	Mussel (<i>Mytilus sp.</i>)
Water	Stubborn Sand	4	n/a
	Toft	2	n/a

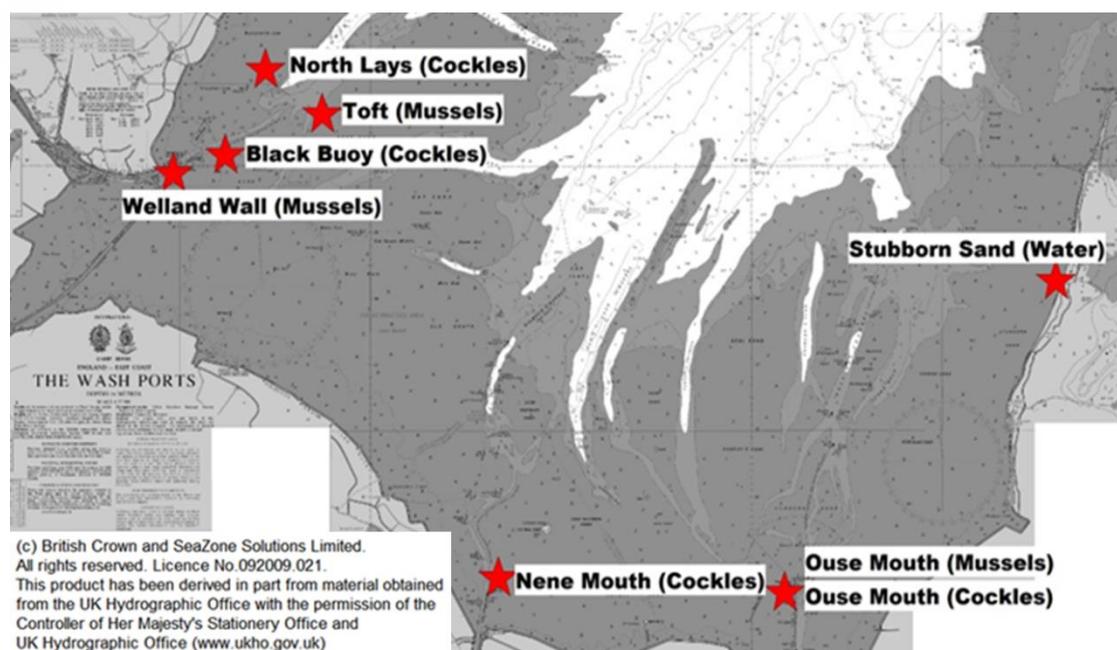


Figure 1. Bacteriological, Biotoxin and water sampling sites in the Wash for 2017-2018.

Results and discussion

The full list of classifications given for 1st September 2017 to 31st August 2018 for shellfish production areas in England and Wales can be found in the table below:

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.cefascas.co.uk/cefascas-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.cefascas.co.uk/cefascas-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.cefascas.co.uk/cefascas-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.cefascas.co.uk/cefascas-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.cefascas.co.uk/cefascas-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.cefascas.co.uk/cefascas-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.cefascas.co.uk/cefascas-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>

Table 4. Designated bivalve mollusc production areas in the eastern-IFCA district. Effective from the 1st September 2017 (Food standards Agency, 2017)

Production area	Classification Zone	Bed Name	Species	Class	Explanatory note
The wash - Boston	Zone 1 South	North Lays	<i>C. edule</i>	B-LT	
	Zone 2 East	Black Buoy	<i>C. edule</i>	B-LT	
<i>Mytilus spp.</i>			B-LT		
	Zone 2 East	Toft	<i>Mytilus spp.</i>	B-LT	
		Welland Wall	<i>Mytilus spp.</i>	Seasonal C	Transition period 1 st November-31 st December Class B season 1 st January-30 th May (reverting to class C at all other times)
The Wash – King’s Lynn	Zone 5	Ouse Mouth	<i>C. edule</i>	B-LT	
			<i>Mytilus spp.</i>	B-LT	
	Zone 5	Nene Mouth	<i>C. edule</i>	B-LT	
			<i>Mytilus spp.</i>	B-LT	
Brancaster		Brancaster	<i>C. edule</i>	B-LT	
			<i>Mytilus spp.</i>	B-LT	
			<i>C. gigas</i>	B-LT	
		Thornham	<i>C. gigas</i>	B-LT	
Blakeney		South Side	<i>C. gigas</i>	B-LT	
		Wells – The Pool	<i>Mytilus spp.</i>	B-LT	
River Alde		???????			
Butley		Butley Oysterage	<i>C. gigas</i>	B-LT	
Deben		Girlings Hard	<i>Mytilus spp.</i>	B-LT	
			<i>O. edulis</i>	B-LT	
			<i>C. gigas</i>	B-LT	

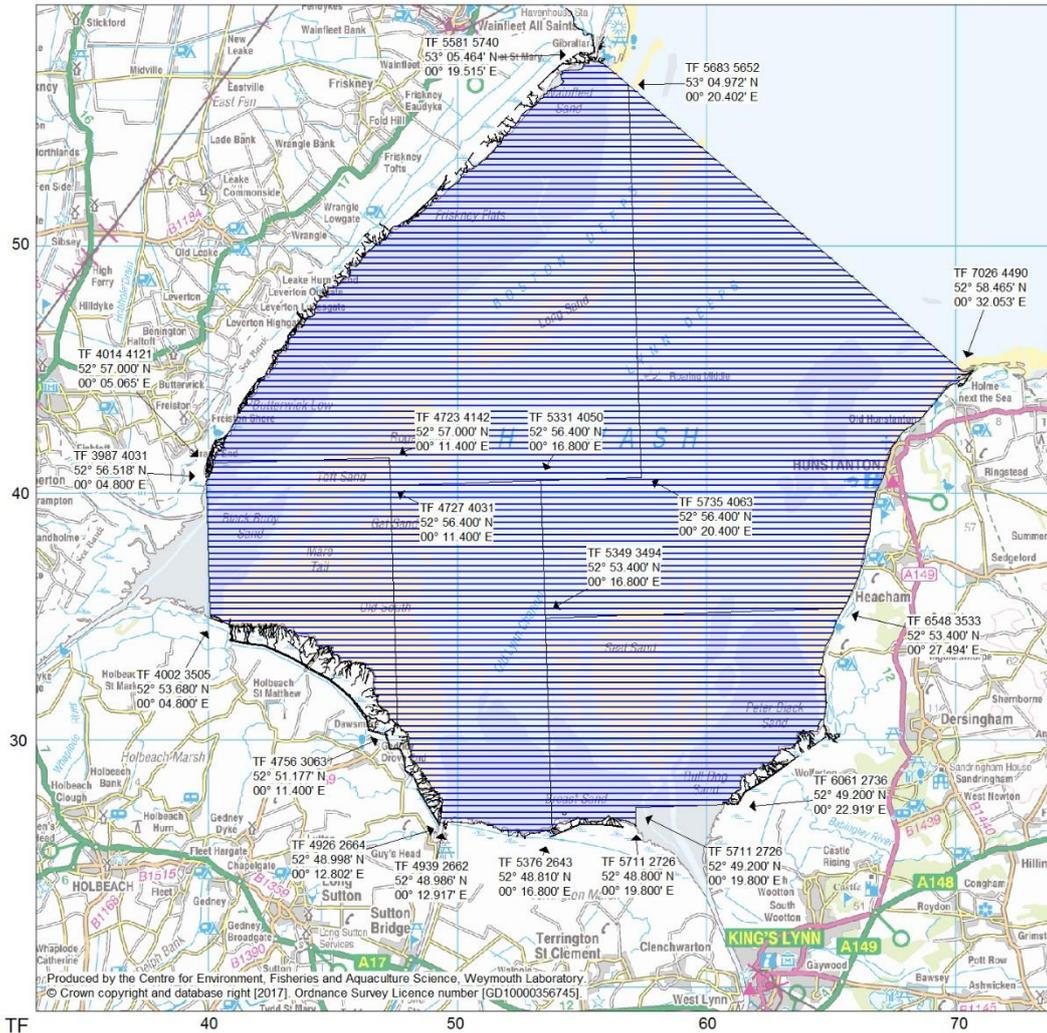
		Shottisham Creek	<i>Mytilus spp.</i>	B-LT	
			<i>C. gigas</i>	B-LT	
		Spinny Marsh	<i>C. gigas</i>	B-LT	
		Stonner Point	<i>C. gigas</i>	B-LT	

Table 4 highlights the classification results of bivalve production areas in the Eastern-IFCA district effective as of 1st September 2017 through to 31st August 2018. The latest classifications (2017-2018) for sites in which Eastern-IFCA collect samples are all B-grade, except for Welland Wall, which falls within the Welland and Witham inner zone, which has been classified as a seasonal class C grade. Any shellfish gathered from a B-grade classification 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. 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 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. The majority of areas have achieved a long-term status with 5 years of compliance data with 90% or better compliance with 4600 *E. coli*/100g. 2017 saw a downgrading of site status for Welland Wall with a seasonal C grade during the summer months. In contrast, Welland Wall has maintained a B grade classification throughout the previous 5-year period. Anecdotal reports relating to deterioration of site classification through water quality have suggested a link between the use of adjacent pasture for bovine grazing, however these are unsubstantiated and would require a robust scientific sampling and monitoring plan to ascertain any negative impacts (Senior IFCO, *pers. Comms.*).

Bed classification results for 2017-2018 are considered generally good, however the aim would be to achieve an A grade classification for all production areas within the Eastern-IFCA district. 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.

Wash - C. edule

Scale - 1:230000



Classification:	Class A	Class B	Class C	Prohibited	
Status	LT Class B	Seasonal Class B/C			

Classification of Bivalve Mollusc Production Areas: Effective from 1 September 2017

The areas delineated above are those classified as bivalve mollusc production areas under EU Regulation 854/2004.

Further details on the classified species and the areas may be obtained from the responsible Food Authority. Enquiries regarding the maps should be directed to: Shellfish Microbiology, CEFAS Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB. (Tel: 01305 206600 Fax: 01305 206601)

N.B. Lat/Longs quoted are WGS84

Unless otherwise stated, non-straight line boundaries between co-ordinates follow the OS 1:25,000 mean high water line.

Separate map available for *Mytilus* spp. for the Wash

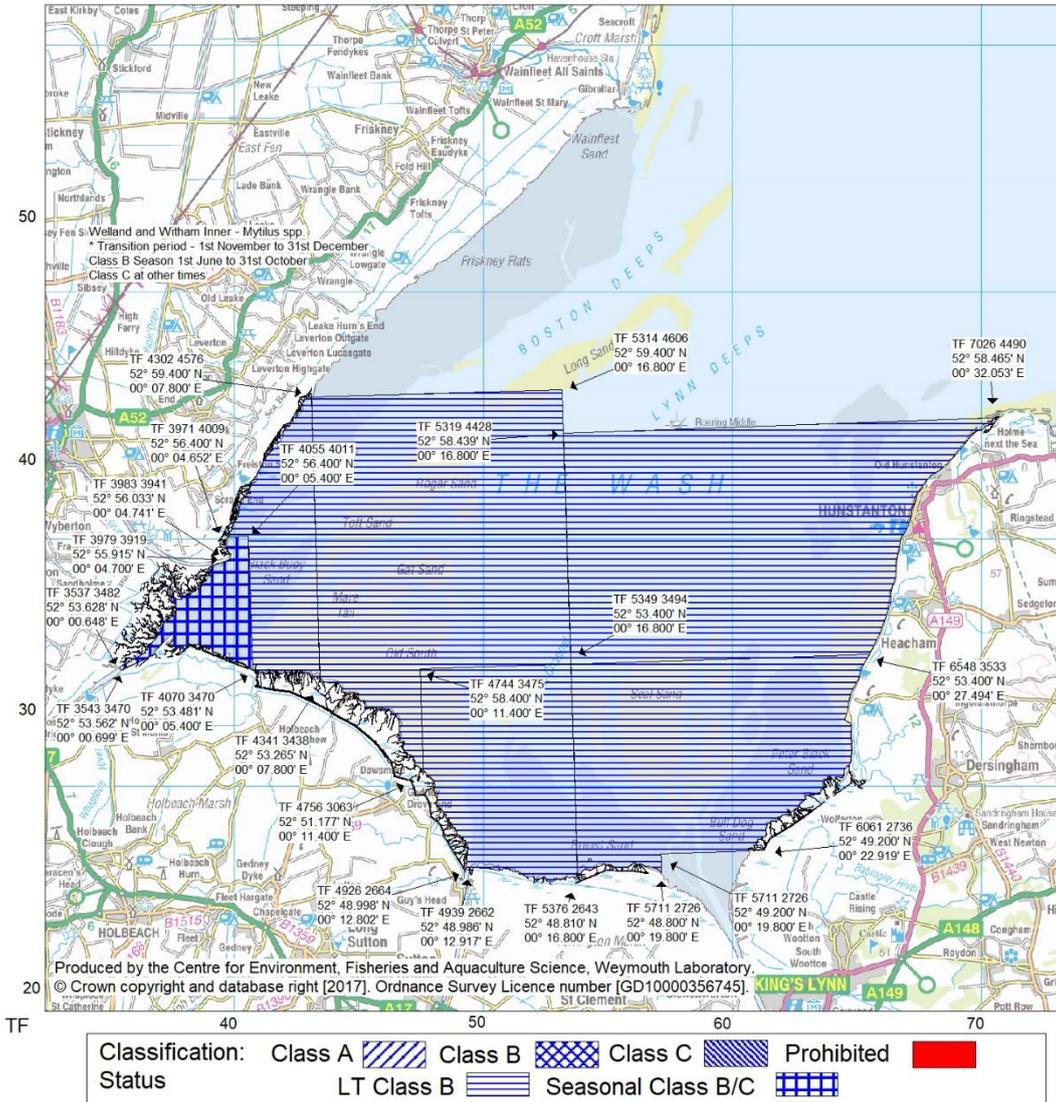
Food Authorities: Borough Council of Kings Lynn and West Norfolk (Heacham and Hunstanton; Ouse Mouth)
Boston Borough Council (Witham and Welland; Freiston to Wainfleet)

Fenland District Council (Nen Mouth)

Figure 2. Classification of Bivalve Mollusc Production Areas. The Wash – C. edule. Effective from the 1st September 2017 (Cefas, 2017)

Wash - *Mytilus* spp

Scale - 1:230000



Classification of Bivalve Mollusc Production Areas: Effective from 1 September 2017

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Separate map available for *C. edule* for the Wash

* Transition period (1st November to 31st December) - Class B season may commence following two samples ≤ 4600 taken at least one week apart

Food Authorities: Borough Council of Kings Lynn and West Norfolk (Heacham and Hunstanton; Ouse Mouth)
 Boston Borough Council (Mare Tail, Gat and Toft; Welland and Witham Outer; Welland and Witham Inner)
 Fenland District Council (Nene Mouth)

Figure 3. Classification of Bivalve Mollusc Production Areas. The Wash – *Mytilus* spp. Effective from 1st September 2017 (Cefas, 2017).

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- Centre for Environment, Fisheries and Aquaculture Sciences (2017). *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 14/11/2017).
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- 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.

