Centre for Environment Fisheries & Aquaculture Science

Cefas

Testing of crustacean tissue samples associated with NE England mortality events for marine biotoxins

Cefas analysis following crustacean sickness and mortalities reported North East England, 2021

REDACTED

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1	REDACTED	15/02/22	1st version
2	REDACTED	22/02/22	Additional information on sample dates and locations
3	REDACTED	23/02/2022	Sample id checks
4	REDACTED	01/03/2022	Update with lobster results
5	REDACTED	01/03/2022	Approved for submission

This report provides a brief summary of the results obtained following the testing of crab and lobster tissue samples received at Cefas in recent weeks. This follows on from a previous report which showed only low concentrations of the marine neurotoxins (domoic acid and saxitoxins) in crabs from the study area. For these samples we conducted analysis for a larger range of marine biotoxins produced naturally at certain times of the year by certain species of marine phytoplankton, using chemical detection methods. There follows, an overall summary of the findings, along with a description of the methods utilised, with additional Annexes to the report providing further details, including example chromatographic outputs.

For further information, the method used for detection of PSP toxins has been fully validated for application to crabs, but no such validation has been performed for ASP, DSP and other lipophilic toxins as well as brevetoxins and brevetoxin metabolites. As such, these results are not reported to ISO17025 standard.

Table 1. Summary of samples received

Sample text	**Species**	**Additional sample id**	**Sampled**	**Tissue tested**
CH1	Brown crab		8th Oct 21, Bran Sands, Tees	Hepatopancreas (HP)
CH2	Shore crab		8th Oct 21, Bran Sands, Tees	HP
CH3	Shore crab		8th Oct 21, Bran Sands, Tees	HP
CH4	Shore crab		8th Oct 21, Bran Sands, Tees	HP
CH5	Shore crab		8th Oct 21, Bran Sands, Tees	HP
CH6	Lobster		8th Oct 21, Bran Sands, Tees	HP
CH7	Brown crab		8th Oct 21, Redcar, Tees	HP
CH8	Brown crab		8th Oct 21, Redcar, Tees	HP
CH9	Brown crab		8th Oct 21, Redcar, Tees	HP
CH10	Brown crab		8th Oct 21, Redcar, Tees	HP
CH11	Brown crab		8th Oct 21, Redcar, Tees	HP
CH12	Brown crab (x2 pooled)		8th Oct 21, Redcar, Tees	HP
CH13	Lobster		22nd Oct 21, Shellfish Merchant, Tees	HP
BS1	Mussels		13th Jan 22, Bran Sands, Tees	Whole
CR1	Shore crab	NZ6621321943	25th Oct 21, Saltburn	HP
CR2	Shore crab	NZ5184431906	25th Oct 21, Seaton Carew	Gill
CR3	Shore crab	NZ6621321946	25th Oct 21, Saltburn	Gill
CR4	Shore crab	NZ5541829042	4th Oct 21, Bran Sands	Gill
CR5	Shore crab	NZ8112416045	8th Oct 21, Runswick	HP
CR6	Brown crab	NZ7274327667	2nd Nov 21, Maezie Belle (Fishing vessel – caught offshore Skinningrove)	Gill
CR7	Brown crab	NZ7274327667	2nd Nov 21, Maezie Belle (Fishing vessel – caught offshore Skinningrove)	HP
CR8	Shore crab	NZ5184431906	25th Oct 21, Seaton Carew	HP
CR9	Shore crab	NZ554987042	4th Oct 21, Bran Sands	HP
CR10	Shore crab	NZ6621321946	25th Oct 21, Saltburn	Gill
CR12	Brown crab	NZ7274327667	2nd Nov 21, Maezie Belle (Fishing vessel – caught offshore Skinningrove)	HP

CR13	Brown crab	NZ7274327667	2nd Nov 21, Maezie Belle (Fishing vessel – caught offshore Skinningrove)	Gill
CR14	Shore crab	NZ5184431906	25th Oct 21, Seaton Carew	Gill
CR15	Shore crab	NZ8112416045	8th Oct 21, Runswick	HP
CR16	Shore crab	NZ8112416045	8th Oct 21, Runswick	HP
CR17	Shore crab	NZ5184431906	25th Oct 21, Seaton Carew	HP
CR18	Shore crab	NZ5184431906	25th Oct 21, Seaton Carew	HP
CR19	Shore crab	NZ8112416045	8th Oct 21, Runswick	Gill
CR20	Shore crab	NZ6621321946	25th Oct 21, Saltburn	HP
CR21	Shore crab	NZ6621321946	25th Oct 21, Saltburn	HP
CR22	Shore crab	NZ554987042	4th Oct 21, Bran Sands	HP
CR23	Shore crab	NZ8112416045	8th Oct 21, Runswick	Gill
CR24	Shore crab	NZ8112416045	8th Oct 21, Runswick	Gill
CR25	Shore crab	NZ554987042	4th Oct 21, Bran Sands	HP
CR26	Shore crab	NZ554987042	4th Oct 21, Bran Sands	HP
CR27	Shore crab	NZ1814431906	25th Oct 21, Seaton Carew	Gill
CR28	Brown crab	NZ7274327667	Maezie Belle (Fishing vessel – caught offshore Skinningrove)	Gill
CR29	Brown crab	PM42124	HP17	HP
CR30	Brown crab	PM42124	HP28 25th Nov Bridlington	HP
CR31	Brown crab	PM42124	HP7 25th Nov Bridlington	HP
CR32	Brown crab	PM42124	HP15 25th Nov Bridlington	HP
CR33	Brown crab	PM42124	HP16 25th Nov Bridlington	HP
CR34	Brown crab	PM42124	HP21 25th Nov Bridlington	HP
CR35	Brown crab	PM42124	HP19 25th Nov Bridlington	HP
CR36	Brown crab	PM42124	HP14 25th Nov Bridlington	HP
CR37	Lobster	PM42124	HP25 25th Nov Bridlington	HP
CR38	Lobster	PM42124	HP22 25th Nov Bridlington	HP
CR39	Brown crab	PM42124	HP20 25th Nov Bridlington	HP
CR40	Brown crab	PM42124	HP27 25th Nov Bridlington	HP
CR41	Brown crab	PM42124	HP26 25th Nov Bridlington	HP
CR42	Brown crab	PM42124	HP12 25th Nov Bridlington	HP
CR43	Brown crab	PM42124	HP2 25th Nov Bridlington	HP
CR44	Brown crab	PM42124	HP8 25th Nov Bridlington	HP
CR45	Brown crab	PM42124	HP4 25th Nov Bridlington	HP
CR46	Brown crab	PM42124	HP11 25th Nov Bridlington	HP
CR47	Lobster	PM42124	HP23 25th Nov Bridlington	HP
CR48	Brown crab	PM42124	HP5 25th Nov Bridlington	HP
CR49	Brown crab	PM42124	HP3 25th Nov Bridlington	HP
CR50	Brown crab	PM42124	HP1 25th Nov Bridlington	HP
CR51	Brown crab	PM42124	HP29 25th Nov Bridlington	HP
CR52	Brown crab	PM42124	HP18 25th Nov Bridlington	HP
CR53	Lobster	PM42124	HP24 25th Nov Bridlington	HP
CR54	Brown crab	PM42124	HP10 25th Nov, Bridlington	HP
CR55	Brown crab	PM42124	HP13 25th Nov, Bridlington	HP

CR56	Brown crab	PM42124	HP9 25th Nov, Bridlington	HP
CR57	Brown crab	PM42124	HP6 25th Nov, Bridlington	HP
CR58White	Brown crab		19th Jan 22, Sea off Saltburn	White meat
CR58Brown				Brown meat
CR59	*L. holsatus*		19th Jan 22 – S. Skinningrove Wick	HP
CR60	*L. holsatus*		19th Jan 22 – S. Skinningrove Wick	HP
CR61	*L. holsatus*		19th Jan 22 – S. Skinningrove Wick	HP
CR62	*L. holsatus*		19th Jan 22 – S. Skinningrove Wick	HP
CR63	*L. holsatus*		19th Jan 22 – S. Skinningrove Wick	HP
CR64	*L. holsatus*		18th Jan 22 – Off Seaton Carew	HP
Lobster 408	*H. gammarus*	Received whole	Received from NE IFCA	Claw
				HP
				Gonad
				Gut
				Tail
				Gills
Lobster 465	*H. gammarus*	Received whole	Received from NE IFCA	Claw
				HP
				Gonad
				Gut
				Tail
				Heart

Table 2. Summary of methods utilised for testing:

Analytes	Method	Status
Paralytic shellfish toxins (PSTs)	Acetic acid extraction, carbon de-salting SPE clean-up, dilution and LC-MS/MS analysis	Validated in shellfish and crab, but not accredited
Domoic acid (DA)	50% aqueous methanol (MeOH) extraction with LC-UV analysis	Developmental method, performance characteristics determined in crab, not accredited
Lipophilic toxins (LT) including Diarrhetic Shellfish Toxins (DST)	100% methanolic extraction followed by LC-MS/MS analysis	Validated in shellfish but not for non-bivalves
Brevetoxins and *Karenia* sp. algal metabolites	100% methanolic extraction followed by LC-MS/MS analysis	Developmental method. Brevetoxin method validated in bivalves only. Other targets not previously tested.

Results

Table 3 summarises the results obtained following each of the methods undertaken at the Cefas laboratory. The full results for each sample are listed in the associated Excel table "Crustacean toxin results summary – for sharing – 1st March 2022.xlsx".

Table 3. Overall summary of results for each class of toxins

Analytes	Findings	Comments
Paralytic shellfish toxins (saxitoxins) by LC-MS/MS	~25% of samples contained low levels of either STX or dcSTX, with maximum PST reaching 188 µg STXeq/kg (<25% maximum permitted limit (MPL) for bivalve mollusc food safety)	LODs range from 0.4 to 13 µg STX eq/kg per analogue for shellfish
Domoic acid by LC-UV	~19% of samples containing peaks indicative of domoic acid with maximum toxin concentration reaching 5 mg/kg (25% of MPL)	LOD estimated around 0.4 mg/kg
Diarrhetic shellfish poisoning (DSP) toxins by LC-MS/MS	~90% of samples containing DSP toxins (okadaic acid and dinophysistoxin 2 ; OA/DTX2), with maximum concentration of 179 µg/kg (above the MPL of 160 µg/kg)	LOD estimated around 1-5 µg/kg
Other regulated lipophilic toxins by LC-MS/MS	~32% samples containing AZA1 above LOD, maximum concentration 35 µg/kg (22% of the MPL of 160 µg/kg). ~30% of samples containing low concentrations of yessotoxin (YTX), maximum concentration of 168 µg/kg (~4.5% of MPL) Trace levels of the spirolide SPX1	LOD estimated around 1-5 µg/kg
Emerging lipophilic toxins (brevetoxins and associated metabolites, cyclic imines)	No pinnatoxins, brevetoxins, brevetoxin metabolites or other associated toxin analogues detected	No validated method – screening test only

[a]STX, dcSTX, NEO, dcNEO, C1-4, GTX1-6, dcGTX1-4

In addition to the archived crab and lobster HP samples, two lobsters were shipped to Cefas Feb 2022, both of which were implicated with poor health (samples 408 and 465). Only trace levels of toxins were detected in these samples, but dissection of the whole animals showed the clear presence of the parasitic lobster louse *Nicothose astaci* in the gills, which is linked to respiratory issues within lobsters.

Implications and interpretation

- These results showed the presence of certain neurotoxins responsible for PSP and ASP syndromes in humans produced naturally by certain species of marine phytoplankton, but only at levels reaching a maximum of 25% of the maximum permitted limits for toxins in bivalve molluscs. With examples of crustaceans accumulating such toxins at much higher levels, with no apparent impact on health, there is no evidence here for PSP or ASP affecting the crab mortality findings.
- Analysis conducted for brevetoxins, toxin potentially produced by certain species of *Karenia* phytoplankton, showed no detectable levels of toxins. Therefore there was no evidence for these toxins impacting upon invertebrate health within the samples assessed in this study.
- Further analysis for the full range of lipophilic toxins showed the presence of DSP toxins (Okadaic acid and dinophysistoxin 2) in approximately 90% of samples tested in this batch. Two of the samples contained quantifiable concentrations above the MPL of 160 µg/kg, with the majority below this limit, so may present a food safety risk to seafood consumers if the hepatopancreas tissue is consumed.
- Okadaic acid and Dinophysis toxins are inhibitors of protein phosphatases 1 and 2A, resulting in rapid increase of phosphorylated proteins in cells and a diarrhetic illness in human consumers. Whilst there are some publications reporting neurotoxic effects of okadaic acid (e.g. Valdiglesias *et al*., 2013), to our knowledge there is no evidence for these toxins to have been previously linked to invertebrate mortalities.

To note:

- These methods are targeted detection methods, i.e. they can only detect specific compounds which are incorporated into the method(s) and are available as certified reference standards. We are not able to conduct non-targeted screening assays – other organisations should be consulted if this approach is required.
- As discussed previously, these methods are validated only in the matrix of bivalve mollusc shellfish (various species), and crab (for PSP toxins only). As such, we have no evidence for toxin recovery and method performance for the analysis of domoic acid in these samples. Consequently, there is the potential for under or over-estimating toxin concentrations, without any such validation of method performance characteristics.

Conclusions

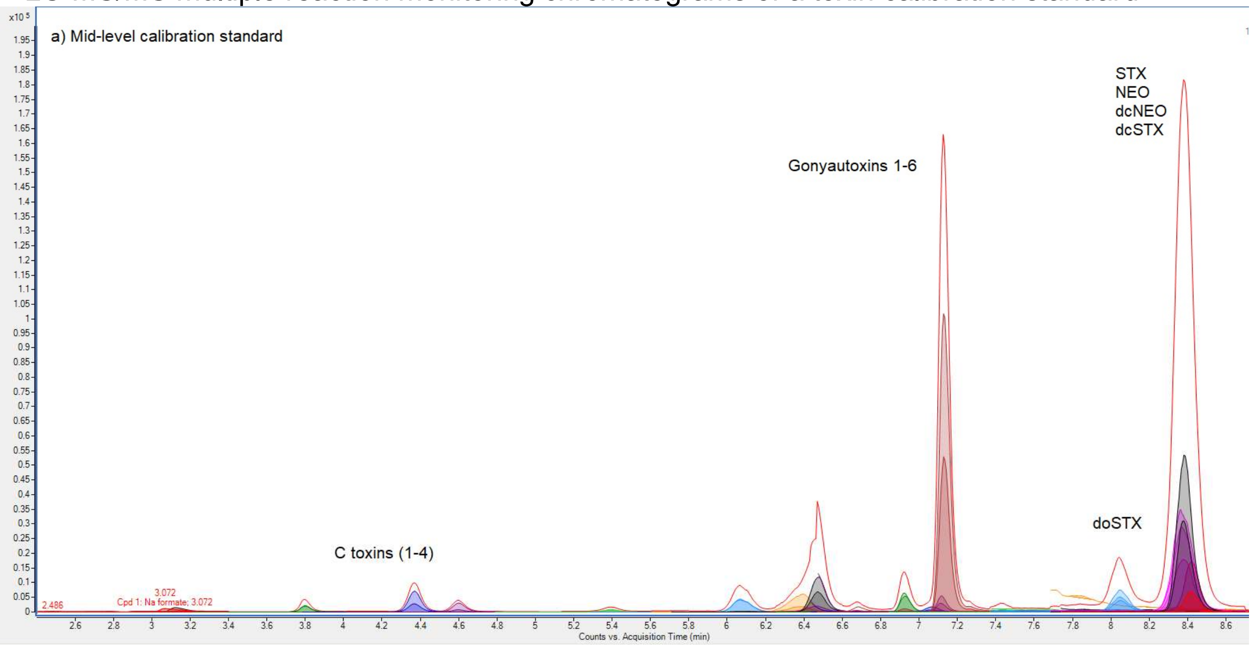
Overall there were no clear indications of marine neurotoxins being present in the samples received at levels which would cause concern. Concentrations of DSP toxins may be present at levels high enough to cause a food safety concern for human consumers, however tests were only conducted in the hepatopancreas for the majority of samples received, and not in the whole crab. Furthermore the potential role of the mode of action of OA/DTX2 in combination with other impacts is unclear.

Annex – methods used for analysis

1. PSTs (saxitoxins) by LC-MS/MS

Extraction of tissues using 1% acetic acid (in boiling water) as per validated method Turner et al., 2020. Centrifuged extracts subjected to desalting step and dilution in acetonitrile, prior to analysis by LC-MS/MS.

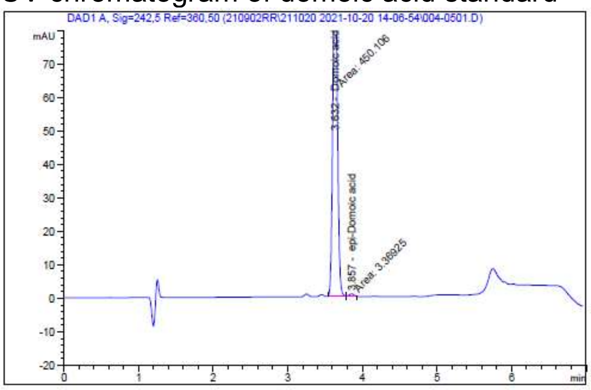
LC-MS/MS multiple reaction monitoring chromatograms of a toxin calibration standard



2. DA by LC-UV

Samples were extracted using 50% MeOH / 50% Water, using the approach described above. After extraction, centrifuged supernatants were filtered (0.2 µm) and subjected to LC with UV detection (LC-UV) without SPE as conducted for routine monitoring of bivalves.

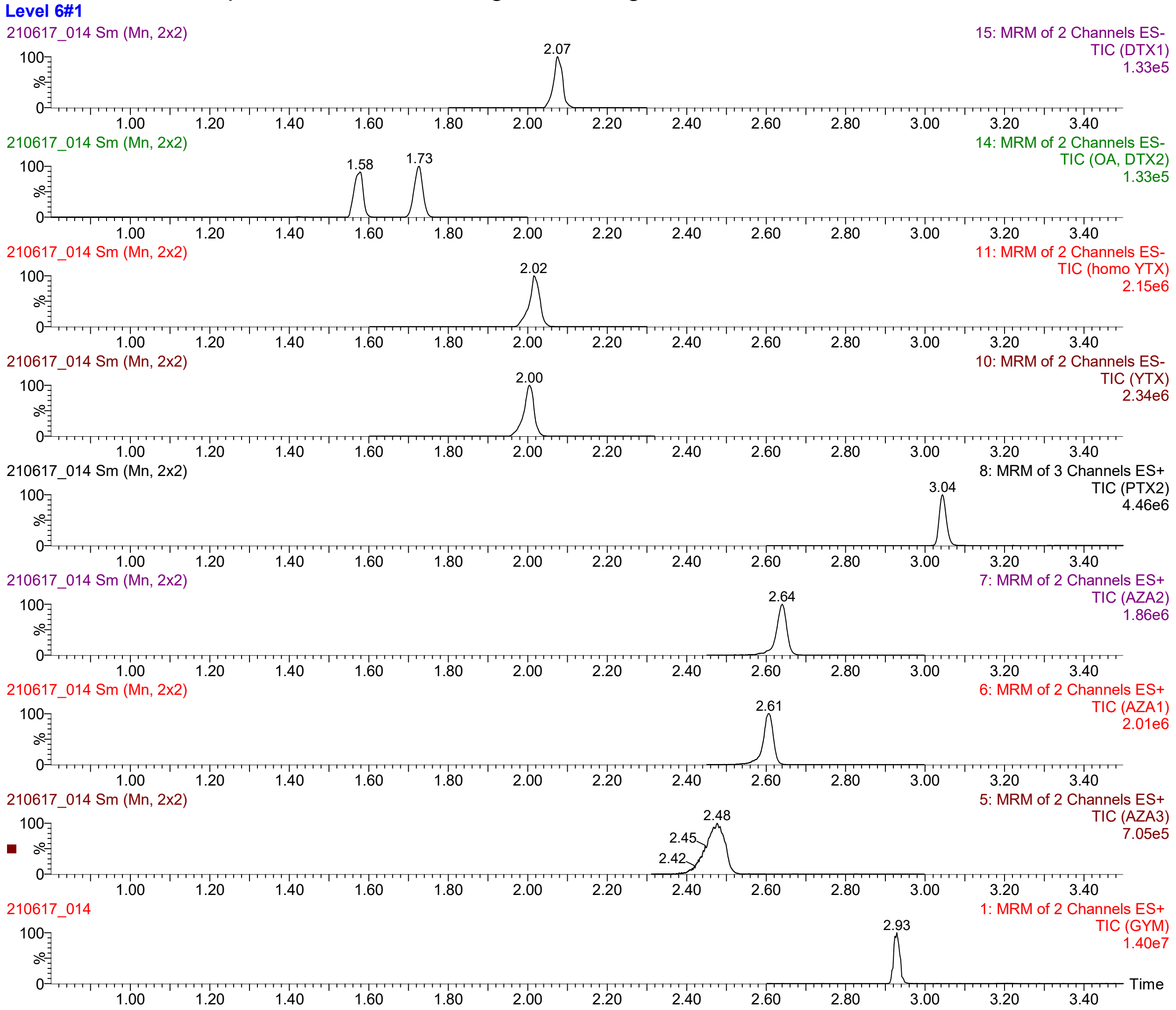
UV chromatogram of domoic acid standard



3. Lipophilic toxins by LC-MS/MS

LC-MS/MS following EU reference laboratory reference method. Extraction of tissues using 100% methanol prior to filtration and LC-MS/MS analysis. An additional aliquot subjected to alkaline hydrolysis to liberate acyl esters of OA-group toxins prior to additional LC-MS/MS analysis.

LC-MS/MS multiple reaction monitoring chromatograms of toxin standards



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[1] Turner, A.D., Dhanji-Rapkova, M., Fong, S.Y.T., Hungerford, J., McNabb, P.S., Boundy, M.J. and Harwood, D.T. (2020). Ultrahigh-performance hydrophilic interaction liquid chromatography with tandem mass spectrometry method for the determination of paralytic shellfish toxins and tetrodotoxin in mussels, oysters, clams, cockles and scallops: collaborative study. *J. AOAC International.* 103, 1-35

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