DSP toxins Analysis

Rapid detection of Okadaic Acid-toxins

B-T Eberhart and Penelope Xian

NOAA Fisheries, NWSFC

Marine Microbes & Toxins Program

Marine Biotoxins Group

DSP Toxins Extraction Method
EU Harmonised SOP

1. 2.5 +/- 0.1 g homogenate
2. 1st extraction with 11 mL MeOH, vortex 1 min
3. Centrifuge 20 min (3,400 rpm)
4. 2nd extraction with 11mL MeOH, homogenize 1 min
5. Centrifuge 20 min (3,400 rpm)
6. Combine supernatants and adjusted to final volume 25 mL

DSP Toxins Extraction Method
Hydrolysis

- Add 125 μL NaOH (2.5M) per 1 mL extract, vortex 30 sec
- Heat in water bath @ 76°C ± 2°C for 40 min
- Adjust weight loss if needed
- Add 125 μL HCl (2.5M) per 1 mL extract, vortex 30 sec
- Filter with 0.2 μm syringe filter (PTFE)
- Perform analyses (LC-MS/MS, PP2A)

OkaTest Kits
Protein Phosphatase 2A

- 96-well plate microplate
- OA-toxins group inhibits phosphatase activity
- PP2A, purified from human red blood cells
- Chromogenic substrate, p-nitrophenyl phosphate
- Resulted color product p-nitrophenyl measured @ 405 nm
- Conc. interpolated from a 5-point OA Standard Curve (0.5-2.8nM)

OkaTest Kit Performance (ZEU)

Parameter	Results
Limit of detection (LOD) n=8. Matrix: mussel	4.4 μg OA equivalents/100 g (blank sample + 3SD)
Limit of Quantification (LOQ) n=8. Matrix: mussel	5.6 ug OA equivalents/100 g (blank mussel + 10SD)
Calibration curve	0.5 nM – 2.8 nM
Working range (5x Extraction)	6.3 to 35.2 μg OA equivalents/100 g
Working range (10x Extraction)	**12.6 to 70.4 μg OA equivalents/100 g**

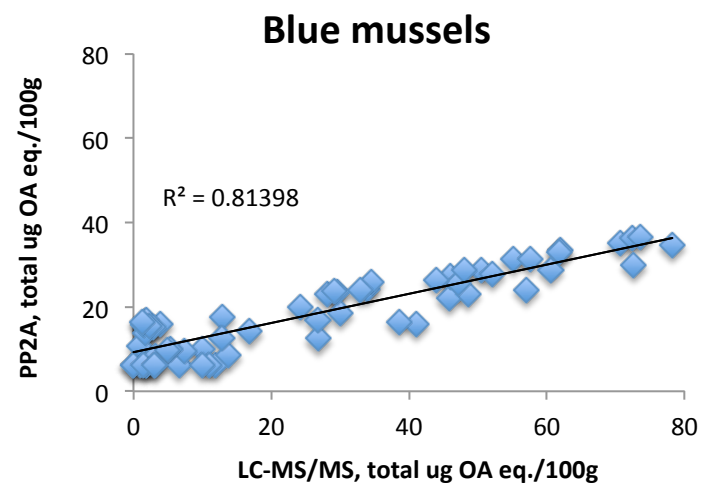
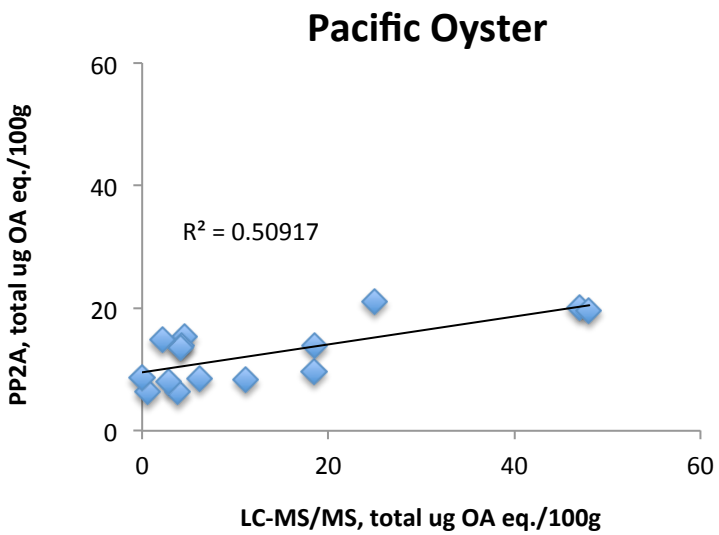
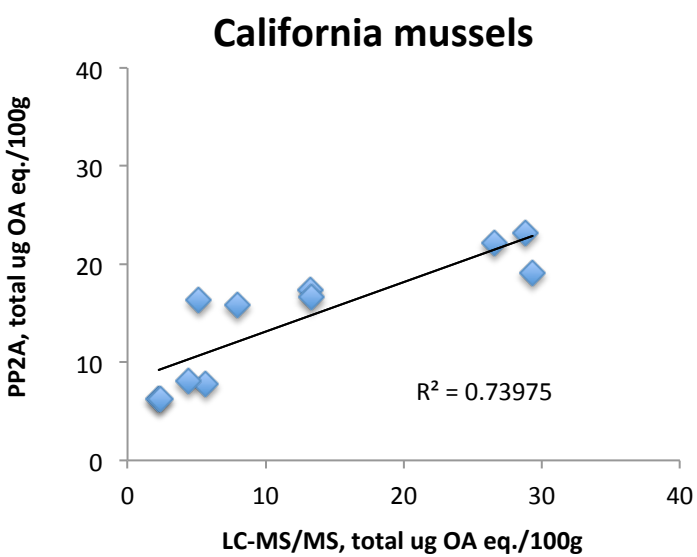
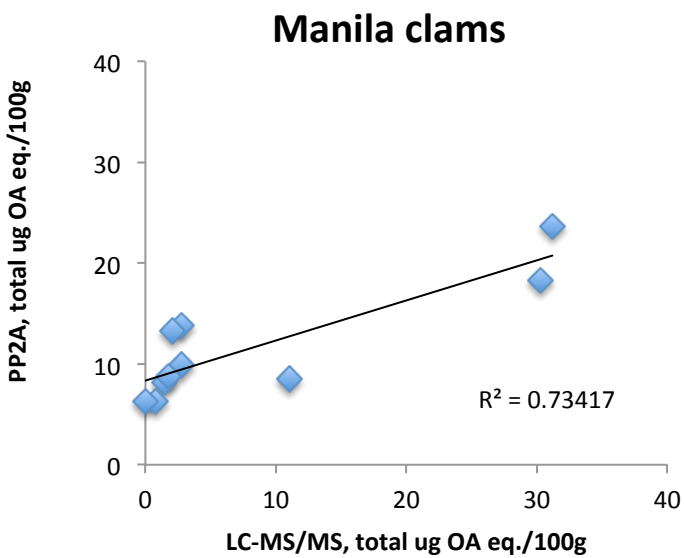
LC-MS/MS vs. PP2A

- Data from set 2

		Expected	Measured Concentration			
		Concentration	LC-MS/MS	PP2A	% Recovery	% Recovery
Sample Nam	Sample Description	spiked(DSTs)	sum (DSTs)	Total DSTs	LC-MS/MS	PP2A
		µg/100 g	µg/100 g	µg/100 g		
138-07	blank mussel	NA	3.85	<LOQ		
138-08	blank mussel	NA	3.88	<LOQ		
138-09	blank mussel	NA	3.97	<LOQ		
138-10	blank mussel - spiked	15.13	17.47	12.95	115	86
138-11	blank mussel - spiked	15.08	20.26	14.93	134	99
138-12	blank mussel - spiked	14.95	19.30	18.28	129	122
138-7PS	blank mussel - post spiked	20.40	26.52	NA	130	
138-8PS	blank mussel - post spiked	20.89	28.48	NA	136	
138-9PS	blank mussel - post spiked	20.92	27.48	NA	131	
138-13PS	solvent blank - post spiked	20.74	18.55	NA		
138-13	solvent blank	NA	NA	<LOD		
138-14	spiked solvent blank	15.05	14.56	17.62	97	121

LC-MS/MS vs. PP2A

- Data from set 3

	Expected Total DSTs	Measured Total DSTs	**LC-MS/MS**		Measured Total DSTs	**PP2A**
	ug OA equiv/100g	ug OA equiv/100g	**%Recovery**	Results, (nM)	ug OA equiv/100g	% recovery
solvent blank	NA	<LOQ		0.376	<LOQ	
solvent blank	NA	<LOQ		0.404	<LOQ	
Zero Mussel CRM	NA	<LOQ		0.308	<LOQ	
Spiked Zero Mussel CRM	15.1	20.19	134	0.668	16.70	111
Zero Mussel CRM	NA	<LOQ		0.39	<LOQ	
Spiked Zero Mussel CRM	15.2	20.01	132	0.707	17.76	117
WDOH low mussel	NA	4.54		0.537	13.17	
WDOH low mussel	NA	4.61		0.522	13.12	
Sequim Bay mussel 8/12/14	36.00	82.05	228	1.531	38.24	106
Sequim Bay mussel 8/19/14	68.00	96.41	142	1.761	43.55	64
Spiked WDOH low mussel	15.0	20.78	138	0.927	23.02	153
Spiked WDOH low mussel	14.9	19.58	131	0.84	20.72	139

DSTs in field samples