

## † Electronic Supporting Information (ESI)

### Multiple charged ions profile in the UHPLC-HRMS analysis of palytoxin analogues from *Ostreopsis cf. ovata* blooms

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**Table S1** Summary of the instrumental systems, electrospray ionization sources and quantification methods used in the comparative of the analytical quantification of the PLTX analogues.

<b>System</b>	<b>System I: Q-Exactive Orbitrap</b>				<b>System II: LTQ-Orbitrap</b>	
<b>Electrospray Ionization Sources</b>	<b>Method 1:</b> HESI-II at 350 °C		<b>Method 2:</b> HESI-II at 25 °C		<b>Method 3:</b> ESI source at 25 °C	
<b>Quantification method</b>	<b>QM-A:</b> the 2 most intense ions	<b>QM-B:</b> group of 13 ions selected	<b>QM-A:</b> the 2 most intense ions	<b>QM-B:</b> group of 13 ions selected	<b>QM-A:</b> the 2 most intense ions	<b>QM-B:</b> group of 13 ions selected

**Table S2** Molecular formulae of PLTX analogues, the ion exact mass ( $m/z$ ) and the monoisotopic mass of the most intense isotope ( $m/z$ ) selected for quantitative analysis by UHPLC-HRMS.

		Iso-PLTX	OVTX-a	OVTX-b	OVTX-c	OVTX-d	OVTX-e	OVTX-f	OVTX-g
Formula		C <sub>129</sub> H <sub>223</sub> N <sub>3</sub> O <sub>54</sub>	C <sub>129</sub> H <sub>223</sub> N <sub>3</sub> O <sub>52</sub>	C <sub>131</sub> H <sub>227</sub> N <sub>3</sub> O <sub>53</sub>	C <sub>131</sub> H <sub>227</sub> N <sub>3</sub> O <sub>54</sub>	C <sub>129</sub> H <sub>223</sub> N <sub>3</sub> O <sub>53</sub>	C <sub>129</sub> H <sub>223</sub> N <sub>3</sub> O <sub>53</sub>	C <sub>131</sub> H <sub>227</sub> N <sub>3</sub> O <sub>52</sub>	C <sub>129</sub> H <sub>223</sub> N <sub>3</sub> O <sub>51</sub>
[M+3H-4H <sub>2</sub> O] <sup>3+</sup>	Exact mass	869.8197	859.1564	873.8318	879.1635	864.4881	864.4881	868.5002	853.8248
	Selected ion*	870.1542	859.4909	874.1663	897.4979	864.8225	864.8225	868.8347	854.1593
[M+3H-3H <sub>2</sub> O] <sup>3+</sup>	Exact mass	875.8232	865.1600	879.8354	885.1670	870.4916	870.4916	874.5037	859.8283
	Selected ion*	876.1577	865.4944	880.1698	885.5015	870.8261	870.8261	874.8382	860.1628
[M+3H-2H <sub>2</sub> O] <sup>3+</sup>	Exact mass	881.8268	871.1635	885.8389	891.1705	876.4951	876.4951	880.5073	865.8318
	Selected ion*	882.1612	871.4979	886.1733	891.5050	876.8296	876.8296	880.8417	866.1663
[M+3H-H <sub>2</sub> O] <sup>3+</sup>	Exact mass	887.8303	877.1670	891.8424	897.1741	882.4986	882.4986	886.5108	871.8354
	Selected ion*	888.1647	877.5015	892.1769	897.5085	882.8331	882.8331	886.8452	872.1698
[M+H+Mg] <sup>3+</sup>	Exact mass	901.1569	890.4937	905.1691	910.5007	895.8253	895.8253	899.8374	885.1620
	Selected ion*	901.4914	890.8281	905.5036	910.8352	896.1598	896.1598	900.1719	885.4965
[M+H+Ca] <sup>3+</sup>	Exact mass	906.4828	895.8195	910.4949	915.8266	901.1511	901.1511	905.1633	890.4879
	Selected ion*	906.8172	896.1540	910.8294	916.1610	901.4856	901.4856	905.4977	890.8223
[M+H+Fe] <sup>3+</sup>	Exact mass	911.1418	900.4785	915.1539	920.4856	905.8102	905.8102	909.8223	895.1469
	Selected ion*	912.1414	901.4781	916.1535	921.4851	906.8097	906.8097	910.8219	896.1464

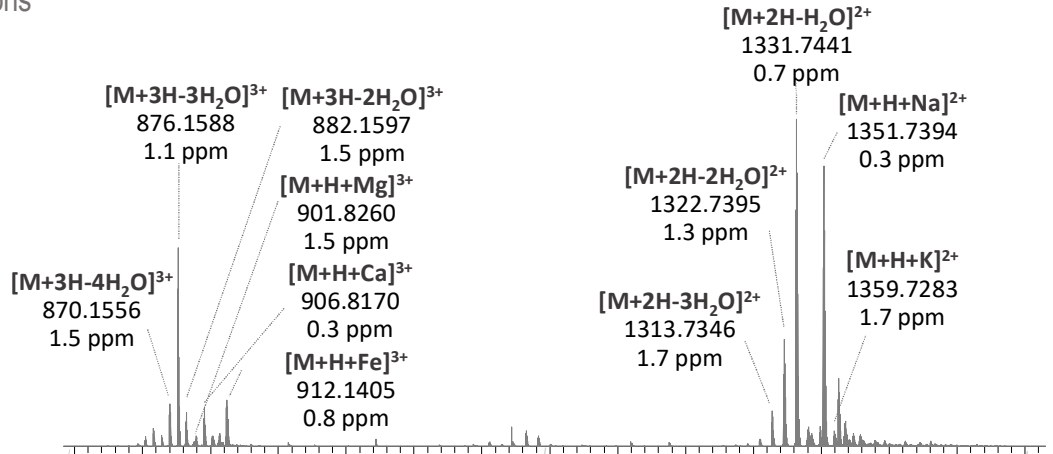
<b>[M+2H-3H<sub>2</sub>O]<sup>2+</sup></b>	<b>Exact mass</b>	1313.2312	1297.2363	1319.2494	1327.2469	1305.2338	1305.2338	1311.2520	1289.2389
	<b>Selected ion*</b>	1313.7329	1297.7380	1319.7511	1327.7486	1305.7355	1305.7355	1311.7536	1289.7405
<b>[M+2H-2H<sub>2</sub>O]<sup>2+</sup></b>	<b>Exact mass</b>	1322.2365	1306.2416	1328.2547	1336.2522	1314.2390	1314.2390	1320.2572	1298.2441
	<b>Selected ion*</b>	1322.7382	1306.7433	1328.7564	1336.7538	1314.7407	1314.7407	1320.7589	1298.7458
<b>[M+2H-H<sub>2</sub>O]<sup>2+</sup></b>	<b>Exact mass</b>	1331.2418	1315.2469	1337.2600	1345.2574	1323.2443	1323.2443	1329.2625	1307.2494
	<b>Selected ion*</b>	1331.7435	1315.7486	1337.7617	1345.7591	1323.7460	1323.7460	1329.7642	1307.7511
<b>[M+2H]<sup>2+</sup></b>	<b>Exact mass</b>	1340.2471	1324.2522	1346.2653	1354.2627	1332.2496	1332.2496	1338.2678	1316.2547
	<b>Selected ion*</b>	1340.7488	1324.7538	1346.7669	1354.7644	1332.7513	1332.7513	1338.7695	1316.7564
<b>[M+H+Na]<sup>2+</sup></b>	<b>Exact mass</b>	1351.2380	1335.2431	1357.2562	1365.2537	1343.2406	1343.2406	1349.2588	1327.2457
	<b>Selected ion*</b>	1351.7397	1335.7448	1357.7579	1365.7554	1343.7423	1343.7423	1349.7605	1327.7474
<b>[M+H+K]<sup>2+</sup></b>	<b>Exact mass</b>	1359.2250	1343.2301	1365.2432	1373.2407	1351.2276	1351.2276	1357.2457	1335.2326
	<b>Selected ion*</b>	1359.7267	1343.7318	1365.7449	1373.7423	1351.7292	1351.7292	1357.7474	1335.7343

\*Selected ion: monoisotopic mass of the most intense isotope selected for quantitative analysis.

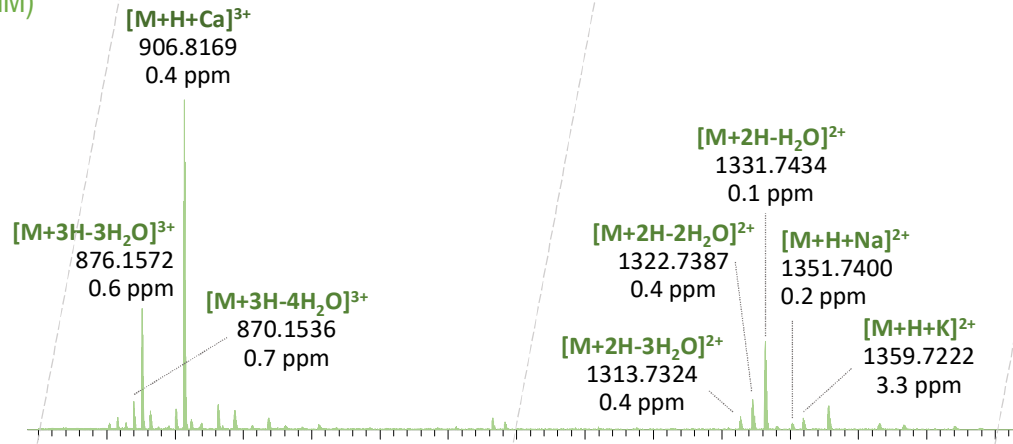
**Table S3** Statistic *p*-values of the paired t-tests to explore the significant differences in the total toxin concentrations as a function of the quantification methods (QM-A or QM-B) and instrumental methods (Method 1, Method 2, or Method 3).

		<i>P</i> -value (one-tail)	<i>P</i> -value (two-tails)
<b>QM-A</b>	<b>Method 1 vs. 2</b>	1.44E-05	2.89E-05
	<b>Method 1 vs. 3</b>	7.47E-06	1.49E-05
	<b>Method 2 vs. 3</b>	7.88E-05	1.58E-04
<b>QM-B</b>	<b>Method 1 vs. 2</b>	1.83E-01	3.67E-01
	<b>Method 1 vs. 3</b>	3.43E-06	6.86E-06
	<b>Method 2 vs. 3</b>	2.86E-05	5.72E-05
<b>QM-A vs. QM-B</b>	<b>Method 1</b>	4.61E-06	9.23E-06
	<b>Method 2</b>	1.08E-04	2.16E-04
	<b>Method 3</b>	3.89E-04	7.78E-04

a) Control conditions



b)  $CaCl_2$  (1 mM)

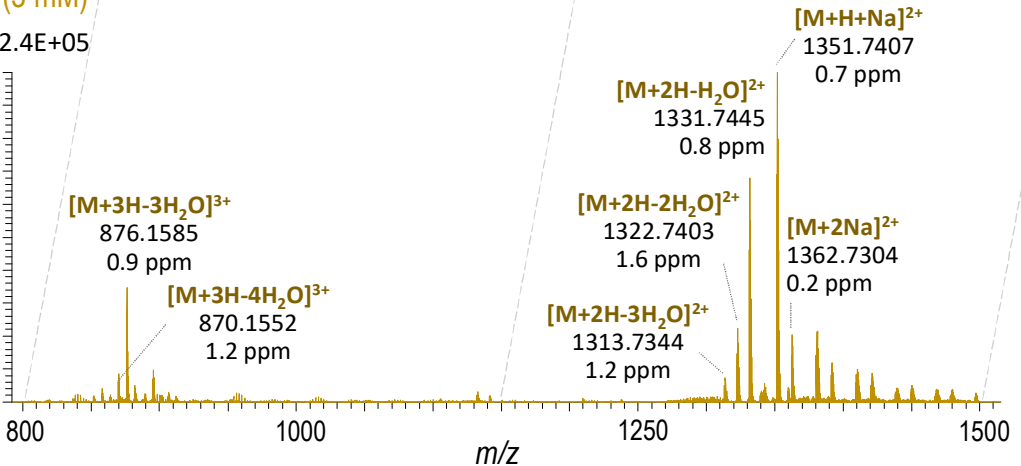


c)  $NaCl$  (3 mM)

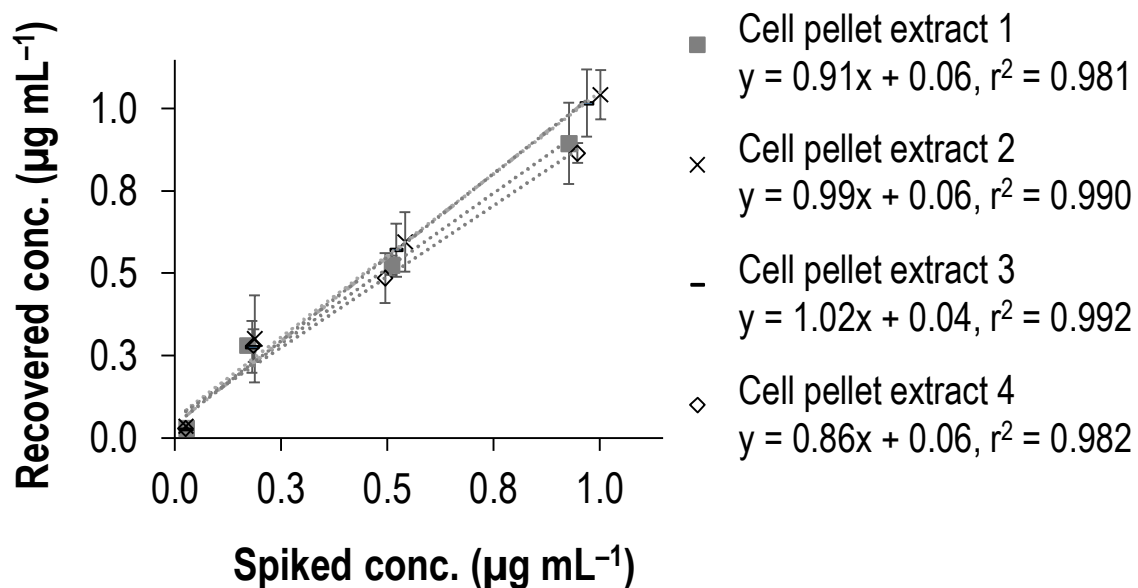
NL: 2.4E+05

Relative Abundance (%)

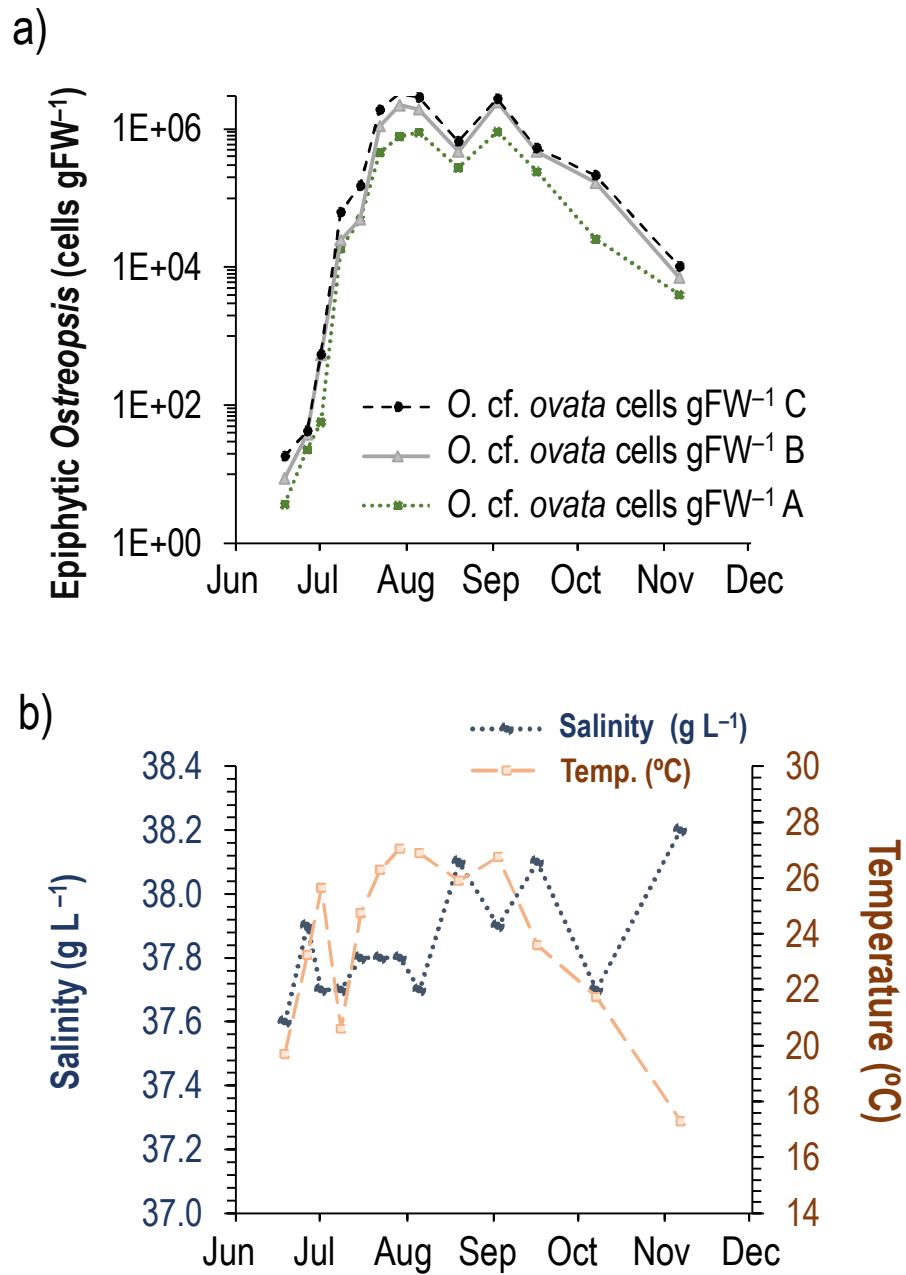
100  
50  
0



**Fig. S1** HRMS spectra obtained from the UHPLC-HRMS analysis of PLTX standard ( $1 \mu\text{g mL}^{-1}$ ) using the post-column addition of (a) control conditions (b)  $CaCl_2$  (1 mM) and (c)  $NaCl$  (3 mM).



**Fig. S2** Calibrations curves for PLTX standard spiked in cell pellets (matrix-matched standards). The vertical bars indicate the standard deviation for three replicate injections.



**Fig. S3** (a) *Ostreopsis cf. ovata* epiphytic abundance during the 2019 proliferation; (b) salinity and temperature measured during the *O. cf. ovata* proliferation.