Analytical approaches used to discover toxins produced by *Ostreopsis* species

CAWTHRON

26 September 2023 – ICMSS Portugal

Cawthron Institute

- Based in Nelson, New Zealand
- Pioneering science for >100 years
- Largest independent research organisation in New Zealand
- Close connection with fisheries and aquaculture industries
- Research, consultancy, commercial testing laboratories

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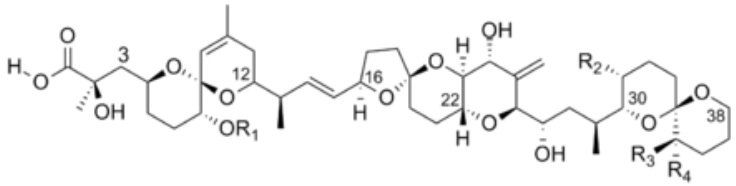
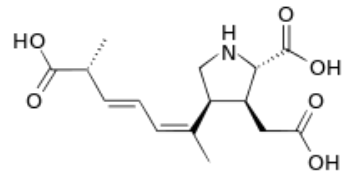
World-class science for a better future.

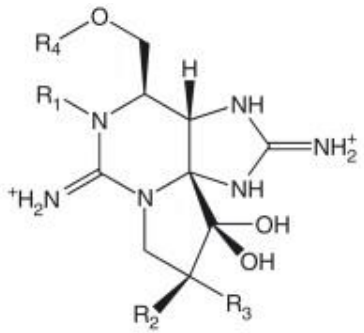
- Healthy eco-systems
- Prosperous blue economy
- Thriving people and communities

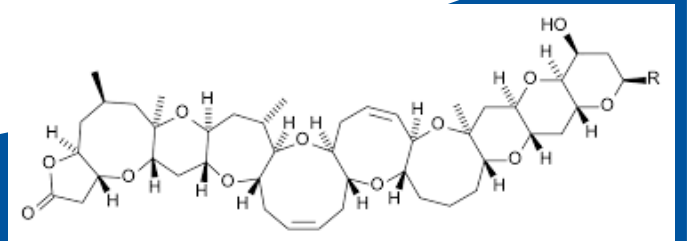
Regulated marine toxins in New Zealand

- Shellfish industry impacted by HABs and regulated marine toxins
- DSP/PSP - most problematic with regular closures of growing areas
- ASP - rarely an issue and only in scallops
- NSP - only one recorded event in the early 1990s
- AZP - no issues yet but occasional very low level detects



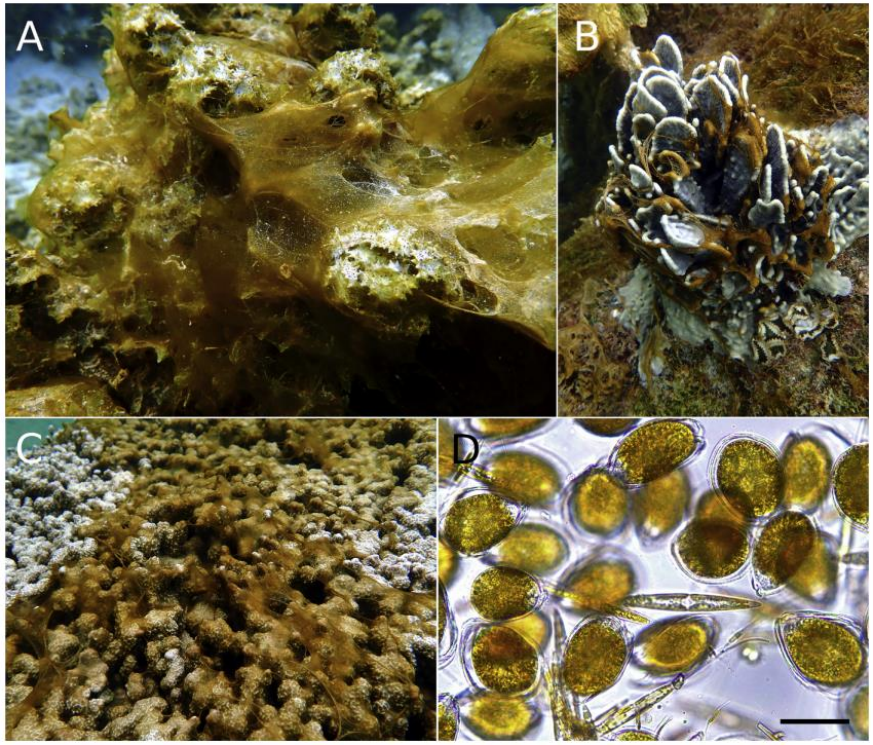




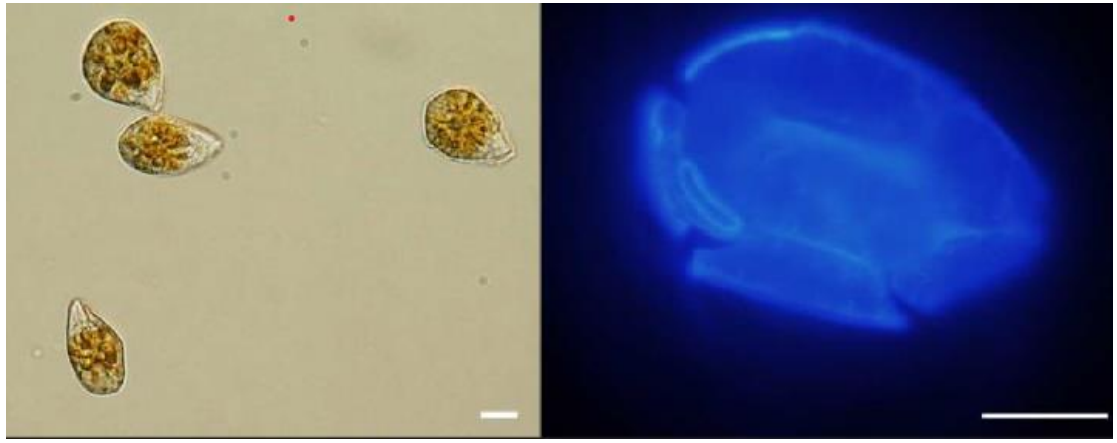


Emerging marine toxins

- Ciguatoxins - CP prevalent in the Sth Pacific with *Gambierdiscus* sp. observed in Kermadec Is
- Tetrodotoxin - detected in NZ shellfish
- Brevisulcenals - novel HAB species *Karenia brevisulcata*
- Ovatoxins? - *Ostreopsis sp.* blooms in New Zealand



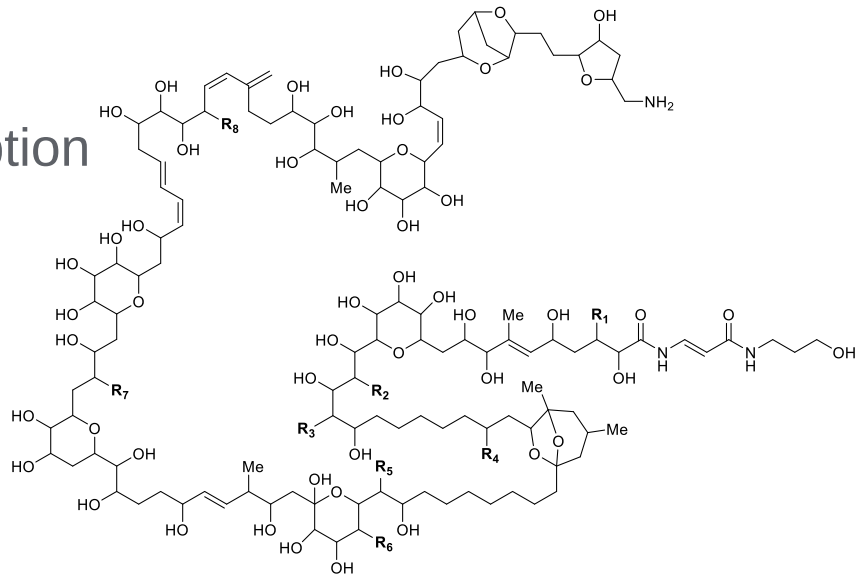
Ostreopsis ovata



- *Ostreopsis ovata* observed in Mediterranean
- Produces suite of large marine toxins known as ovatoxins/ostreocins
- Blooms associated with human respiratory distress and dermal effects
- Human illness also noted from consumption of seafood containing palytoxin
- EFSA may add palytoxin to the list of regulated biotoxins, despite oral toxicity<<IP

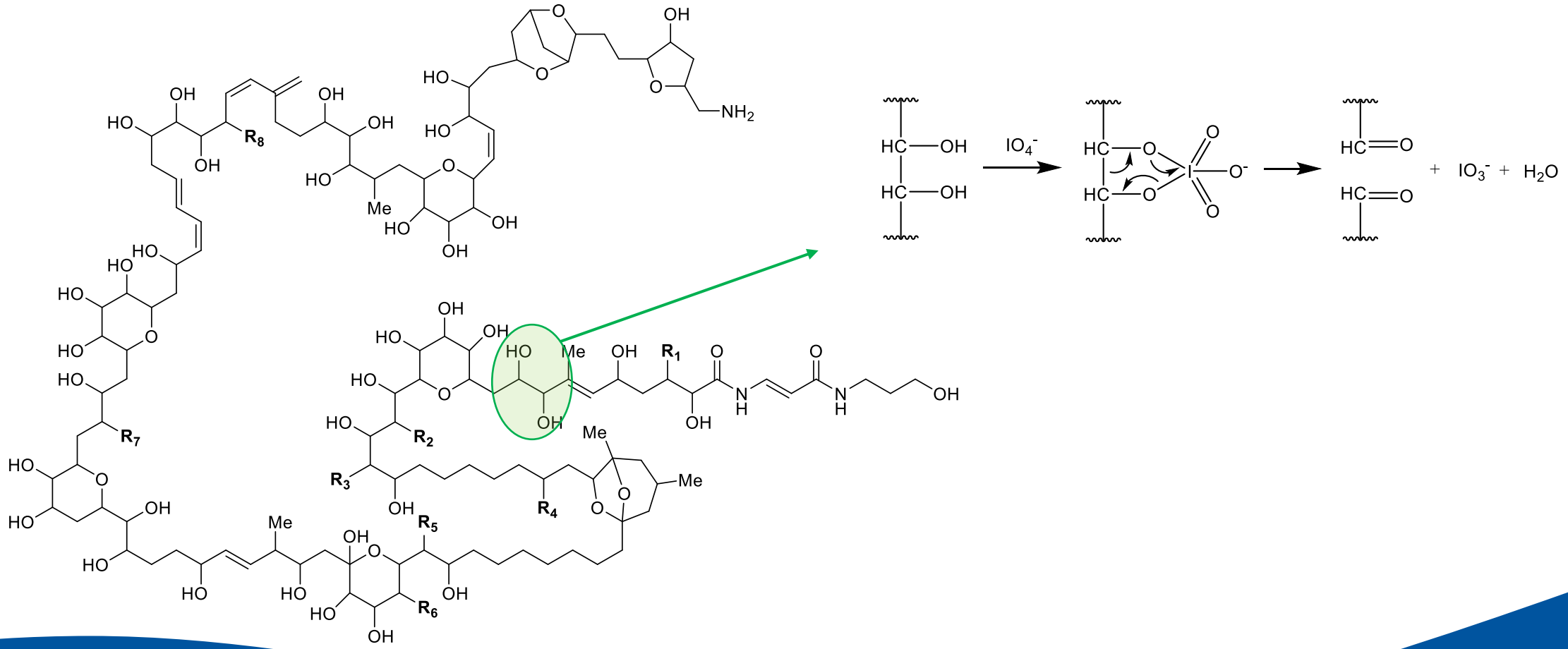
Methods of analysis

	R_1	R_2	R_3	R_4	R_5	R_6	R_7
PLTX	Me	OH	OH	Me	H	OH	OH
OVTX-a	Me	H	OH	Me	OH	H	H
OST-D	H	OH	H	H	OH	H	OH



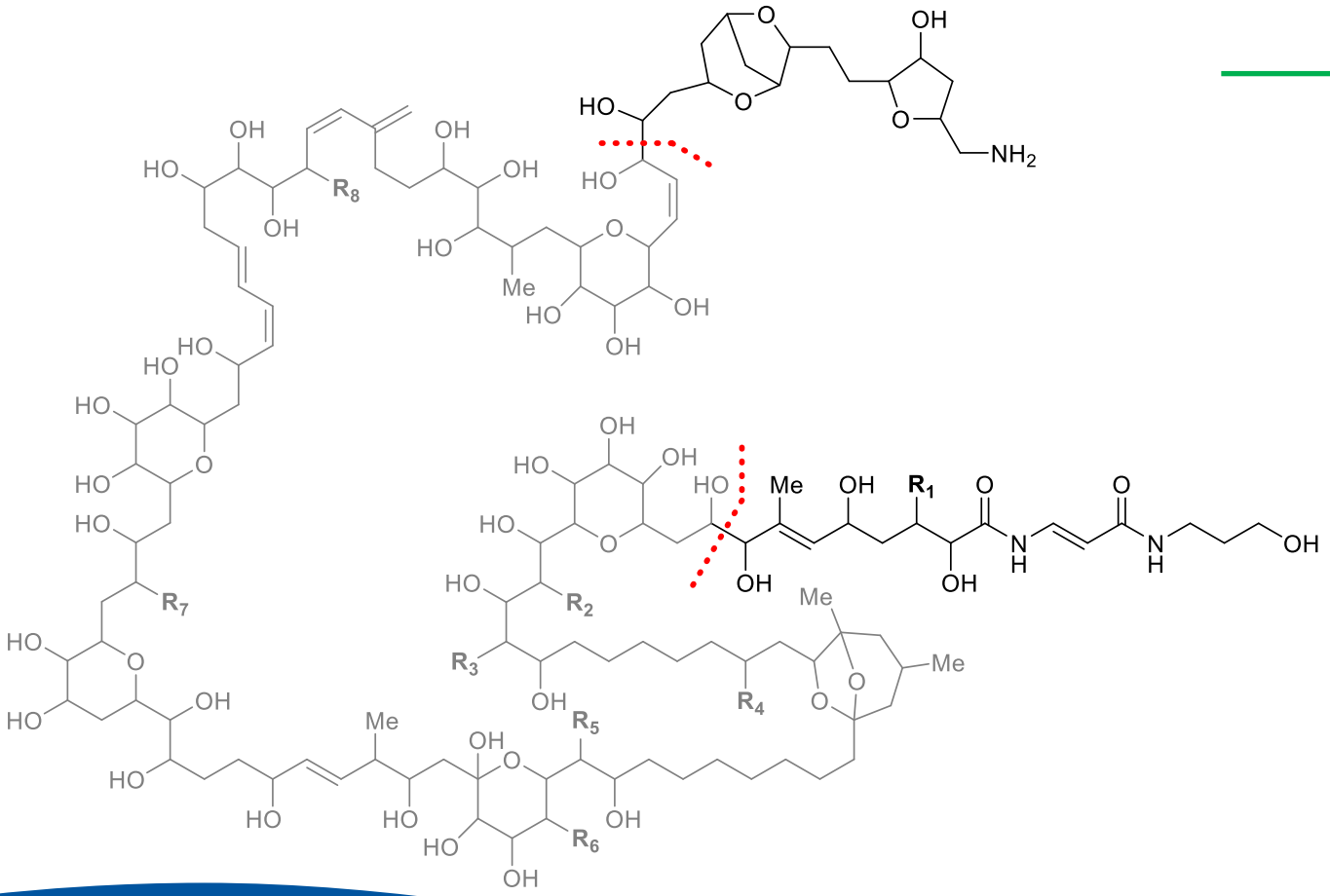
- No UV chromophore and does not fluoresce → LC-MS best option
- LC-MS challenging:
  - large molecules with multiple charge states
  - mixed cationic species (H^+, Na^+, K^+, NH_4^+)
  - large ^{13}C contributions
- Leads to ambiguities of identification with low and variable sensitivity and specificity
- We developed an oxidative cleavage method to break the molecule into smaller pieces

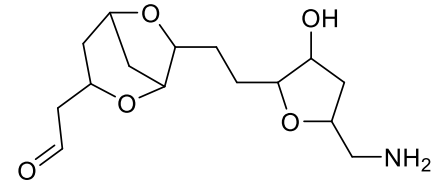
[O] cleavage method



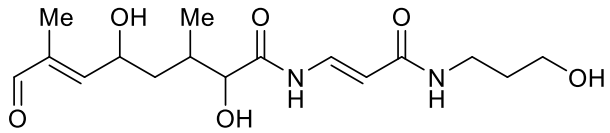
Selwood *et al*., 2012

[O] cleavage method

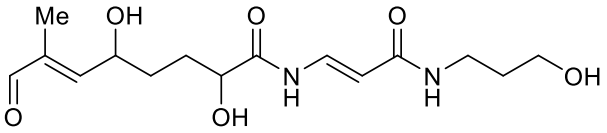




Common - PLTXs, OVTXs, OSTs

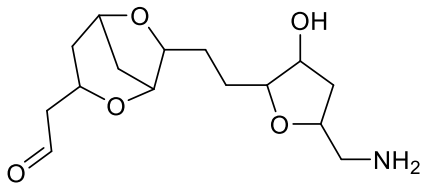


PLTXs, OVTXs



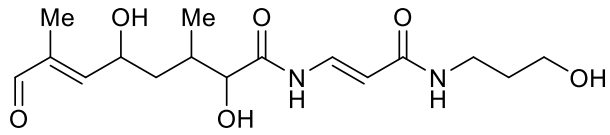
OSTs

Selwood *et al*., 2012

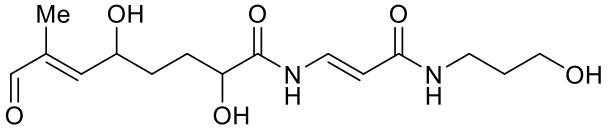


Common - PLTXs, OVTXs, OSTs

Acronym	Chemical formula	Amino [O] frag	Amide [O] frag
PLTX	C129H223N3O54	300	343
	C129H221N3O53	300	325
42-OHPLTX	C129H223N3O55	300	343
PLTX-b	C131H225N3O55	300	343
honoPLTX	C130H225N3O54	300	357
bishomoPLTX	C131H227N3O54	300	371
neoPLTX	C129H221N3O53	300	325
deoxyPLTX	C129H223N3O53	300	343
isobPLTX	C129H223N3O54	300	343
OVTX-a	C129H223N3O52	300	343.2
OVTX-b	C131H227N3O53	300	387.2
isoOVTX-b	C131H227N3O53	300	343.2
OVTX-c	C131H227N3O54	300	387.2
OVTX-d	C129H223N3O53	300	343.2
OVTX-e	C129H223N3O53	300	359.2
OVTX-f	C131H227N3O52	300	343.2
OVTX-g	C129H223N3O51	300	343.2
OVTX-h	C129H225N3O51	300	343.2
OVTX-i	C131H225N3O53	300	343.2
OVTX-j1	C131H225N3O54	300	343.2
OVTX-j2	C131H225N3O54	300	343.2
OVTX-k	C131H225N3O55	300	343.2
OVTX-a IK2	C129H223N3O52	300	343.2
OVTX-d IK2	C129H223N3O53	300	343.2
OVTX-e IK2	C129H223N3O53	300	359.2
OST-A	C127H219N3O54	300	329
OST-B	C127H219N3O54	300	329
OST-D	C127H219N3O53	300	329
OST-E1	C127H217N3O52	300	329



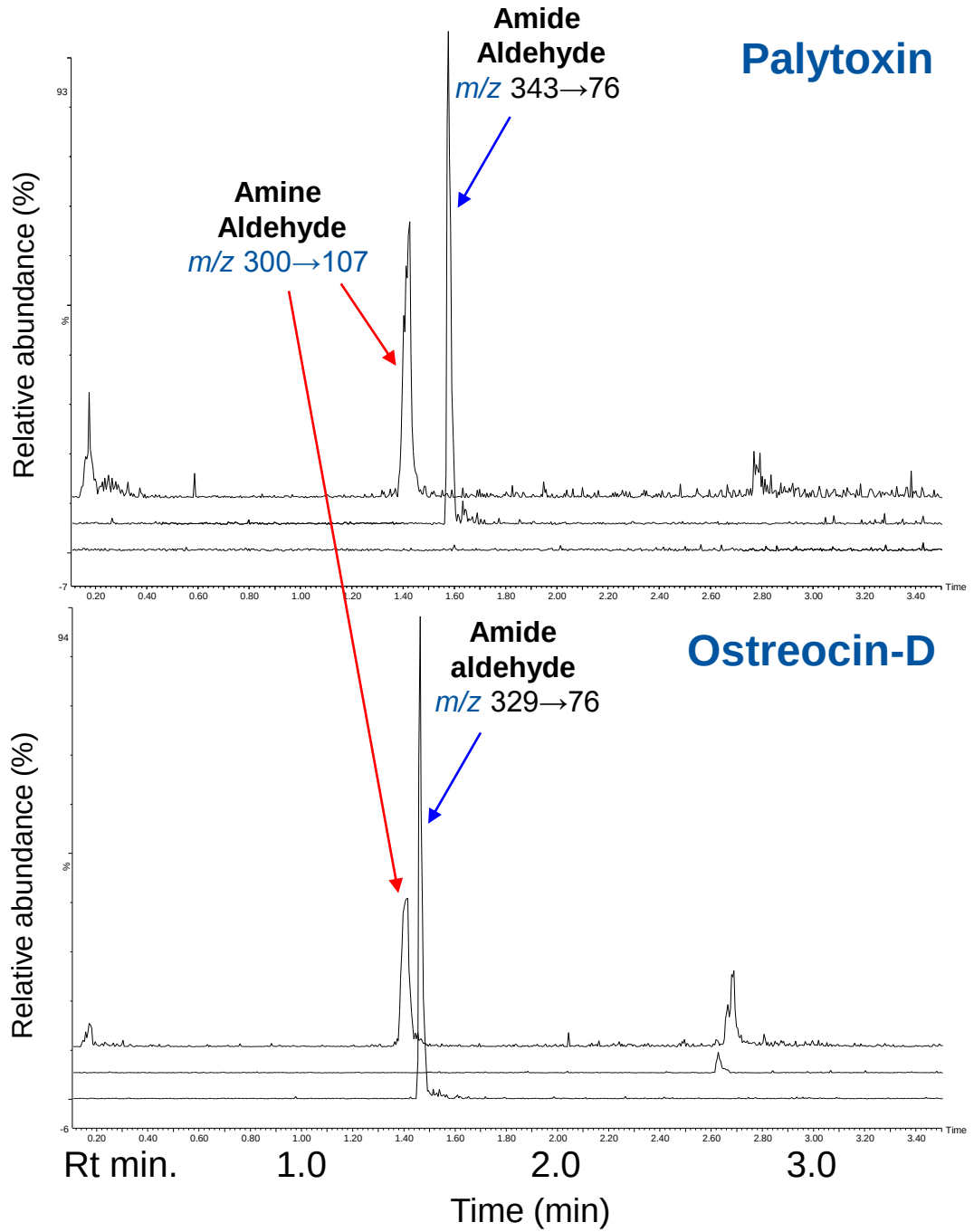
PLTXs, OVTXs



OSTs

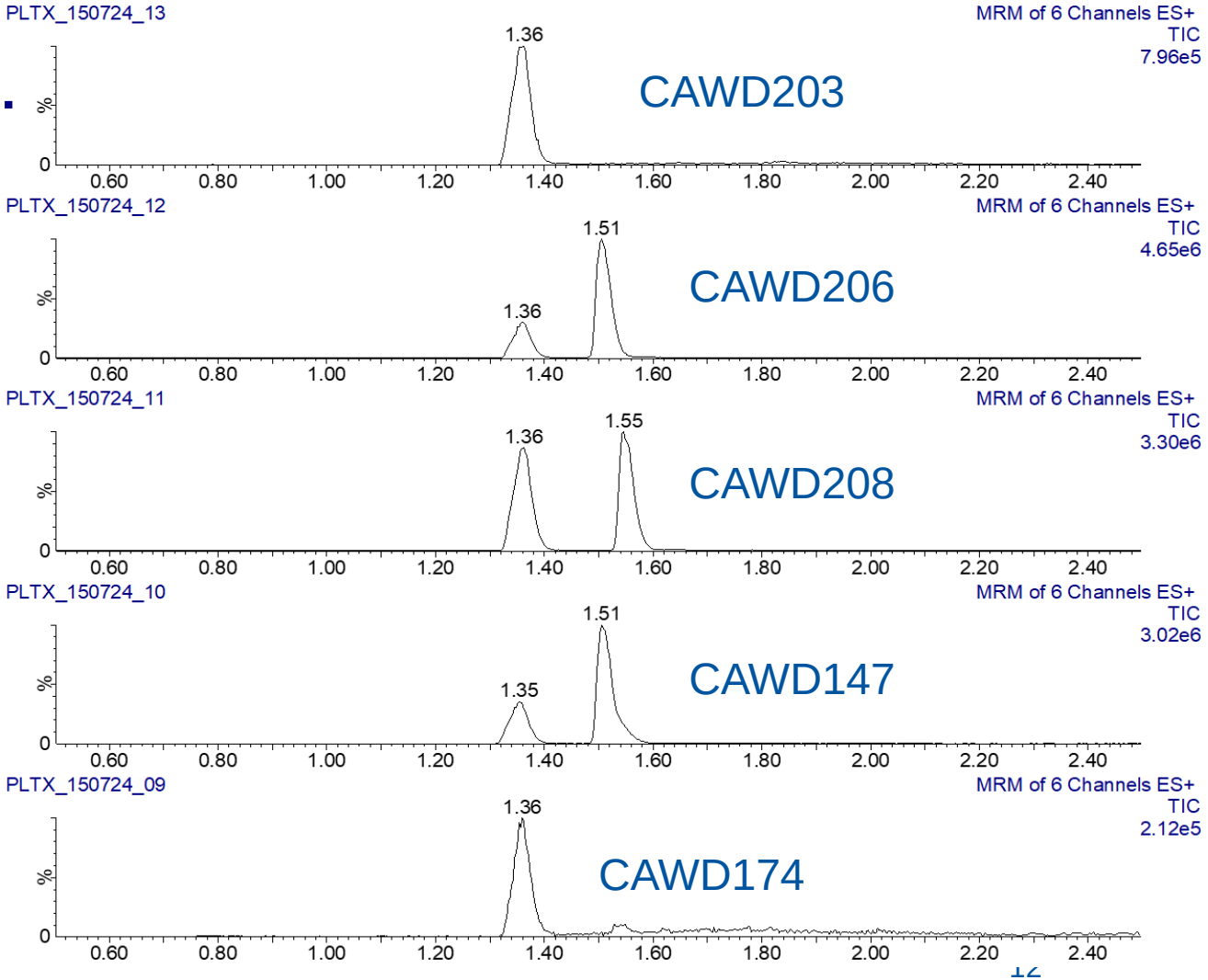
[O] cleavage method

- Sample preparation straight forward
- Common amine fragment clearly identified
- Amide fragment elutes at different RT



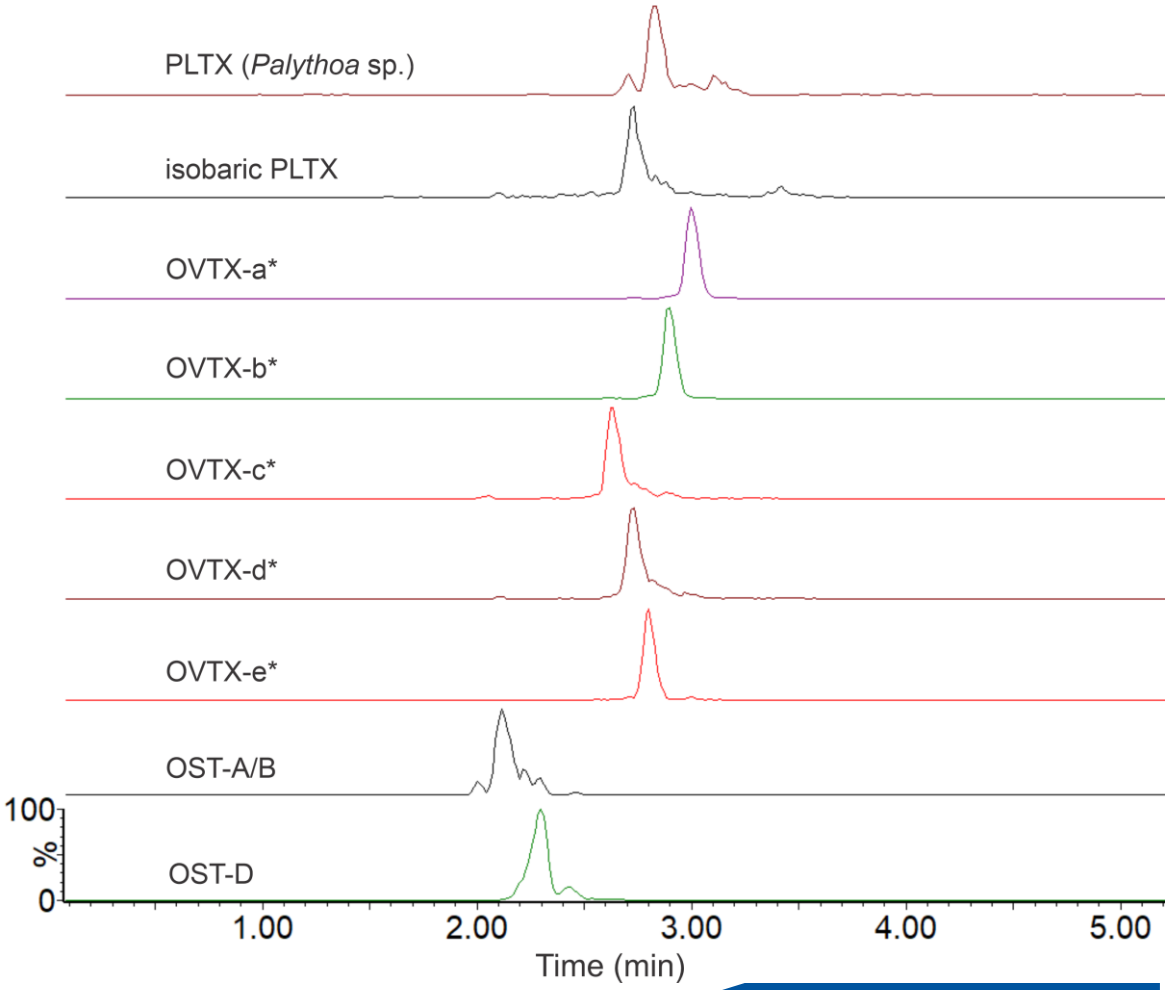
Screening *Ostreopsis* extracts

- Isolates from Cawthron culture collection
- Not *O. ovata* but show biological activity
- Amine fragment observed in all samples
- Amide fragment observed in some
- Intact method needed for confident ID
- No amide fragment = novel compound?



Intact method

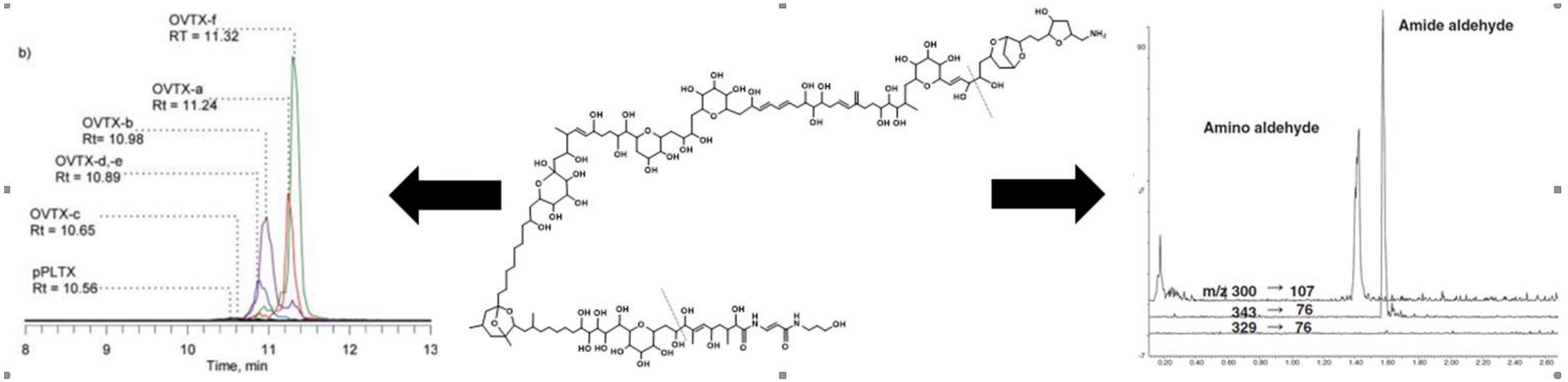
- LC-MS method developed for intact toxins
- Used reference material + isolates (Japan + Italy)
- Able to clearly identify most analogues
- Peak shapes impacted by isomerisation
- Sensitivity < [O] cleavage method but still good



Method performance

- SLV for [O] cleavage method completed and published 2012
- Need to assess performance of intact method: linearity, sensitivity, accuracy and precision
- Fortify mussel, oyster, fish flesh, algal extracts with PLTX, OVTX-a, OST-d (2 levels - low/high)
- SLV for intact method underway at Cawthron
- MLV will involve collaboration between three labs – Cawthron (UK), Cefas (UK), UoNapoli (IT)

Conclusions and next steps



- [O] cleavage method allows rapid & sensitive screening for palytoxin-like compounds
- Intact method allows confirmation of compounds observed
- Used together = useful tools to screen and identify palytoxin-like compounds
- Need to complete performance assessment of intact method
- Need toxicity information for analogues – what risk do they pose to consumers?

Acknowlegements

- Cawthron - Emillie Passfield, Andrew Selwood, Sam Murray
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