MARBiOFEED

Enhanced biorefining methods for the production of marine biotoxins and microalgae fish feed

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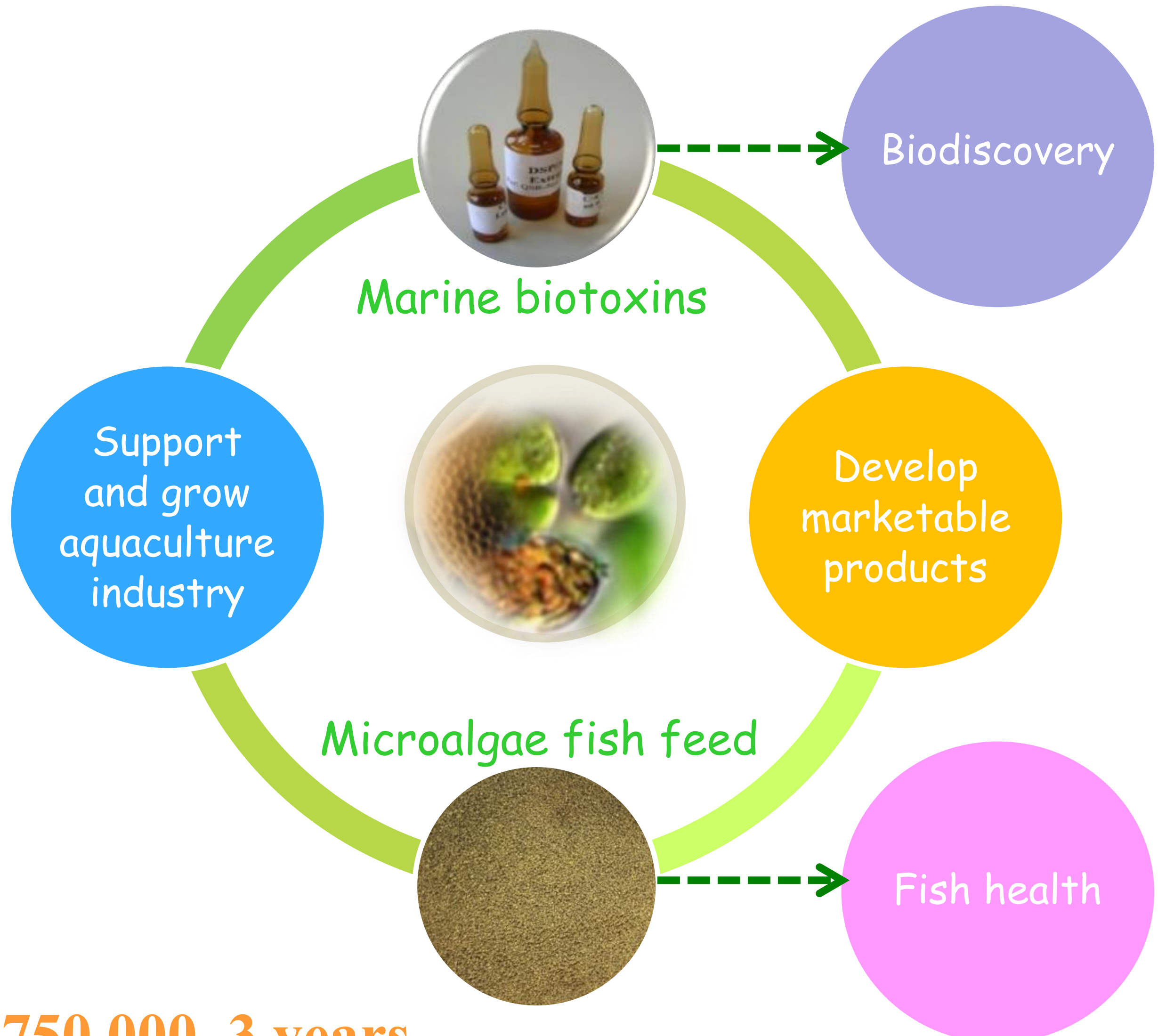
Enhanced biorefining methods for the production of marine biotoxins and microalgae fish feed



Funding: €750,000, 3 years

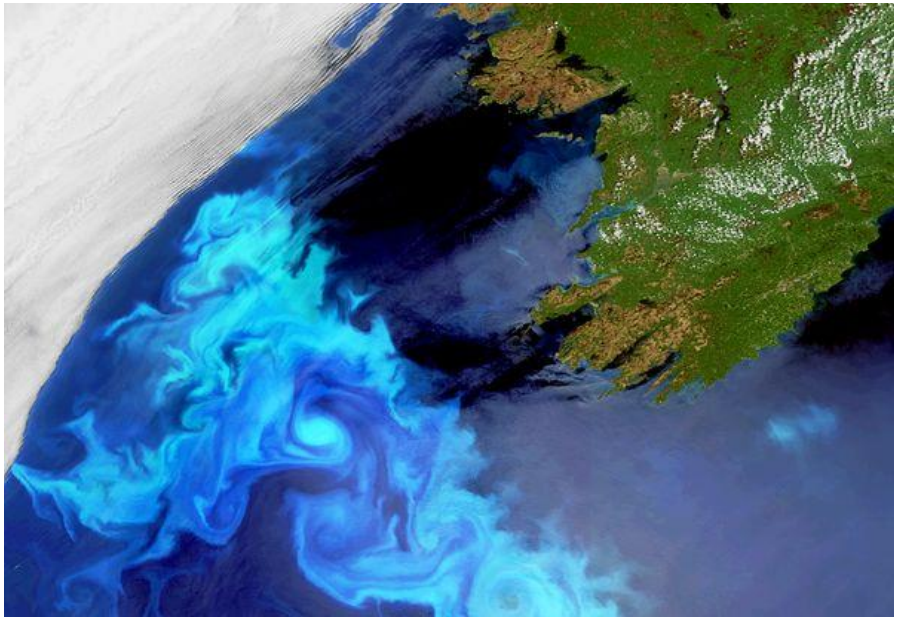
Aquaculture


- Worth €3.4 billion (Europe) in 2012
- Employs >85,000 (EU)
- Important sector for coastal/rural communities
- Fastest growing food sector globally
- Huge growth potential for Europe in the production of sustainable and high quality food for growing populations

Top species produced in EU:

1. **Mussel**
2. **Trout**
3. **Salmon**
4. **Oyster**
5. **Carp**
6. **Sea Bream**
7. **Sea Bass**


Shellfish Industry and Harmful Algal Blooms



- Shellfish are filter feeders

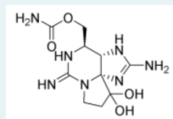
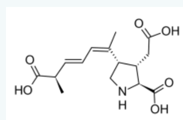
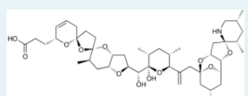
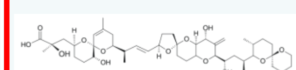
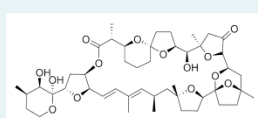
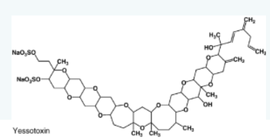
- At certain times of the year phytoplankton blooms can produce biotoxins which can accumulate in shellfish

➢ Impact on commercial aquaculture globally: stock losses ~ €200 M per annum*

➢ threat to human health: illnesses €75 M per annum*

*Maguire et al, Harmful algae, 2016

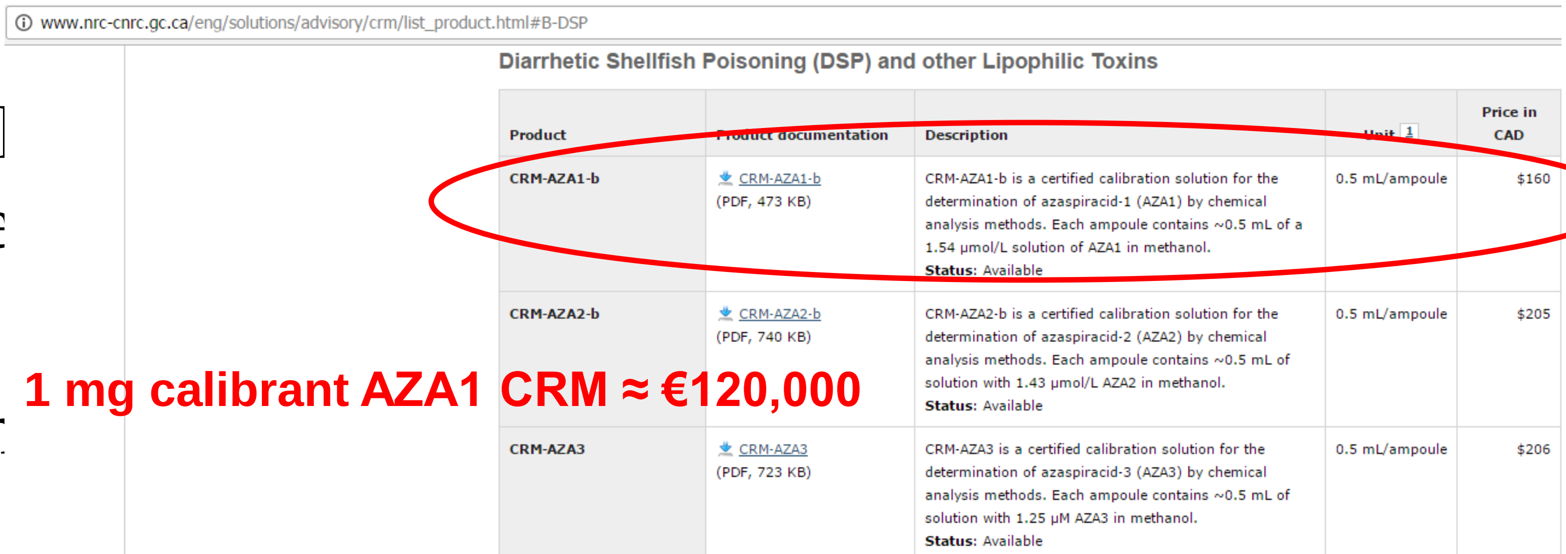
EU regulated toxins (in shellfish)

Classification	Regulated toxin	Causative organism(s)	Poisoning syndromes	Regulatory detection methods
Hydrophilic	**Saxitoxins** 	*Alexandrium* spp., *Gymnodinium catenatum, Pyrodinium bahamense*, certain cyanobacteria	Paralytic shellfish poisoning – **potent neurotoxin**	LC-FD
	Domoic Acid 	*Pseudo-nitzschia* spp., benthic diatoms	Amnesiac shellfish poisoning - **neurotoxin**	LC-PDA
Lipophilic	**Azaspiracids** 	*Azadinium* spp. *Amphidoma languida*	Azaspiracid shellfish poisoning	LC-MS/MS
	Okadaic acid group 	*Dinophysis* spp. and *Prorocentrum* spp.	Diarrhetic shellfish poisoning – **neurotoxic, immunotoxic and embyrotoxic**	LC-MS/MS
	Pectenotoxin 2 	*Dinophysis* spp. and *Protoperidinium* spp.	Diarrhetic shellfish poisoning – toxic by i.p. but not orally	LC-MS/MS
	Yessotoxin 	*Lingulodinium polyedrum, Protoceratium reticulatum*	Diarrhetic shellfish poisoning – toxic by i.p. but not orally	LC-MS/MS

Why isolate?

- Calibration standards (for accurate quantitation and detection)
- Limited supplies

- Toxicology studies
- Furt
  deve
- Con



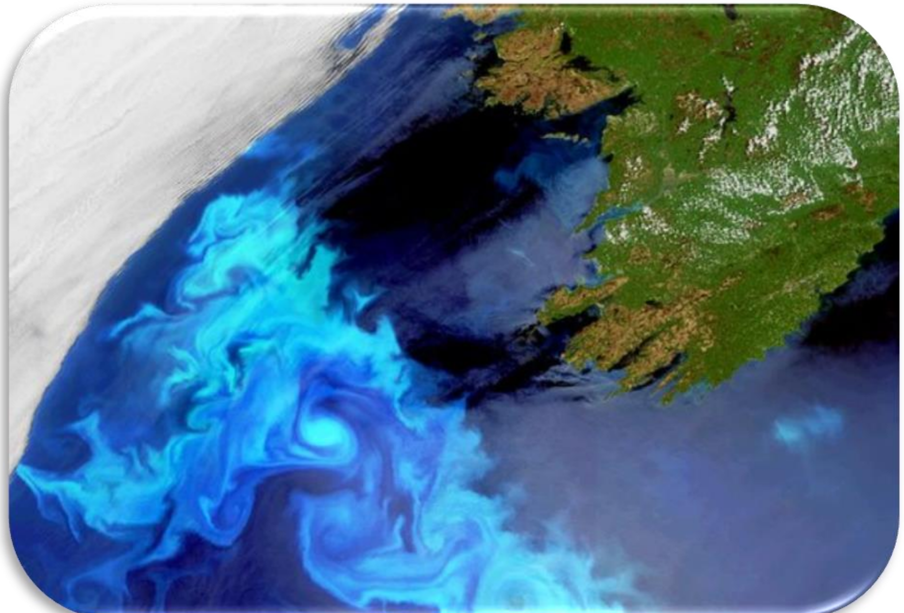
www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/list_product.html#B-DSP

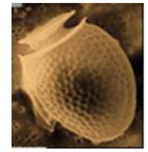
Diarrhetic Shellfish Poisoning (DSP) and other Lipophilic Toxins

Product	Product documentation	Description	Unit 1	Price in CAD
CRM-AZA1-b	CRM-AZA1-b (PDF, 473 KB)	CRM-AZA1-b is a certified calibration solution for the determination of azaspiracid-1 (AZA1) by chemical analysis methods. Each ampoule contains ~0.5 mL of a 1.54 µmol/L solution of AZA1 in methanol. **Status:** Available	0.5 mL/ampoule	$160
CRM-AZA2-b	CRM-AZA2-b (PDF, 740 KB)	CRM-AZA2-b is a certified calibration solution for the determination of azaspiracid-2 (AZA2) by chemical analysis methods. Each ampoule contains ~0.5 mL of solution with 1.43 µmol/L AZA2 in methanol. **Status:** Available	0.5 mL/ampoule	$205
CRM-AZA3	CRM-AZA3 (PDF, 723 KB)	CRM-AZA3 is a certified calibration solution for the determination of azaspiracid-3 (AZA3) by chemical analysis methods. Each ampoule contains ~0.5 mL of solution with 1.25 µM AZA3 in methanol. **Status:** Available	0.5 mL/ampoule	$206

1 mg calibrant AZA1 CRM ≈ €120,000

Toxin sources?



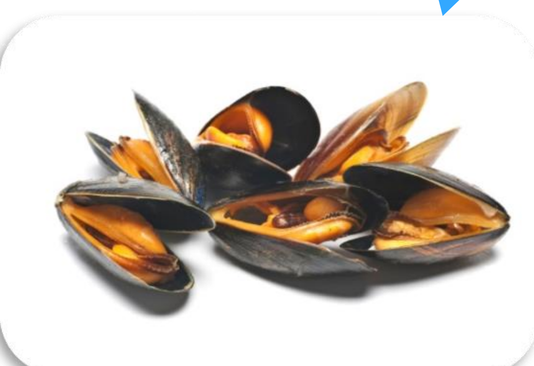


Dinophysis acuta (OA, DTX2)

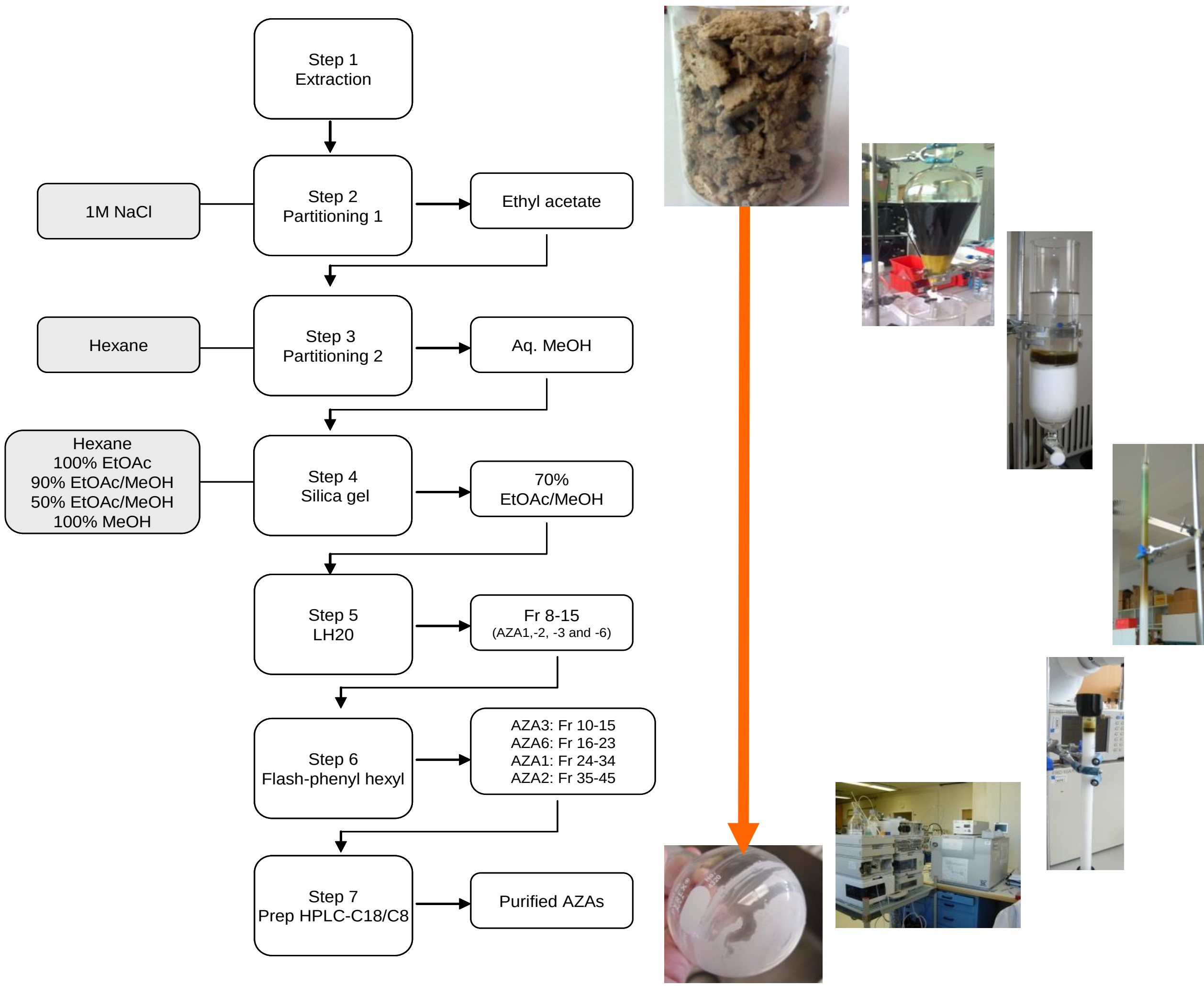
Prorocentrum lima (OA, DTX1 and DTX2)

Azadinium spinosum (AZA1 and AZA2)

Pseudo-nitzchia australis (DA)



Isolation of toxins from shellfish



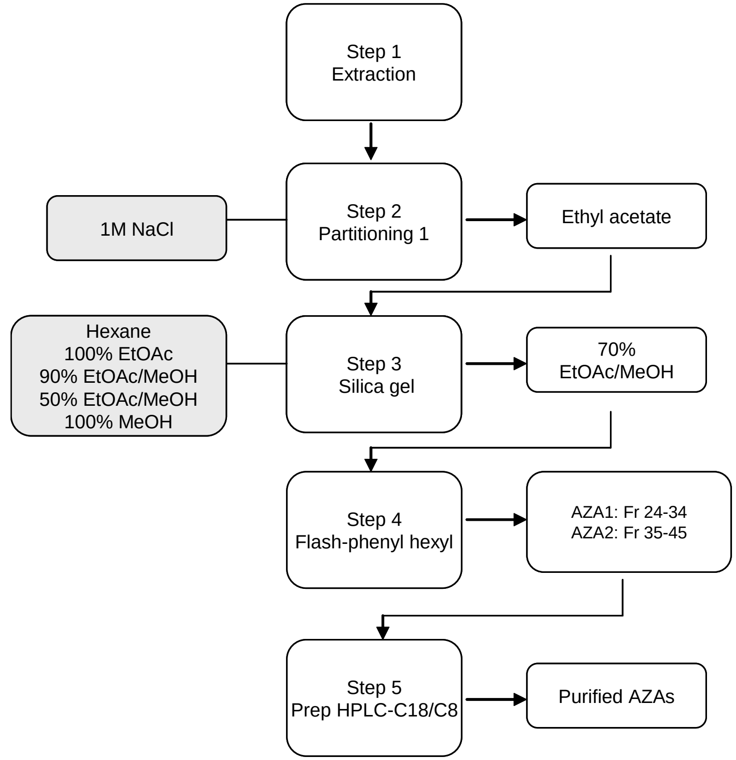
- Lengthy and labour intensive.
- µg–mg amounts isolated (dependant on levels in shellfish).

Yields:
52% (AZAs)*
40% (OA group)**

*Kilcoyne, J., Keogh, A., Clancy, G., LeBlanc, P., Burton, I., Quilliam, M. A., Hess, P., and Miles, C. O. (2012) *J. Agric. Food Chem. 60*, 2447–2455.

**Beach, D. G.; Crain, S.; Lewis, N.; LeBlanc, P.; Hardstaff, W. R.; Perez, R.; Giddings, S. D.; Martinez-Farina, C. F.; Stefanova, R.; Burton, I. W.; Kilcoyne, J.; Melanson, J.; Quilliam, M. A.; McCarron, P. (2016) *J. AOAC Int. 99*, 1–12.

Isolation of toxins from microalgae



- Fewer steps
- Greater recoveries

Yields:
70% (AZAs)*

*Jauffrais, T., Kilcoyne, J., Séchet, V., Herrenknecht, C., Truquet, P., Hervé, F., Bérard, J. B., Nulty, C., Taylor, S., Tillmann, U., Miles, C. O., and Hess, P. (2012) *Mar. Drugs 10*, 1360–1382.

Bulk culturing of microalgae

Dinophysis acuta (OA, DTX2)

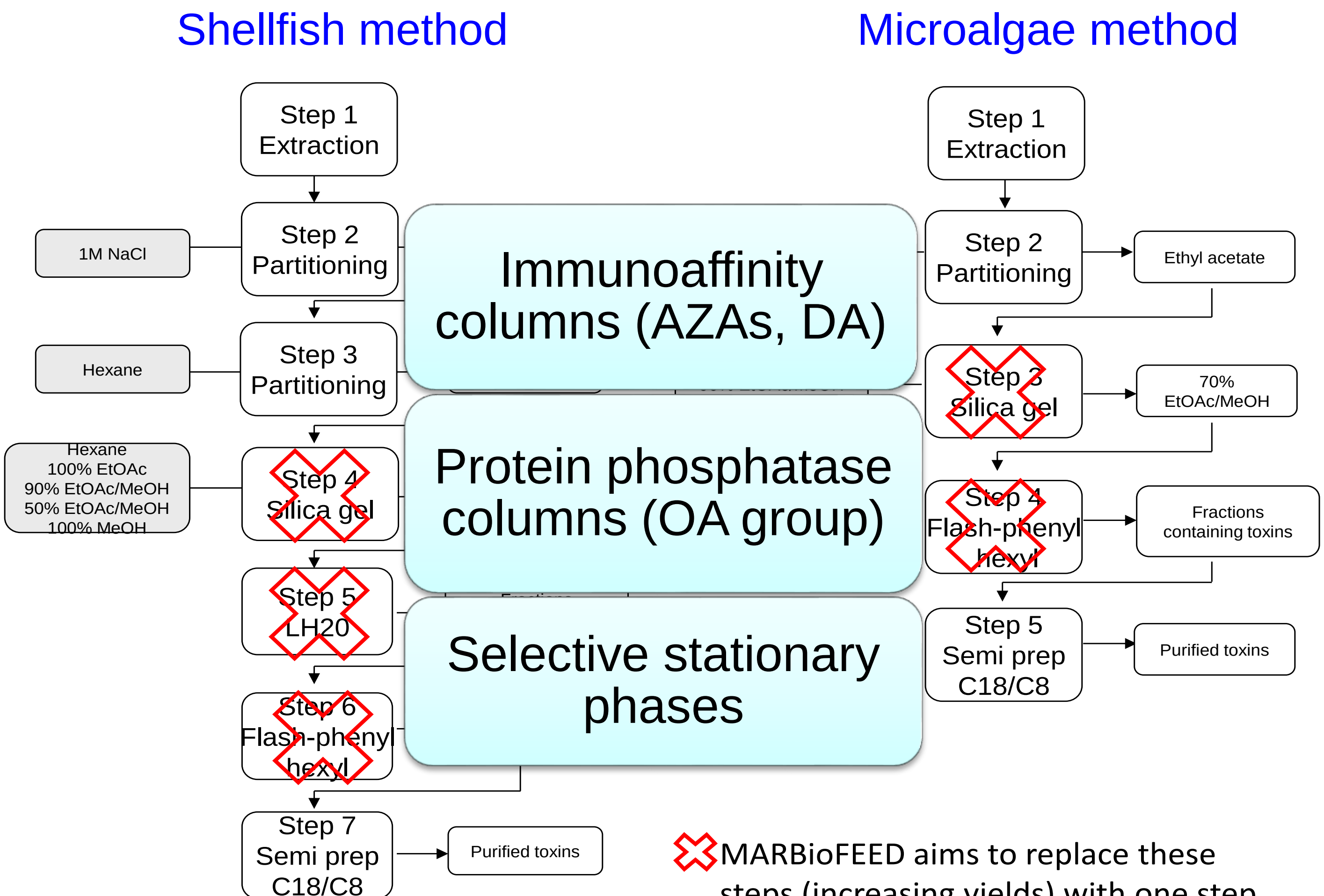
Prorocentrum lima (OA, DTX1 and DTX2)

Azadinium spinosum (AZA1 and AZA2)

Pseudo-nitzchia australis (DA)

Optimisation of cell growth and toxin production

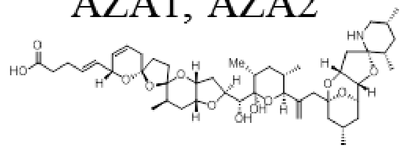
Enhanced biorefining



MARBioFEED aims to replace these steps (increasing yields) with one step that uses selective stationary phases

In vitro production of shellfish metabolites

AZA1, AZA2

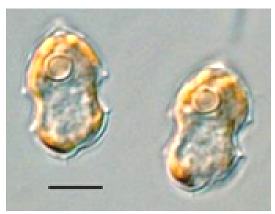


Accumulation

Shellfish (*M. edulis*)

Production

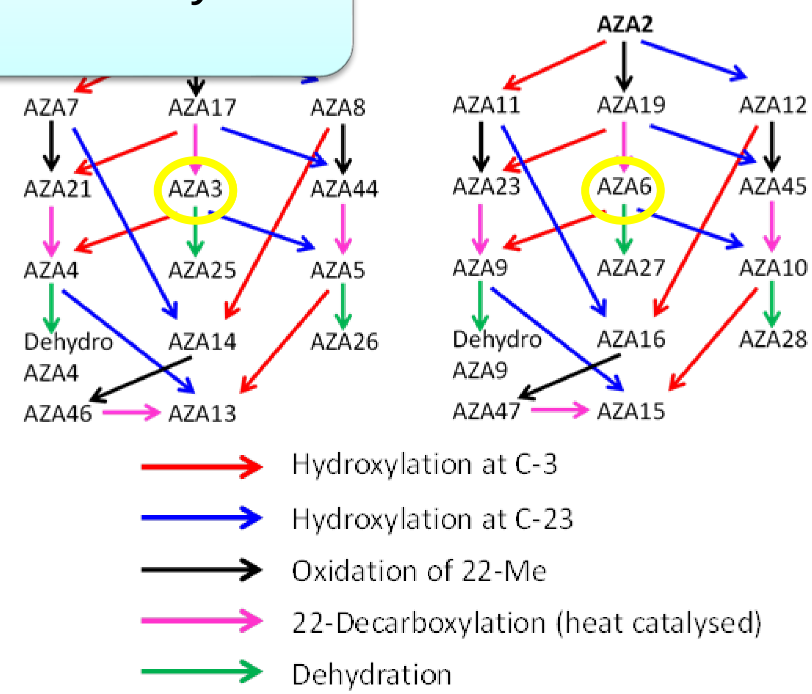
160 µg/kg regulatory li



CYP P450 enzymes

Metabolism

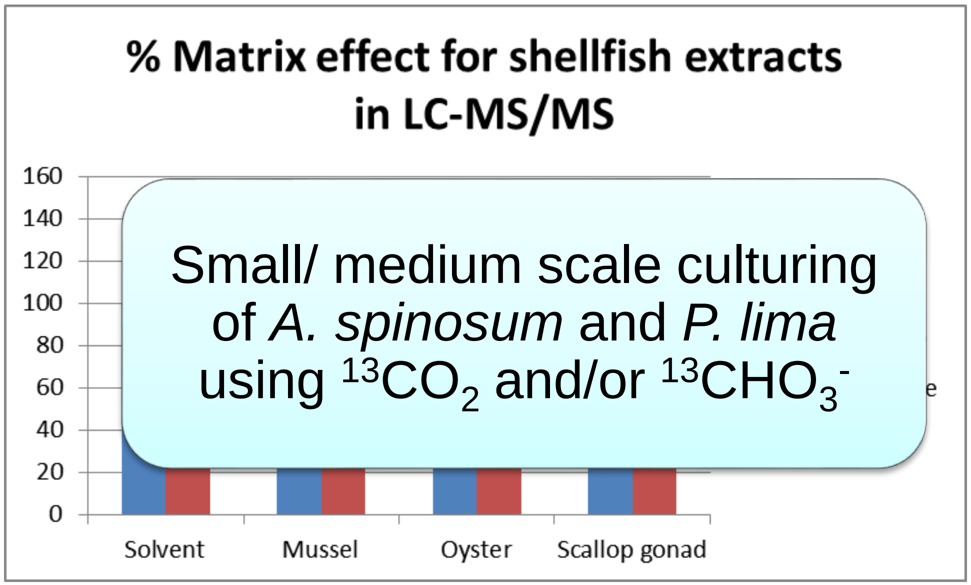
Processing, Cooking, etc.



Severe vomiting, diarrhea

Production of isotope-labelled toxins

- Regulatory method of analysis (LC-MS) is hindered by matrix interferences (analysis of crude samples)



Small/ medium scale culturing of *A. spinosum* and *P. lima* using $^{13}CO_2$ and/or $^{13}CHO_3^-$

- Use of isotopically labelled standards would correct for the inaccuracies caused by these interferences

- No supplies for isotopically labelled marine biotoxins (DA, AZAs and OA group) available

Fish Farming and Fish Feed

Marine life

Fish to shrink by up to a quarter due to climate change, study reveals

Scientists predict 14-24% reduction in fish size by 2050 as ocean temperatures increase

Damian Carrington

@dpcarrington

Sunday 30 September 2012 18.00 BST

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Glassfish on a coral reef in Egypt. Scientists have examined the effect of rising ocean temperatures on the growth and distribution of more than 600 species of fish. Photograph: Tarik Tinazay/AFP/Getty Images

Fish Farming and Fish Feed

- Terrestrial and marine sourced ingredients

- Marine sourced ingredients typically include small fish e.g. sardines, anchovies and herring that are rich in nutrients such as omega-3 fatty acids

- Issues with long term sustainability and rising costs

PERSPECTIVE

Does aquaculture add resilience to the global food system?

Max Troell[a,b,1], Rosamond L. Naylor[c], Marc Metian[b], Malcolm Beveridge[d], Peter H. Tyedmers[e], Carl Folke[a,b], Kenneth J. Arrow[f], Scott Barrett[g], Anne-Sophie Crépin[a], Paul R. Ehrlich[h], Åsa Gren[a], Nils Kautsky[i], Simon A. Levin[j], Karine Nyborg[k], Henrik Österblom[b], Stephen Polasky[l], Marten Scheffer[m], Brian H. Walker[n], Tasos Xepapadeas[o], and Aart de Zeeuw[p]

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Edited by Bonnie J. McCay, Rutgers, The State University of New Jersey, New Brunswick, New Brunswick, NJ, and approved July 23, 2014 (received for review March 13, 2014)

Fish Farming and Fish Feed

European Commission

JRC SCIENTIFIC AND POLICY REPORTS

Microalgae-based products for the food and feed sector: an outlook for Europe

Authors: Christien Enzing, Matthias Ploeg, Maria Barbosa,
Lolke Sijtsma

Editors: Mauro Vigani, Claudia Parisi, Emilio Rodríguez Cerezo

2014

- Microalgae great source of nutritious compounds e.g. fatty acids, proteins and vitamins

- Microalgae products (Europe) only ~ 5% of global market

- Sector is in its infancy

- Requirement for more research – increasing scale and reducing costs

Team makes breakthrough toward fish-free aquaculture feed

June 3, 2016

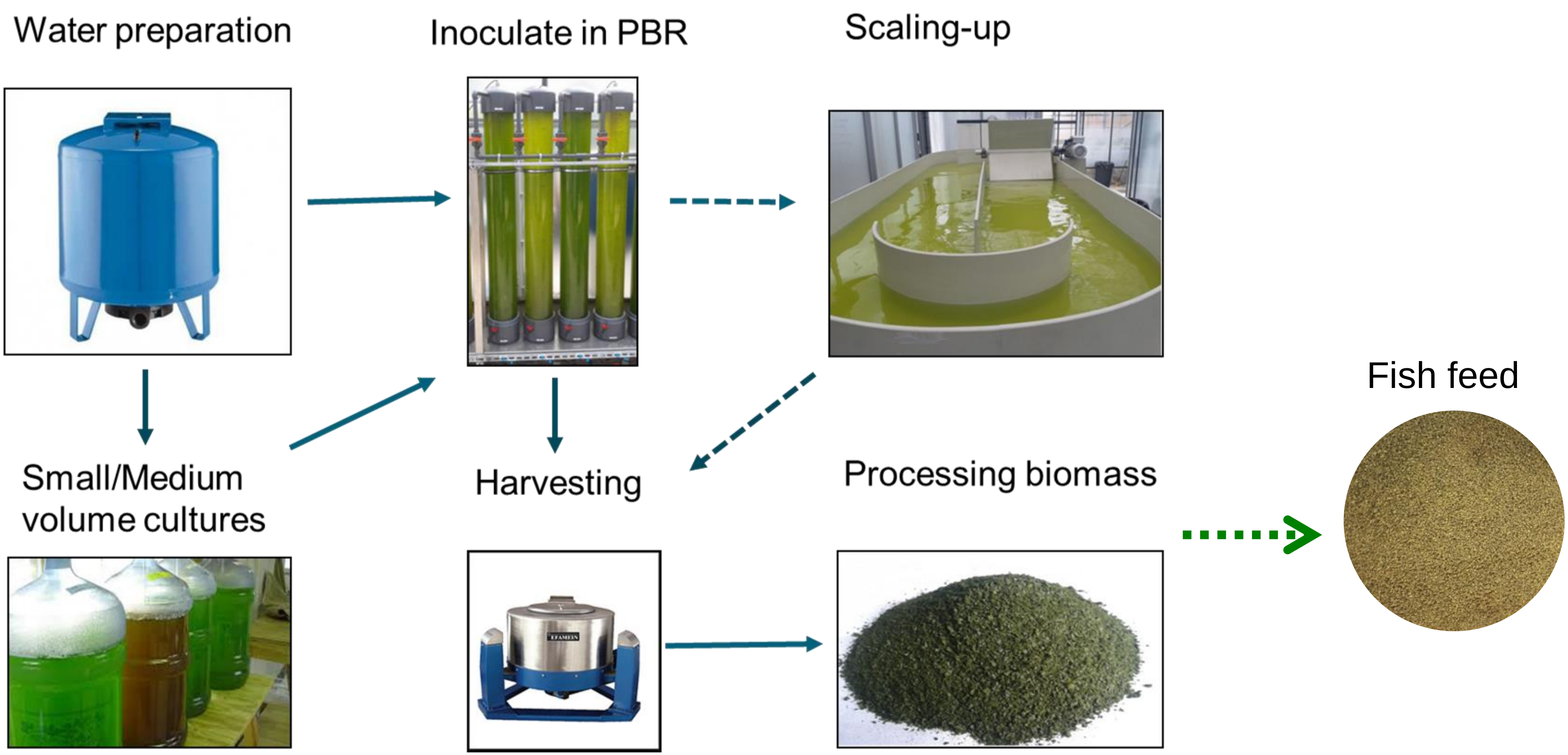
Dartmouth Research Assistant Professor Pallab Sarker (left) and Professor Anne Kapucinski conduct an experiment on the use of microalgae as a sustainable feed ingredient for aquaculture of tilapia. Credit: Dartmouth College

Dartmouth College scientists have discovered that marine microalgae can completely replace the wild fish oil currently used to feed tilapia, the second most farmed fish in the world and the most widely farmed in the United States.

Using the microalgae *schizochytrium* to feed juvenile Nile tilapia - higher weight gain and better food conversion compared to a control diet containing fish oil, and no significant change in survival and growth rates among all diets. The fish-oil-free microalgae diet also had the highest content of omega-3 fatty acids in tilapia fillets.

Microalgae Fish Feed

neo
ALGAE
micro seaweed products



Shellfish as Fish Feed?

• Shellfish waste could be used in fish feed formulations

• Product that has levels of toxins over the regulatory limit could still have value

• Necessary to assess impacts of feeding e.g. salmon contaminated shellfish – requirement for large amounts of purified toxins (available at low cost) to perform such studies.

Thanks for listening!

The Research Council of Norway

Marine Institute
Foras na Mara

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

neo ALGAE
micro seaweed products

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