Ichthyotoxic microalgae from the French coasts: investigating links between biological toxicity, chemodiversity and genetic diversity within the genus *Karlodinium*

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Poster / speed talk
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Potentially ichthyotoxic *Karlodinium* spp. in France

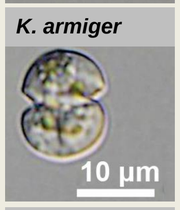
- Ichthyotoxic microalgae produce compounds that lead to fauna mortality, with ecological and economical impacts, particularly in the aquaculture industry (e.g. Hallegraeff et al. 2017).
- *Karlodinium* spp. blooms have induced fish and shellfish kills in several regions of the world. But toxicity is variable among species and strains (Bachvaroff et al. 2009, Krock et al. 2017).
- In France: presence of *Karlodinium* species (Nézan et al. 2014), but toxicity of French strains is unknown.

→ Are French *Karlodinium* spp. at ichthyotoxic risk?

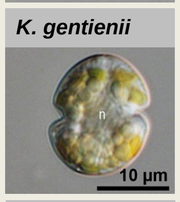
Karlodinium spp. in France

K. veneficum

K. armiger



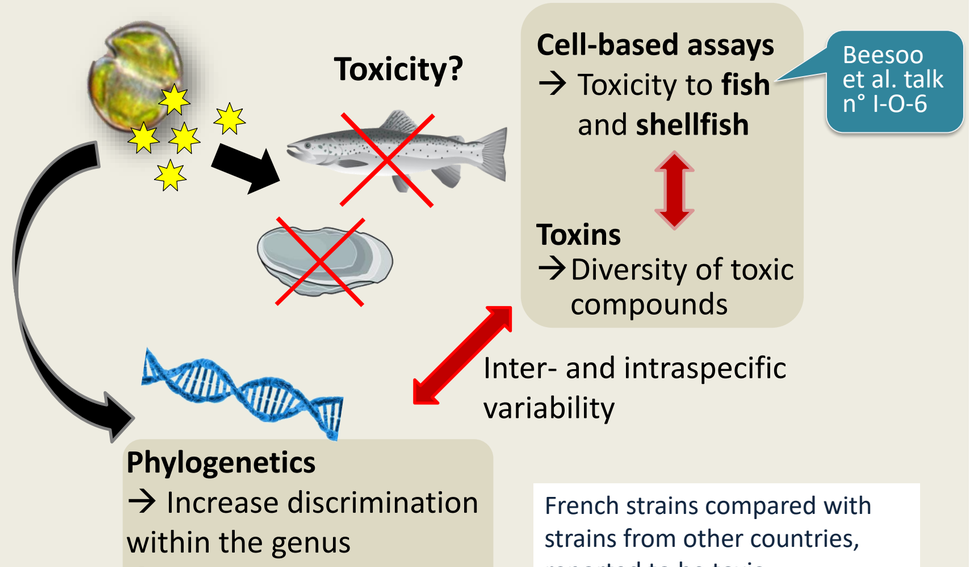
K. gentienii



K. ballantinum

photo: Benico et al 2020

Project:



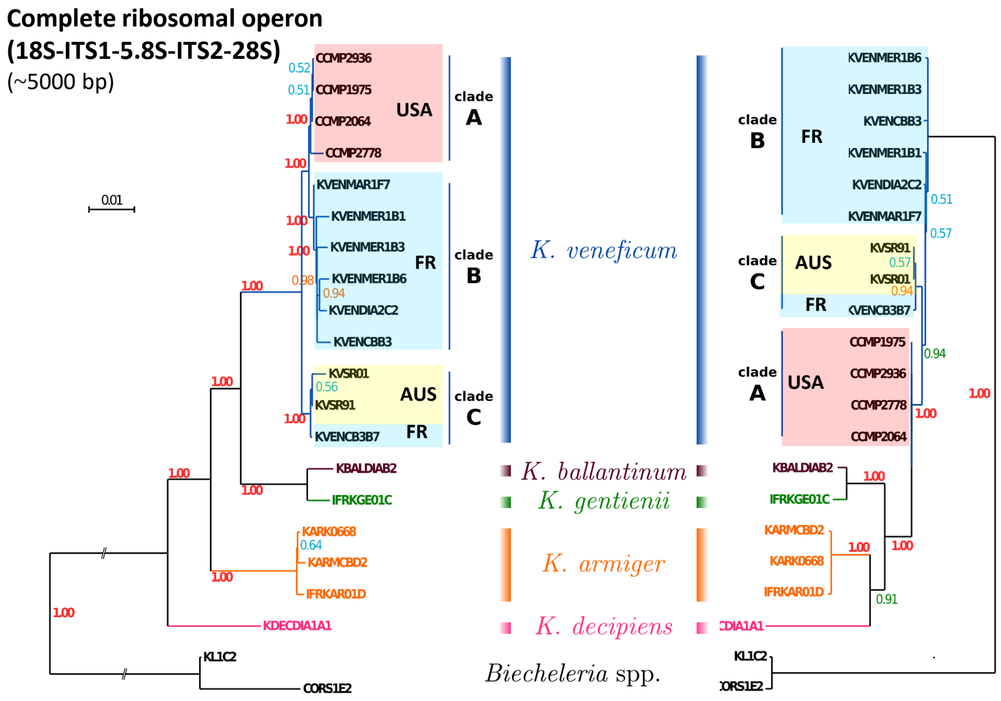
French strains compared with strains from other countries, reported to be toxic
(Bachvaroff et al. 2009, Mooney et al. 2009, Van Wagoner et al. 2010, Rasmussen et al. 2017, Krock et al. 2017)

Phylogenetics

- Sequencing: whole ribosomal operon of rDNA (MinION Nanopore: long sequences but errors, Mi-Seq Illumina : low error rate but fragmentation).
- Bioinformatic analysis and phylogenetic trees (Bayesian method)

Complete ribosomal operon (18S-ITS1-5.8S-ITS2-28S)
(~5000 bp)

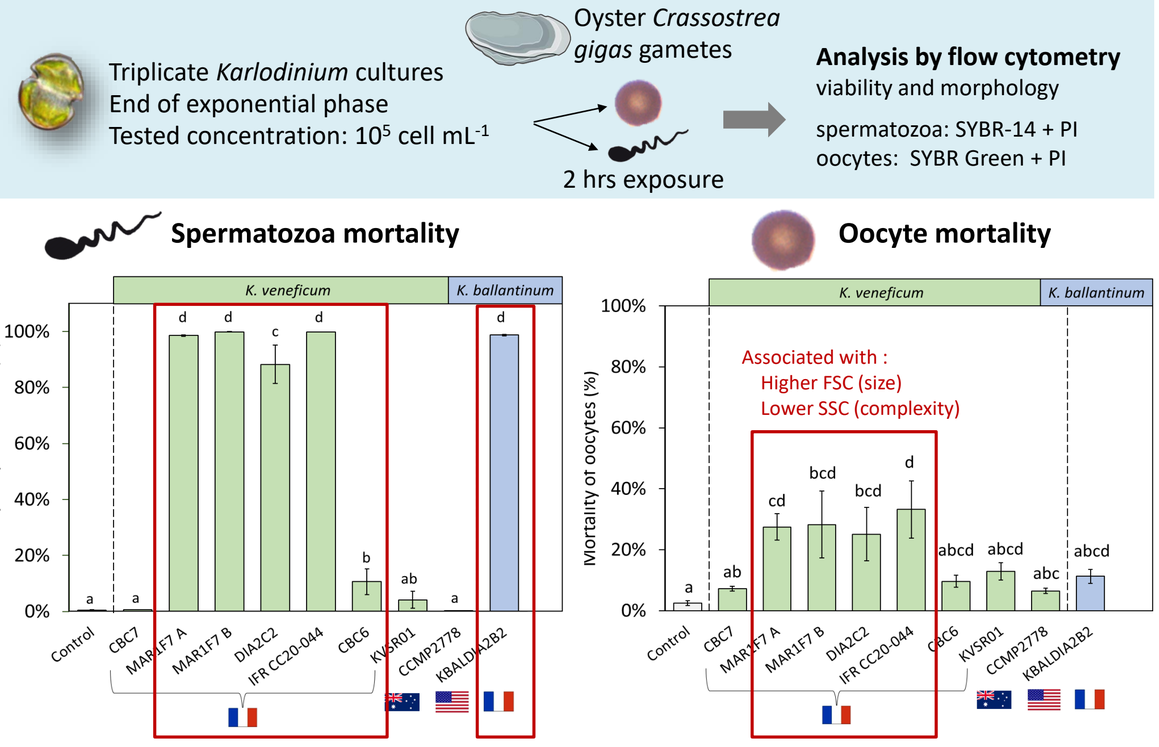
ITS region (ITS1-5.8S-ITS2)
(~700 bp)
Usual marker for *Karlodinium* spp.



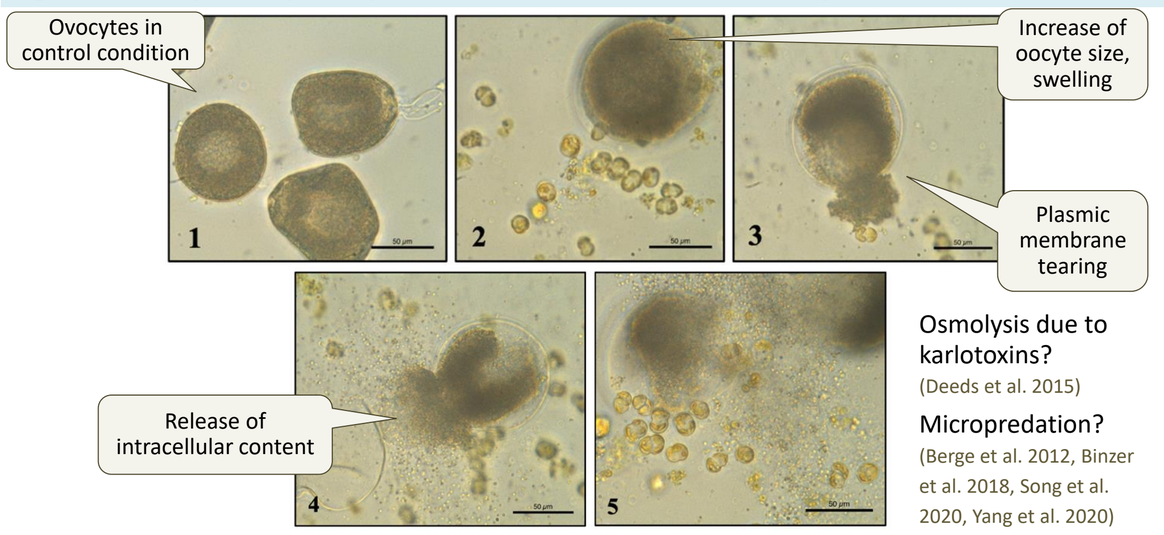
→ Better inter- & intraspecific discrimination using whole ribosomal operon vs ITS region
- Interspecific variability: essentially in ITS 1 & 2, but also in the rest
- Intraspecific variability: all along the operon (24 sites discriminative of a clade)

→ *K. veneficum*: 3 clades according to geographical origin

Toxicity to bivalve cells



Light microscope: oocytes exposed to Control or to French *K. veneficum* MAR1F7strain



Osmolysis due to karlotoxins?
(Deeds et al. 2015)

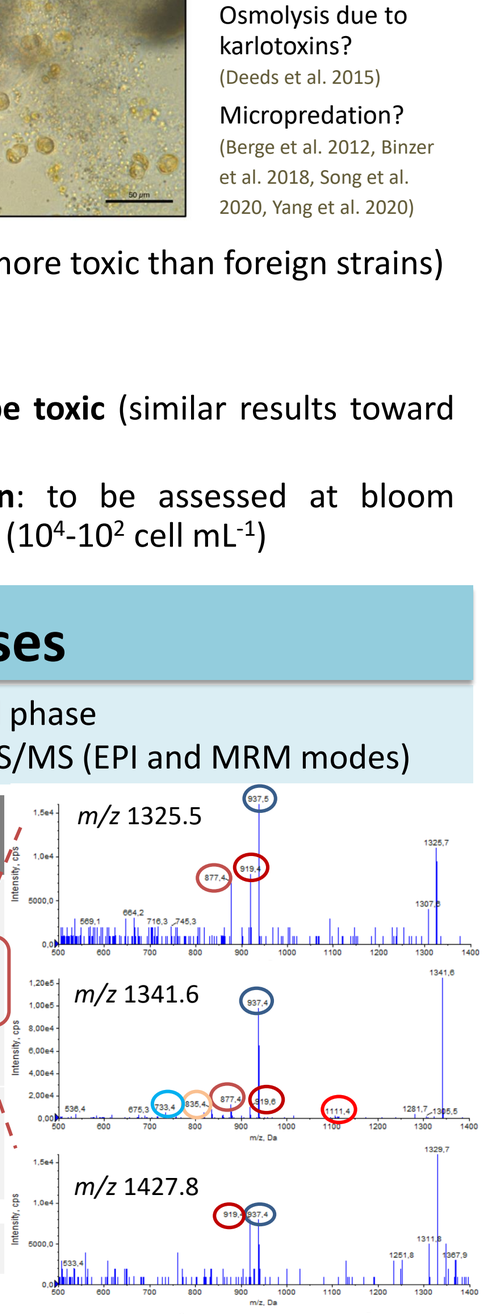
Micropredation?
(Berge et al. 2012, Binzer et al. 2018, Song et al. 2020, Yang et al. 2020)

→ Identification of cytotoxic French strains (more toxic than foreign strains)
→ High intraspecific variability
→ Role of karlotoxins *vs* micropredation?
→ First time *K. ballantinum* is reported to be toxic (similar results toward hemocytes – not presented)
→ Potential effects on oyster reproduction: to be assessed at bloom realistic concentrations of *Karlodinium* spp. (10^4-10^2 cell mL^{-1})

Toxin analyses

- *Karlodinium* spp. cultures, end of exponentiel phase
- Methanolic extractions and analyses by LC-MS/MS (EPI and MRM modes)

Strain	Toxin profile from literature	References	Identified toxins	Potential new analogues
CCMP 2936 *K. veneficum*	KmTx-1 65-E-chloro-KmTx-1 10-O-sulfo-KmTx-1 KmTx-2 KmTx-3 64-E-chloro-KmTx-3 10-O-sulfo-KmTx-3	Van Wagoner et al. 2010 Fu et al. 2010 Krock et al. 2017	KmTx-1 65-E-Cl-KmTx-1 KmTx-2 44-OH-KmTx-2 4,5 dihydro-KmTx-2 KmTx-3 64-E-Cl-KmTx-3 KmTx-9	*m/z* 1325.5 *m/z* 1341.6 *m/z* 1427.8
CCMP 2064 *K. veneficum*	KmTx-2 44-OH-KmTx-2	Bachvaroff et al. 2009 Adolf et al. 2009 Peng et al. 2010	-	*m/z* 1395.6 *m/z* 1369.7
K-0668 *K. armiger*	Karmitoxin	Rasmussen et al. 2017	Karmitoxin	-



→ First analyses of USA and Spanish strains suggest **putative new analogues and variation of karlotoxin composition** compared to profiles previously described in the literature
→ To be continued

Conclusions and perspectives

- Better phylogenetic discrimination within *Karlodinium* genus using whole ribosomal operon compared with usual ITS and LSU regions
- Different phylogenetic clades within *K. veneficum,* according to geographical origin and unrelated to toxicity
- French strains of *Karlodinium* spp. can affect bivalve cells as much as other foreign strains previously reported to be toxic
- Risk for bivalve reproduction needs to be evaluated at bloom realistic concentrations
- Toxicity of *K. ballantinum* and *K. veneficum* due to different mechanisms? → **Toxin analyses to complete and micropredation to study**

Fundings
Let It Bi, Biotox, Biotox-cell, Mecatox projects

References: Adolf et al 2009, J. Phycol. 45, 176–192; Bachvaroff et al 2009, J. Phycol. 45, 137–153; Berge et al 2012, ISME J. 6, 1926–1936; Binzer et al 2018, PLoS One 13, e0199306; Deeds et al 2015, Aquat. Toxicol. 159, 148–155; Fu et al 2010, Aquat. Microb. Ecol. 59, 55–65; Hallegraeff et al 2017, Proceedings of the 17th ICHA. pp. 148–153; Krock et al 2017, Mar. Drugs 15, 391; Mooney et al 2009, J. Phycol. 45, 164–175; Nézan et al 2014, Harmful Algae 40, 75–91; Peng et al 2010, J. Am. Chem. Soc. 132, 3277–3279; Song et al 2020, Harmful Algae 99, 101926.; Yang et al 2020, Harmful Algae 93, 10177.

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