Ecole doctorale n°304 Sciences et environnements — Université de Bordeaux — EPOC

MASARYK UNIVERSITY — Research centre for toxic compounds in the environment

RECETOX DOCTORAL CONFERENCE MAY 2020

Environmentally relevant pesticide mixture: a risk for non-target aquatic organisms?

Laboratory and *in situ* approaches

Eliška ROZMÁNKOVÁ

SUPERVISORS: Patrice GONZALEZ, Luděk BLÁHA (cotutelle thesis)

Introduction

Herbicides

Insecticides

Fungicides

Pesticide cocktail

+ presence of metabolites
→ interactions between the molecules

Objectives of the thesis

Evaluation of sublethal effects and mechanisms of action of the embryo-larval stages of non-target aquatic organisms exposed to environmental concentrations of pesticides alone or mixed:

- Fish *Danio rerio*
- Oyster *Magallana gigas*

***+ In situ* approach with encaged oysters in the Bay**

Thesis outcomes

+ thesis defense December 2020

- *Danio rerio*
  - Herbicides

Aquatic Toxicology 221 (2020) 105444

Environmentally relevant mixture of S-metolachlor and its two metabolites affects thyroid metabolism in zebrafish embryos

Eliška Rozmánková[a,b], Marek Pípal[a], Lucie Bláhová[a], Naveen Njattuvetty Chandran[a], Bénédicte Morin[b], Patrice Gonzalez[b], Luděk Bláha[a,*]

  - Pesticide mixture (5 pesticides) – lab work in progress
- *Magallana gigas* **(presented in this poster)**
  - Individual compounds + prospective nanopesticide comparison – ↑ manuscript finished ↑
  - Pesticide mixture (5 pest.) + *in situ* approach – manuscript in preparation

„Comparative effects of environmentally relevant concentrations of imidacloprid, propiconazole, and its nanoformulation on the development of the early-life stages of the Pacific oyster (*Magallana gigas*)"

- Complementary cooperation studies
  - *Chironomus riparius*

Chemosphere 209 (2018) 568–577

Acute and (sub)chronic toxicity of the neonicotinoid imidacloprid on *Chironomus riparius*

Naveen Njattuvetty Chandran, Dana Fojtova, Lucie Blahova, Eliska Rozmankova, Ludek Blaha*

  - Fish cell line – pesticide mixture focus – lab work in progress with Marie Mlnarikova

Materials & methods

1. Laboratory approach

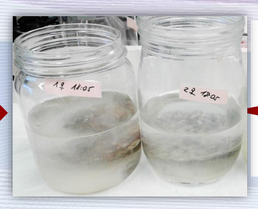
Insecticides — Imidacloprid

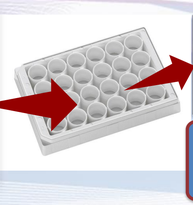
Fungicides — Propiconazole

Herbicides — S-metolachlor, Metolachlor OA, Metolachlor ESA

Focus: environmental concentrations of the most detected pesticides in the Bay

MIXTURE	PROPI	IMIDA	S-M	MOA	MESA	Total concentration
C1	10 ng/L	100 ng/L	10 ng/L	100 ng/L	100 ng/L	0.32 µg/L
C2	50 ng/L	500 ng/L	50 ng/L	500 ng/L	500 ng/L	1.6 µg/L
C3	0.25 µg/L	2.5 µg/L	0.25 µg/L	2.5 µg/L	2.5 µg/L	8 µg/L





D-larve (24 hpf)

Stimulation with heat shocks (18°C & 28°C) 1. → Gametes → fecondation 2. → Development 12h-40h at 24°C 3.

normalized biotest (NF ISO 17244, 2015)

2. *In situ* approach

20 µm mesh NITEX filters

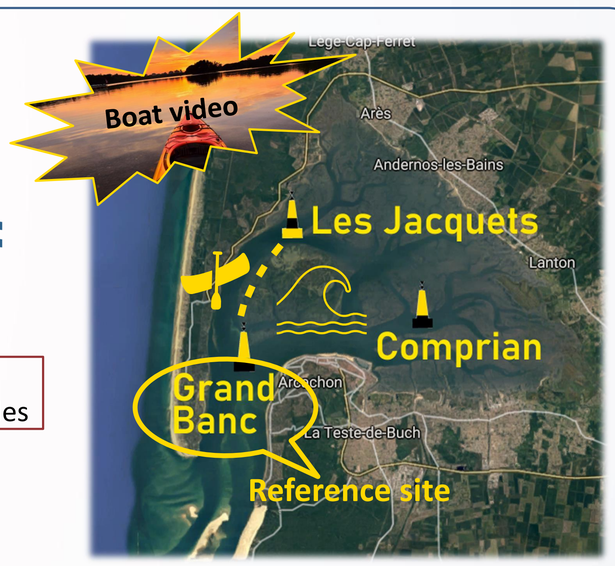
Cut-open cap

Welded HDPE bottles

KG

Underwater video

4 devices per buoy
± 666,000 larvae per device



Endpoints

- Developmental malformations
- Swimming behavior
- Gene expression

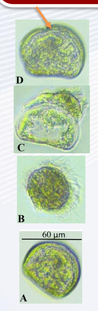
Sampling video

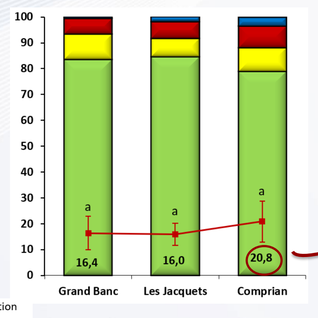
Pesticide concentrations at the beginning and at the end of the *in situ* experiment in the Arcachon Bay (LC-MS/MS (pesticides) and ICP-MS (copper))

Sampling site	Sampling time h	Salinity psu	Propiconazole ng/L	Imidacloprid ng/L	S-metolachlor ng/L	Copper µg/L
Grand Banc	0	28.8	6.30	26.96	72.59	0.59
	48	34.6	5.67	9.14	72.54	0.54
Les Jacquets	0	22.4	12.69	14.63	97.20	0.42
	48	23.5	8.73	10.08	92.75	0.79
Comprian	0	17.1	9.27	10.89	119.79	0.65
	48	18.7	16.74	16.83	138.29	0.49

Results & discussions

1. Developmental malformations





D Shell malformation
C Mantle malformation
B Developmental arrest
A Well-formed
Sum abnormal larvae

Compare with the laboratory conditions

Laboratory conditions	
Negative control	16.4 ± 3.1 %
Mixture exposure „C1" (see mat. & met. 1.)	20.6 ± 3.8 %*

High variability in the field...

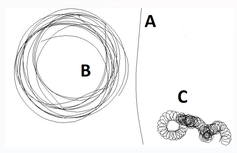
* Kruskal-Wallis + Mann-Whitney, p=0.01, N=7 ± SD

Individual compounds LOECs

S-metolachlor: 10 ng/L (Gamain et al., 2016)
Metolachlor OA: 100 ng/L (Mai et al., 2014)
Metolachlor ESA: 100 ng/L (Mai et al., 2014)
Propiconazole: 200 µg/L (Rozmankova et al., not published yet)
Imidacloprid: 200 µg/L (Rozmankova et al., not published yet)

2. Swimming behavior

Trajectory paths: rectilinear (A), circular (B), motionless (C)

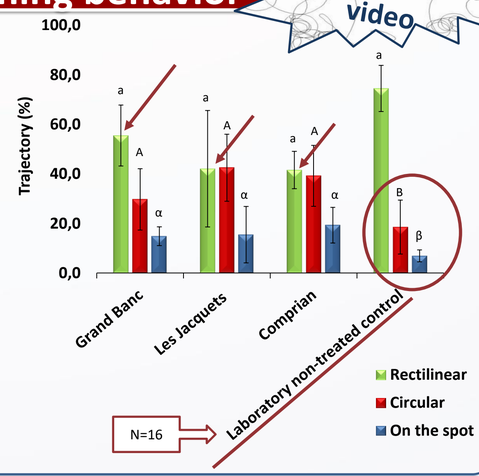


Rectilinear trajectory = **normal** (Gamain et al. 2019 and His et al. 1999)

Sampling site	Speed (µm/s) maximal	Speed (µm/s) average
Grand Banc	304 ± 49	187 ± 43
Les Jacquets	428 ± 63	263 ± 34
Comprian	341 ± 74	204 ± 43

However high, not stat. sign.

Swimming larvae video



3. Gene expression

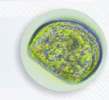
	Function	Mitochondrial metabolism		Apoptosis regulation		Regulation of the cell cycle/ apoptosis	Biotranfor-mation	Oxidative stress defense				Growth arrest and DNA damage	DNA reparation	Detoxification	
	Gene	*12S*	*cox1*	*bax*	*casp3*	*p53*	*cyp1a*	*cat*	*sodCu*	*sodMn*	*gpx*	*gadd45*	*rad51*	*mt1*	*mt2*
In situ: compared to the reference site	Les Jacquets / Grand Banc	-	1.3 ± 0.3**	-	-	-	-	3.4 ± 1.1***	-	-	0.7 ± 0.1***	-	0.8 ± 0.2*	-	0.8 ± 0.5*
	Comprian / Grand Banc	1.3 ± 0.2**	-	-	-	-	-	2.7 ± 1.2***	-	1.2 ± 0.2*	0.6 ± 0.1***	-	-	0.6 ± 0.5***	0.5 ± 0.3***
Laboratory exposure with the mixture (see mat. & met. 1.)	C1	0.74 ± 0.2**	-	-	-	-	0.70 ± 0.2***	-	-	-	-	0.75 ± 0.4*	-	-	-
	C2	0.95 ± 0.1*	-	-	-	-	0.78 ± 0.2***	-	-	-	-	0.68 ± 0.1***	-	-	-
	C3	-	-	0.81 ± 0.01**	-	-	0.85 ± 0.1*	-	-	-	-	0.72 ± 0.2**	-	-	0.71 ± 0.3*

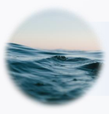
Fold changes; induction emphasized in green, repression in red. *p < 0.05; **p < 0.01; ***p < 0.001

Conlusions

✓ Succesfull use of the designed caging device.

High sensitivity of the early-life stages of oyster.





Relatively good water quality in the Arcachon Bay. But was the reference site a reference one?

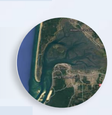
Reference site just outside of the Bay in the ocean could be a better solution, however, that was logistically not feasible. ← Slow boat! :)

1st study assessing the locomotion of oyster larvae exposed *in situ.*

Different impact on the molecular level of larvae in the field and in those exposed to the reconstituted mixture in the laboratory.

The complexity of the environmental mixture (pesticides, metals, drugs, personal care products, hydrocarbons...) cannot be explained by the effect of the most abundant herbicides, insecticide; and fungicide in the Bay)

Assessment of the quality of coastal waters.



Could be used by environmental agencies, marine stations, ...

For a live commentary, please see SETAC SciCon presentation 1.02.3.

SciCon Available till 6 June!