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Effects of two fungicides, Boscalid and Imazalil on the development of *A. ruthenus* embryos based on an experimental approach



Eric ROCHARD

Blandine DAVAIL, Clémentine ÉLY, Christophe ROBINSON, Patrick CHÈVRE, Fabrice VÉTILLARD, Iulia GRECU, Lorena DEDIU, Jérôme CACHOT.



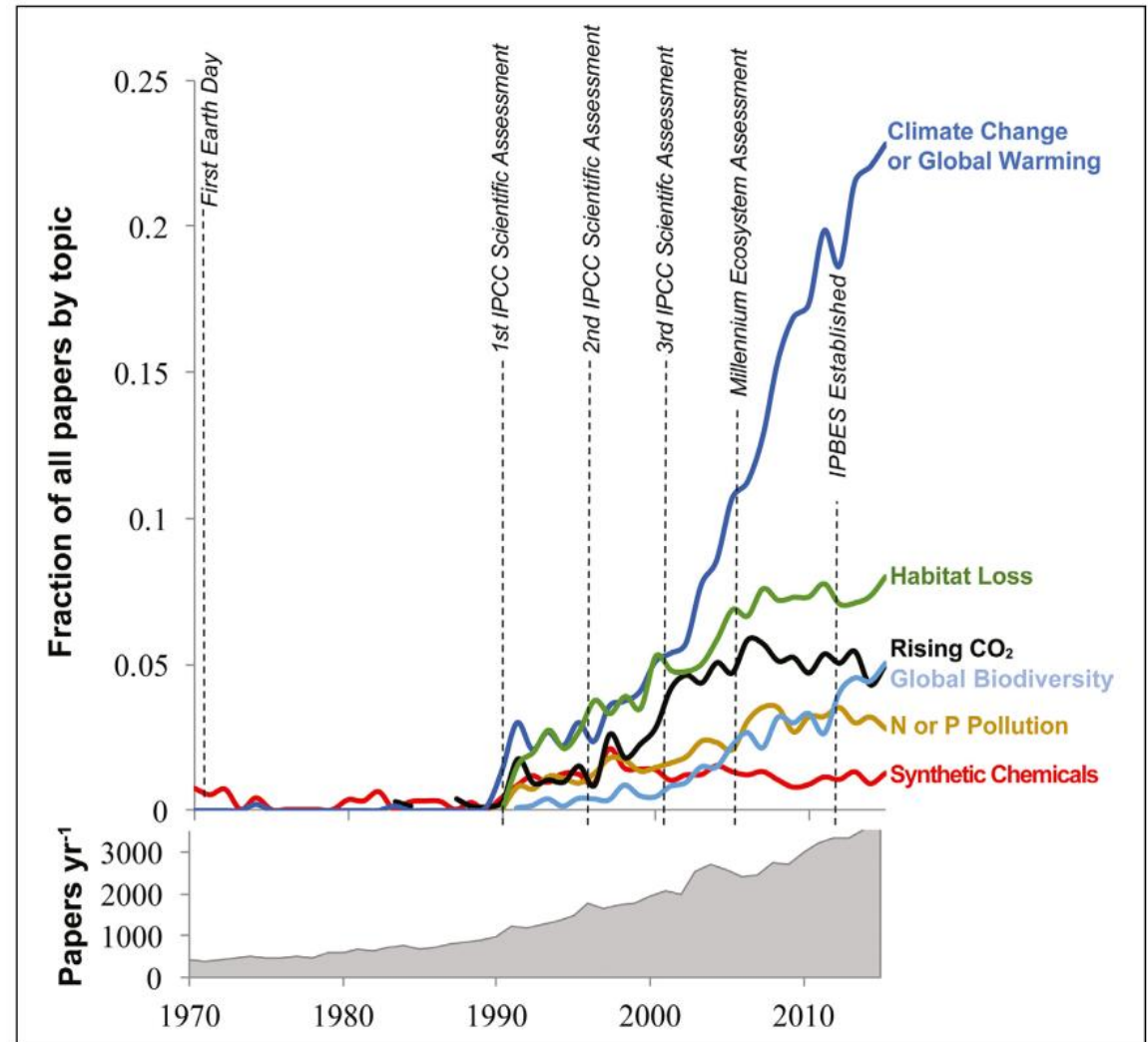
Chemical contamination

- A threat for fish populations?
- To detect such effect: focus on embryos and larvae, or possibly maturation rates.
- As there are so many chemicals in so many different environments, it is difficult to address this issue in the wild (Bancel et al., 2024, 2025)
- What has been studied in terms of toxicity of chemical contamination of water on young sturgeon?
 - Embryos and larvae of *A. brevirostrum* (Kocan et al., 1996) and juveniles *A. gueldenstaedti* (Bickham et al., 1998) exposed to extremely contaminated sediments.
 - Embryos of *A. brevirostrum* and *A. oxyrinchus* exposed to environmental concentrations of PCB (Chambers et al. 2012)
 - Embryos of *A. sturio* exposed to natural spawning grounds substrate (Delage, 2015)
 - Larvae of *A. medirostris* and *A. transmontanus* exposed to selenium (Silvestre et al. 2010) and *A. ruthenus* embryos exposed to lead (Cristina et al. 2018).
 - Embryos of *A. gueldenstaedti* exposed to herbicide Ordram (Tikhonova & Shekhanova, 1983) and embryos of *Huso huso*, *A. stellatus* and *A. persicus* exposed to Glyphosate at different concentrations (Filizadeh and Rajabi Islami, 2011)
 - Embryos of *A. ruthenus* exposed to the neonicotinoïd Imidaclopride (Croc et al, poster ISS10)



Chemical contamination

Surprisingly *chemical contamination has not been included in most analysis of global change, even if increases in synthetic chemical production and diversification outpaced other agents of global change* (Bernhardt et al., 2017)



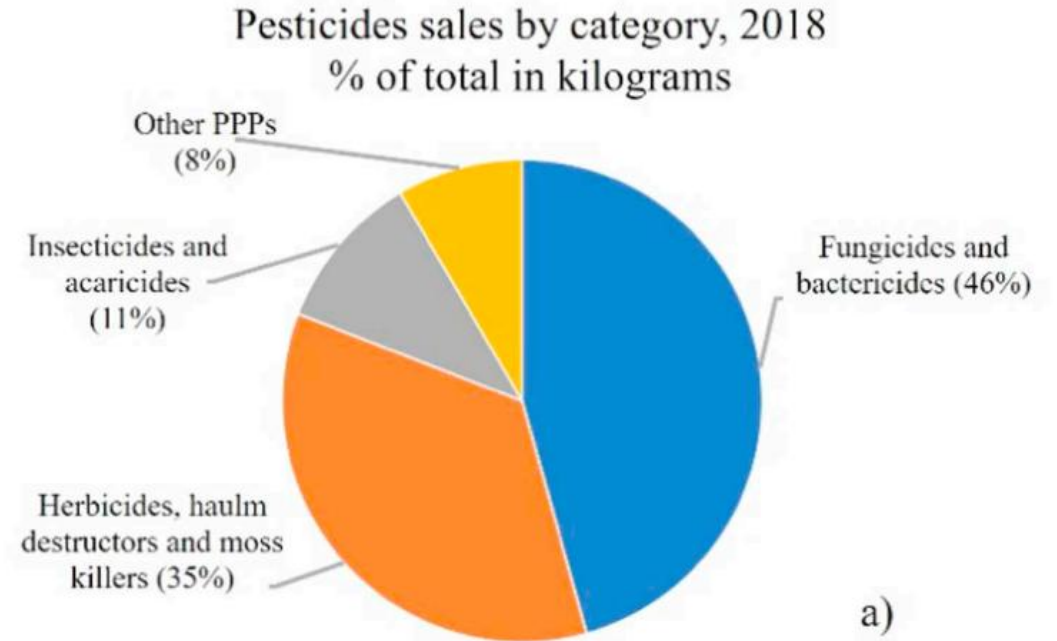
(Bernhardt et al., 2017)

Unfortunately, chemical contamination plays a minor role in conservation ecology research (Sigmund et al., 2023).



Pesticides

- Increased use from 2.3 to 4.6 million tonnes per year between 1990 and 2018 (FAOSTAT, 2020)
- Pesticides and their residues can enter water and soil through surface runoff and rainwater infiltration, and then end up in other environmental compartments far from where they were applied (Belenguer et al., 2014 ; Masiá et al., 2015 ; Ccanccapa et al., 2016a).
- Detected in biota and impact on the health of aquatic organisms (Münze et al., 2017 ; Ernst et al., 2018 ; Liu et al., 2020).
- Fungicides account for about half of all pesticides sales (Tieuova et al., 2020)

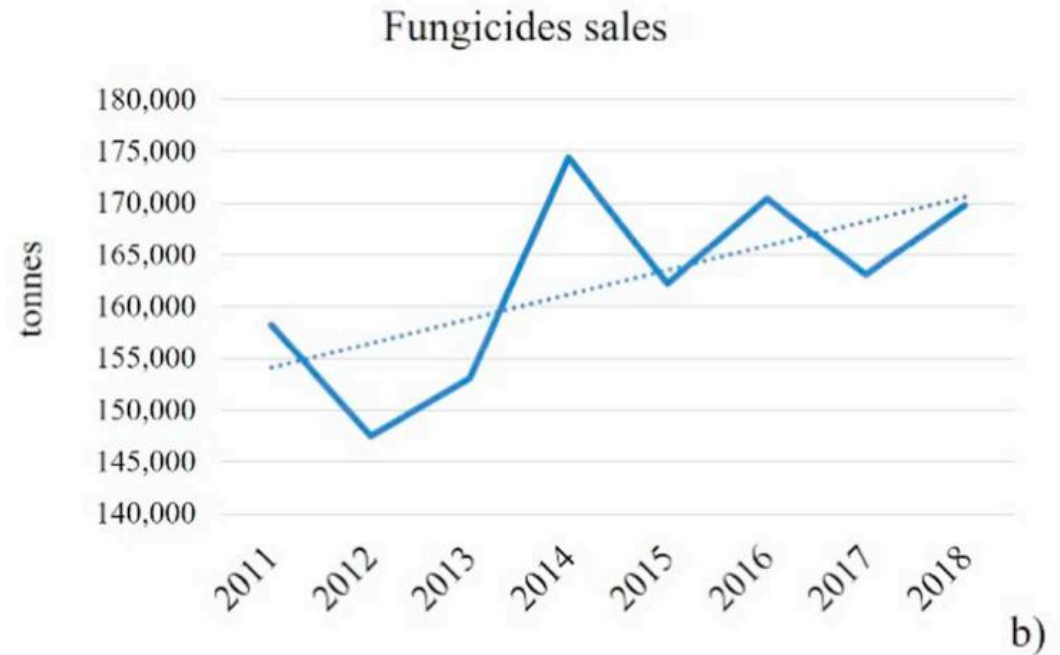


Tieuova et al., 2020



Fungicides

- Fungicides are used against fungus and rot infections, to preserve the freshness of fruit and vegetables after harvesting.
- Demand has risen sharply in recent years
- They have made a major contribution to increasing agricultural productivity, but their excessive use has had repercussions for health and the environment (Rohr et al., 2017; Pimentao et al., 2024).



Tleuova et al., 2020

b)

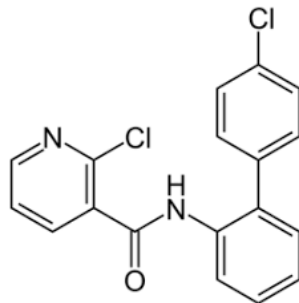


Hypothesis and approach

1. At the current environmental concentrations observed:
 - H0: fungicides protect sturgeon eggs and embryos against the development of fungi without any harmful effects
 - H1: fungicides have harmful effects
- To test this hypothesis we carried out some experiments with two fungicides: Boscalid and Imazalil.

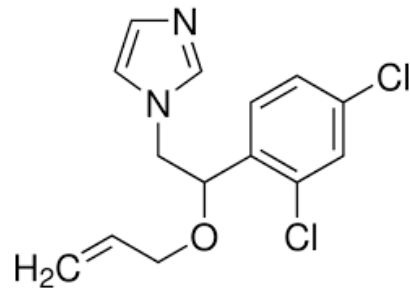


Boscalid



- Carboxamide family.
- Recent fungicide to treat a wide range of crops such as cereals, fruit, vegetables and vines.
- It is commonly found in freshwater and estuarine aquatic environments as well as in sediments and aquatic organisms (Reilly et al., 2012 ; Smalling et al., 2013; Mauser et al., 2025)
- A maximum concentration of **36 µg/l** was detected in three large estuaries in California (Smalling & Orlando, 2011).
- In the Garonne basin, the average concentration in water is 0.087 µg/l, with maximum values of **0.88 µg/l** (Adour-Garonne Water Agency DB)

Imazalil



- Azole family
- Widely used from the beginning of the 70s on a variety of crops, particularly fruit, vegetables and seed potatoes to protect them from fungal infections and rot
- It is commonly found in surface water samples, as well as in non-target aquatic organisms
- Imazalil concentrations detected in water samples reach **0.41 µg/l** from the Ebro basin in Spain (Ccanccapa et al., 2016b) and **0.08 µg/l** from the Danube basin in Romania (Chitescu et al., 2015).
- In highly polluted agricultural areas, they have been detected at **1 mg/l** (Belenguer, et al., 2014 ; Ccanccapa, et al., 2016b).

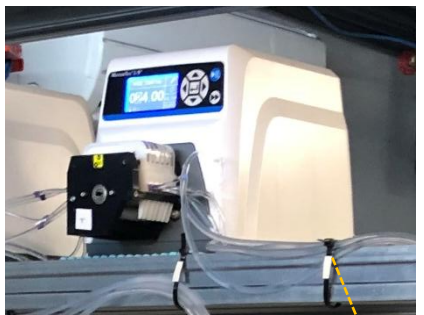


Experimental design

- Fungicides stock solutions diluted to obtain concentrations of **1 $\mu\text{g/l}$** and **10 $\mu\text{g/l}$** of boscalid and imazalil.
- DMSO added to the solutions used (Kais et al., 2013)
- **Experiment A:** contamination phase with contaminants before experiment in order to saturate the device with the desired concentrations.
- **Experiment B :** contamination phase with contaminants before experiment in order to saturate the device with the desired concentrations.
 - † continuous addition of a small quantity of contaminants during exposure.

Experimental device

- Dechlorinated tap water + DMSO
- $15 \pm 1^\circ\text{C}$
- $9 \pm 0.8 \text{ mg/l } [\text{O}_2]$
- $\text{pH } 8.4 \pm 0.1$
- Nitrite below 0.015 mg/l
- 12h/12h photoperiod

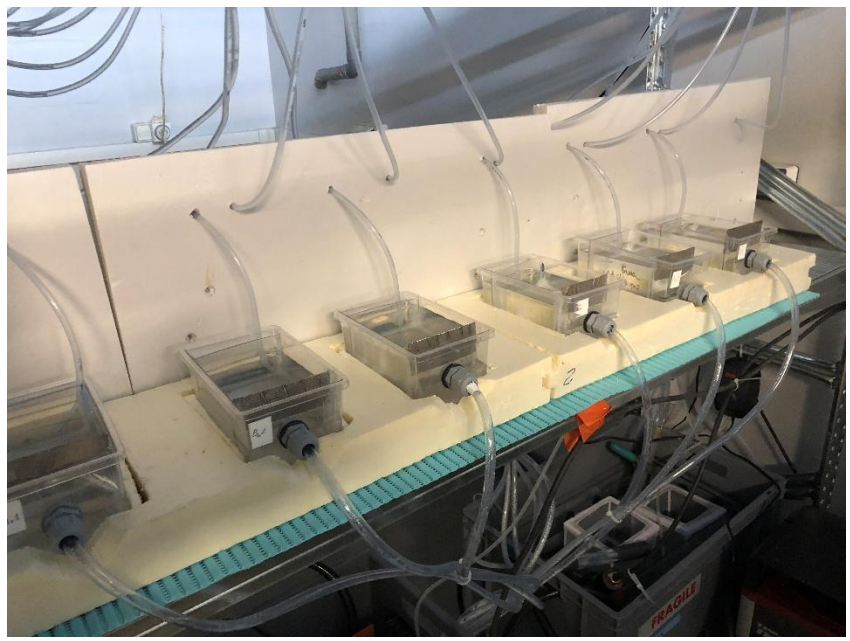
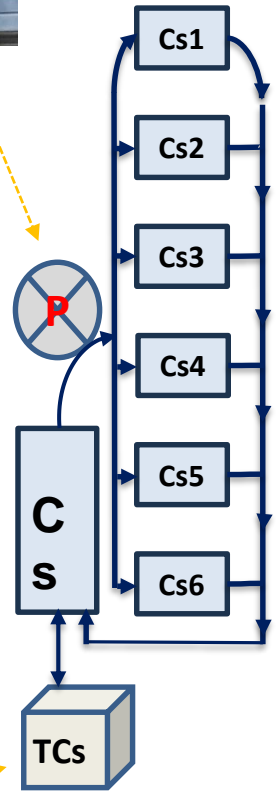


Peristaltic pump

e.g. Control solvent circuit



Buffer tank



6 incubators

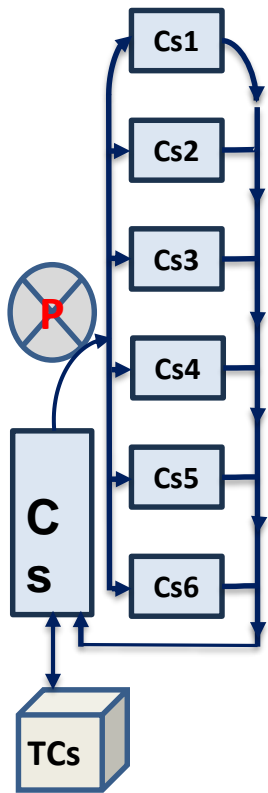


Temperature controller device

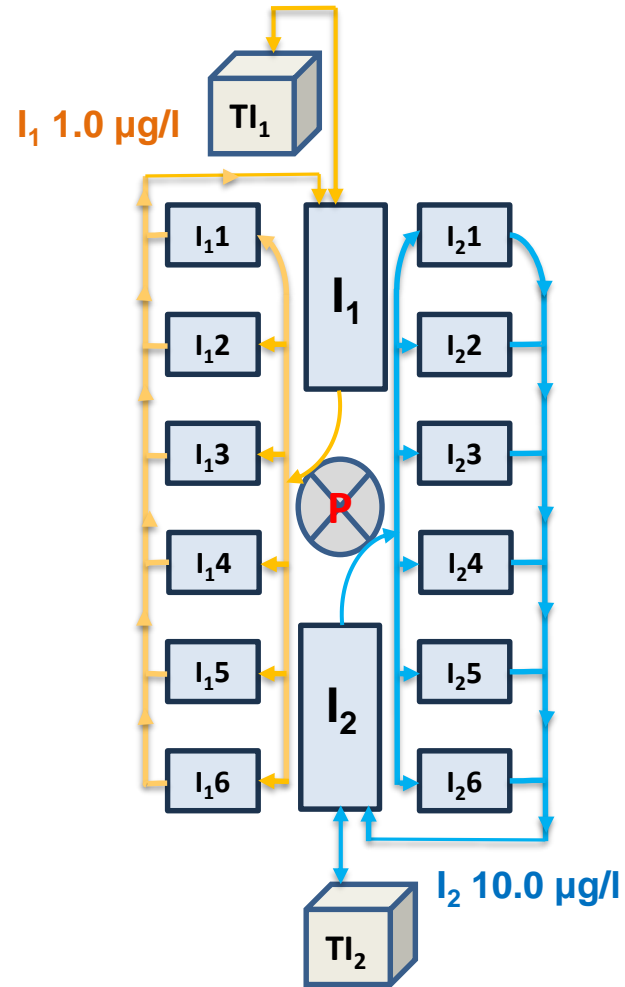


Experiment A: 5 conditions and 6 incubators for each condition

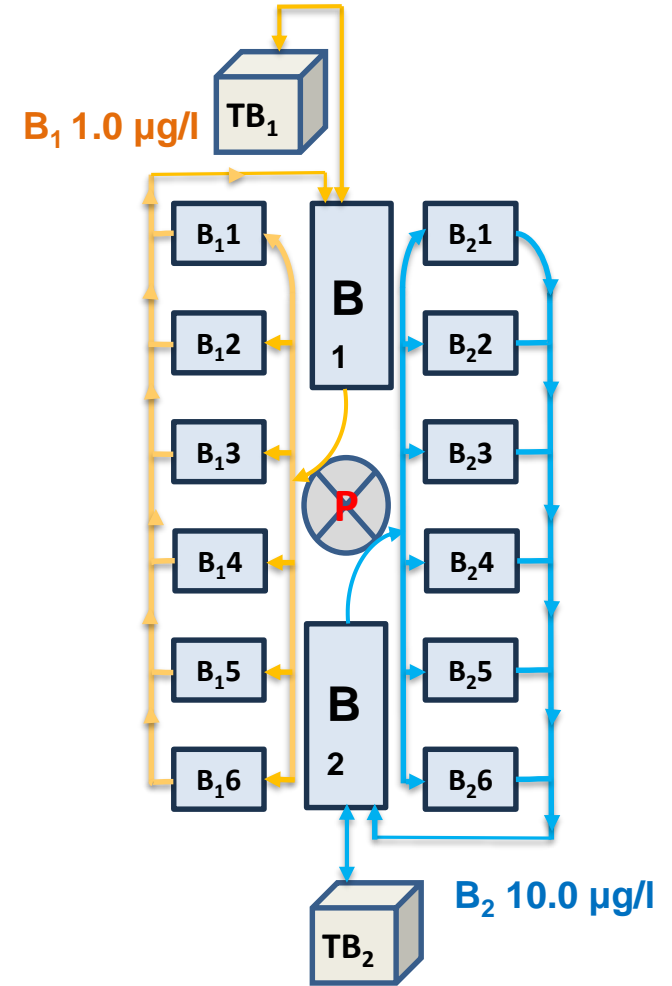
Solvent control



Imazalil



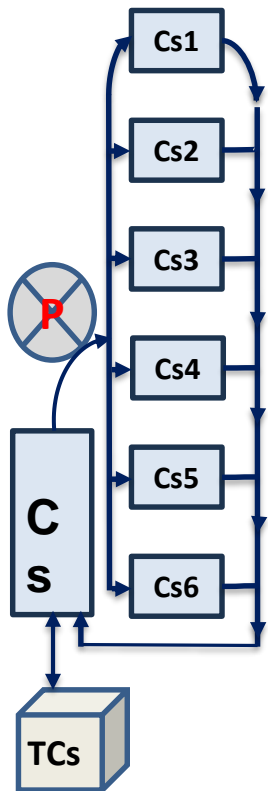
Boscalid



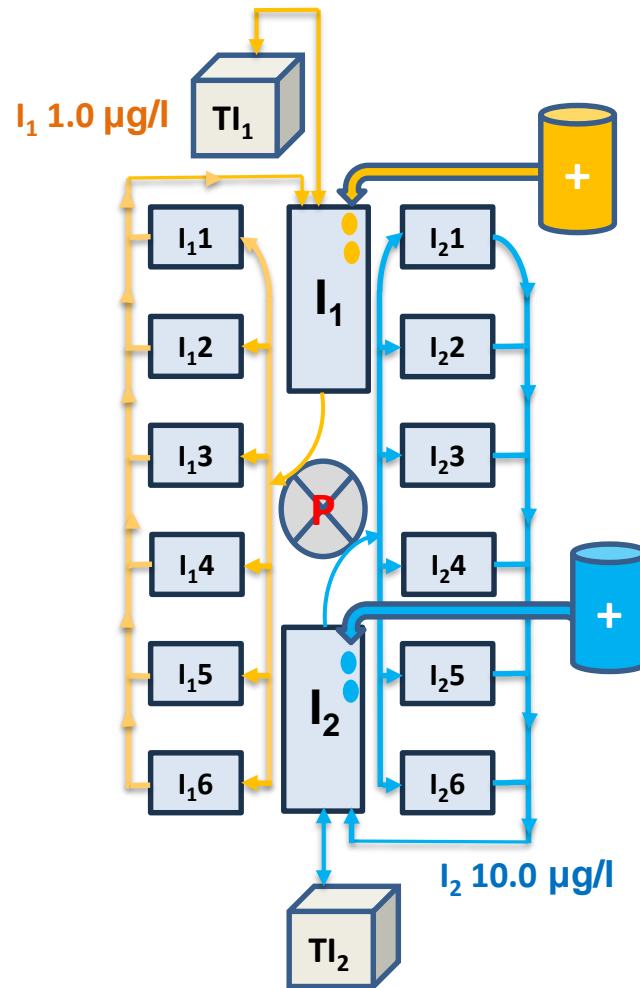


Experiment B: 5 conditions and 6 incubators for each condition

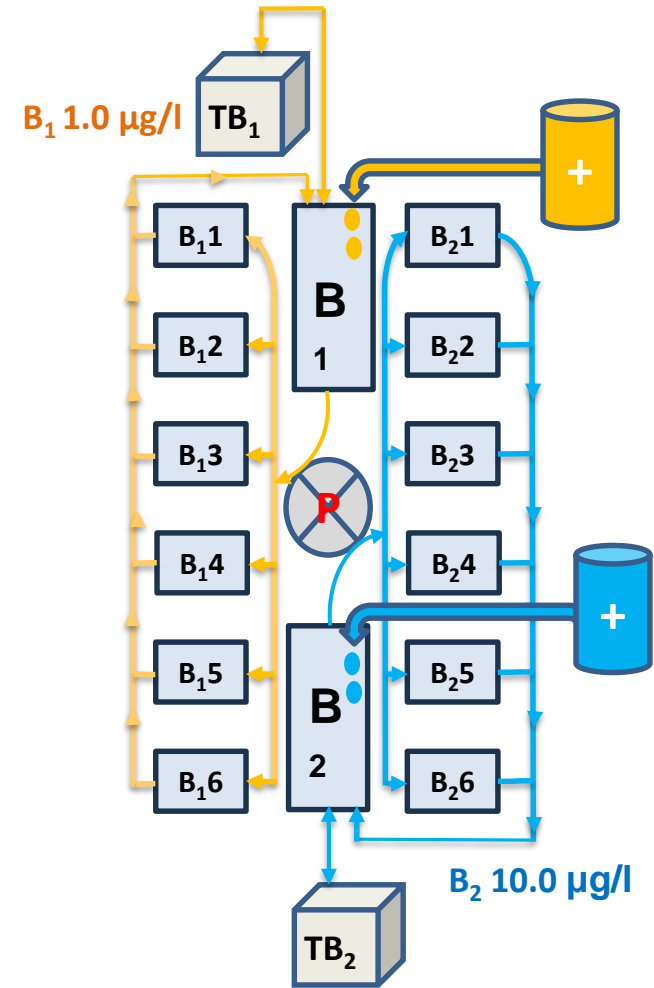
Solvent control



Imazalil



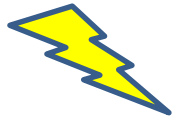
Boscalid





Obtaining the eggs (Exp. A and B)

Hormonal stimulation



4 females

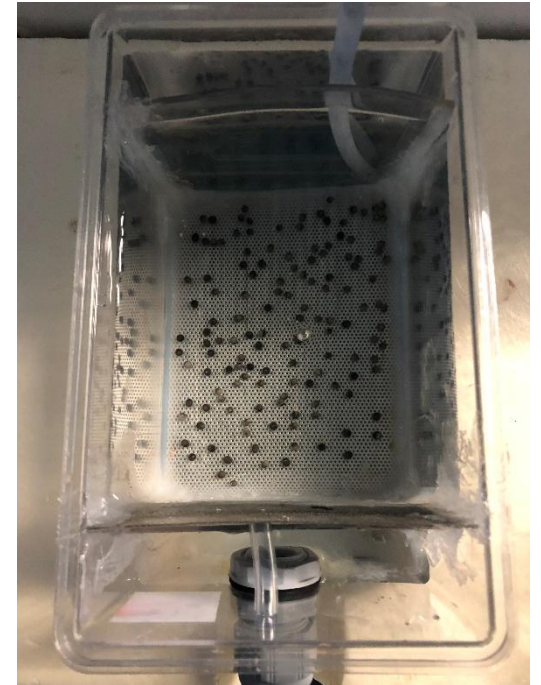


4 males

100 eggs quickly placed in incubator



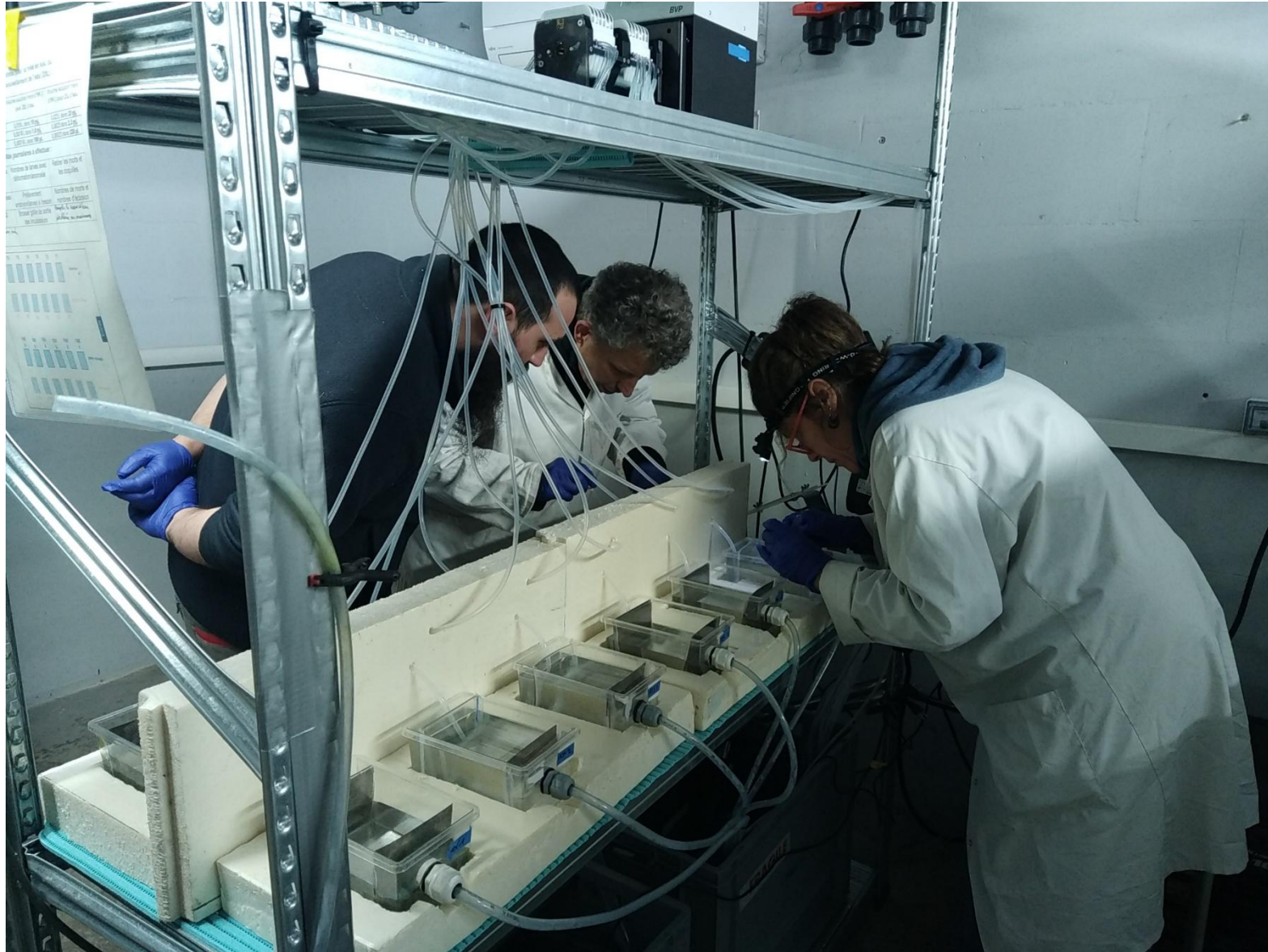
- Pool of oocytes and sperms
- Fertilisation in small batches (100 oocytes + 125 μ l sperm + 10 ml water)





Twice-daily development and survival monitoring (Exp. A and B)

- Mortality
- Hatching

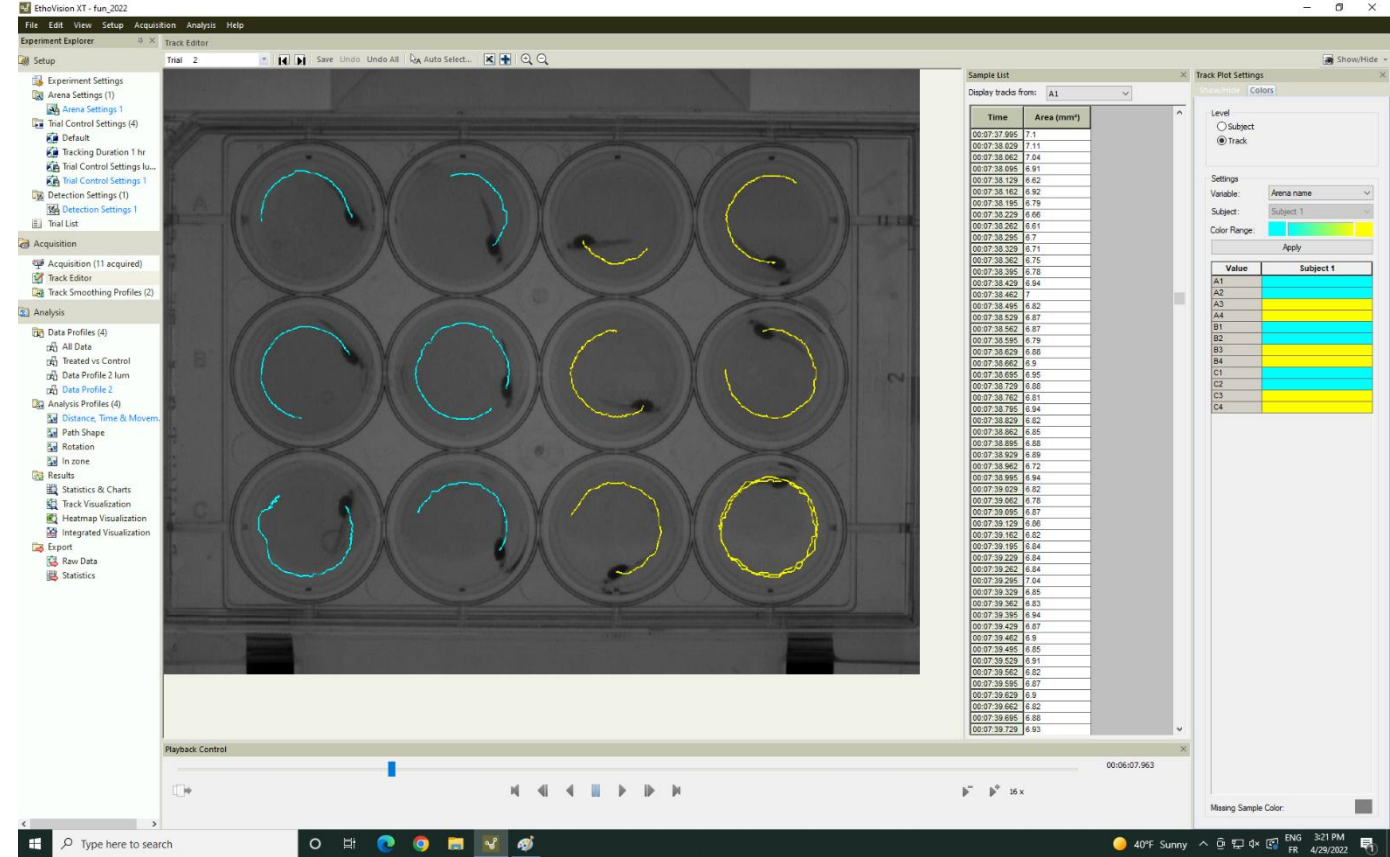




Behavior (EXP. A)



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- **At 1dph**
- **6 larvae and 5 replicates by condition**

- Recording chamber from the DanioVision system equipped with an infrared camera.
- Videos acquired and processed with Ethovision XT tracking software 16.0
- (Noldus Information Technology B.V ®)



Biometry and heart rate measurement (Exp. A)

- **At 5 dph heart rate recording of individuals**
 - 6 anaesthetised larvae, 5 replicates by condition
 - 3 video records (Nikon DS-Fi3 and NIS-elements D 4 40 imaging software)
 - 30 seconds
- **At 6 dph, biometric analysis**
 - 6 anaesthetised larvae, 5 replicates by condition
 - sacrificed using an isoeugenol solution
 - binocular magnifier (Nikon SMZ1270, with Nikon DS-Fi3 camera and NIS-Elements D 4.40.00 Imaging Software)
 - measurement scale using a graticule (100 x 0.1 mm, Tonbridge, Kent, UK).
 - Total length, head and body
 - Identification of abnormal individuals



Genetics (Exp. B)

- Five embryos or larvae for each condition were collected at different times: 4, 72, 180, 216, 240 and 288 hpf.
- Samples stored in RNAlater in the refrigerator at 5°C overnight and then frozen at -20°C.



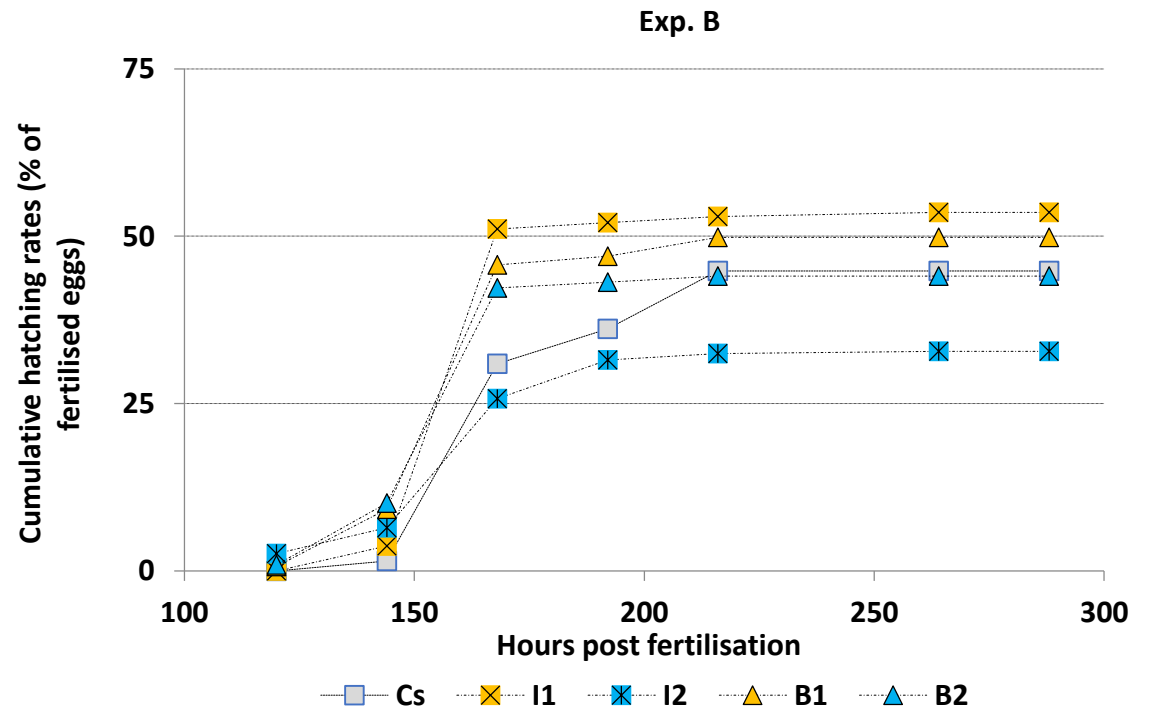
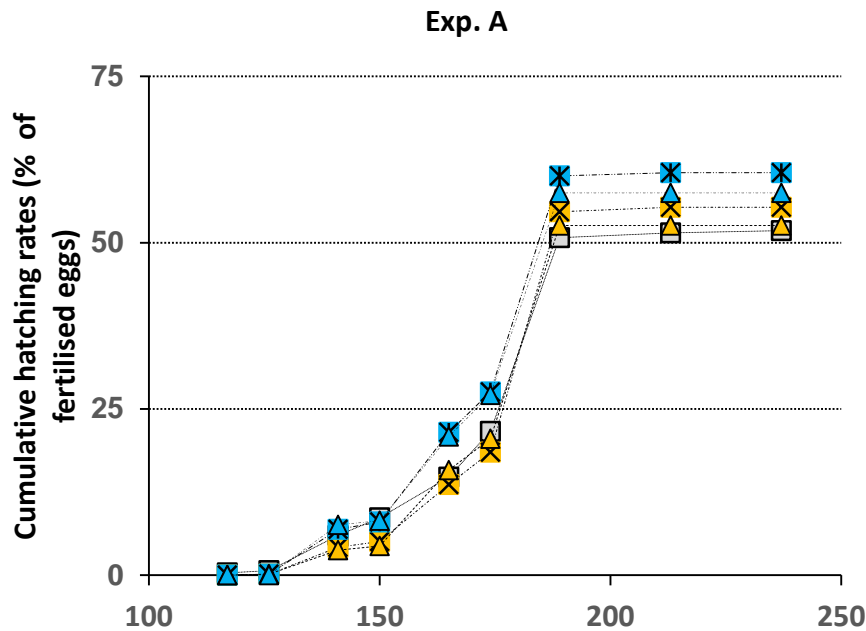
Concentrations actually measured

$\mu\text{g/l}$	Imazalil		Boscalid	
Target	1.00	10.00	1.00	10.00



Kinetics and hatching rates (Exp. A and B)

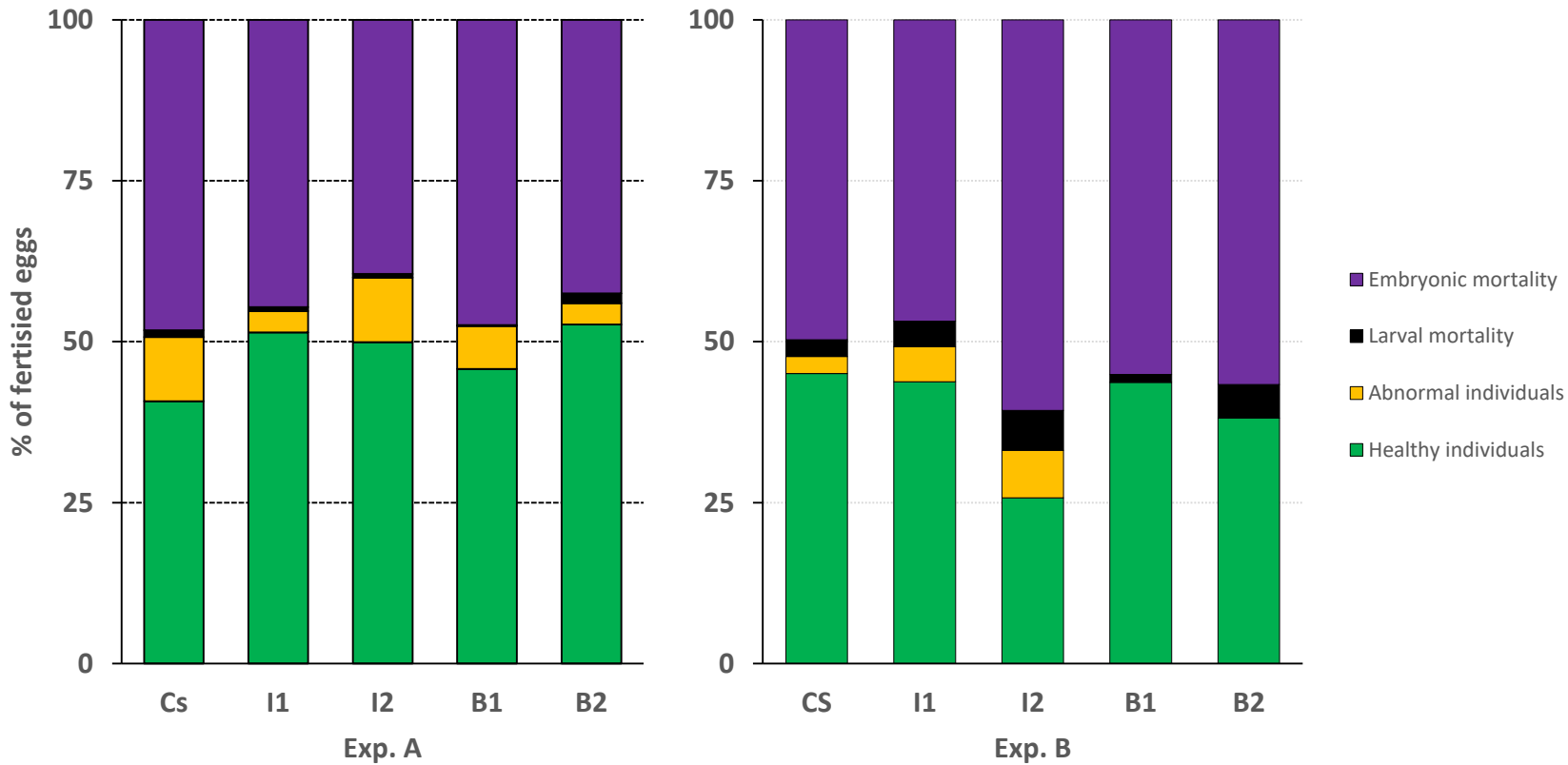
- Similar kinetics in both experiments (from 5 to 7 dpf)
- No difference between conditions in Exp. A
- **Significantly lower hatching rate with the highest imazalil concentration in Exp. B (I2_B)**





Fate of the eggs (Exp. A and B)

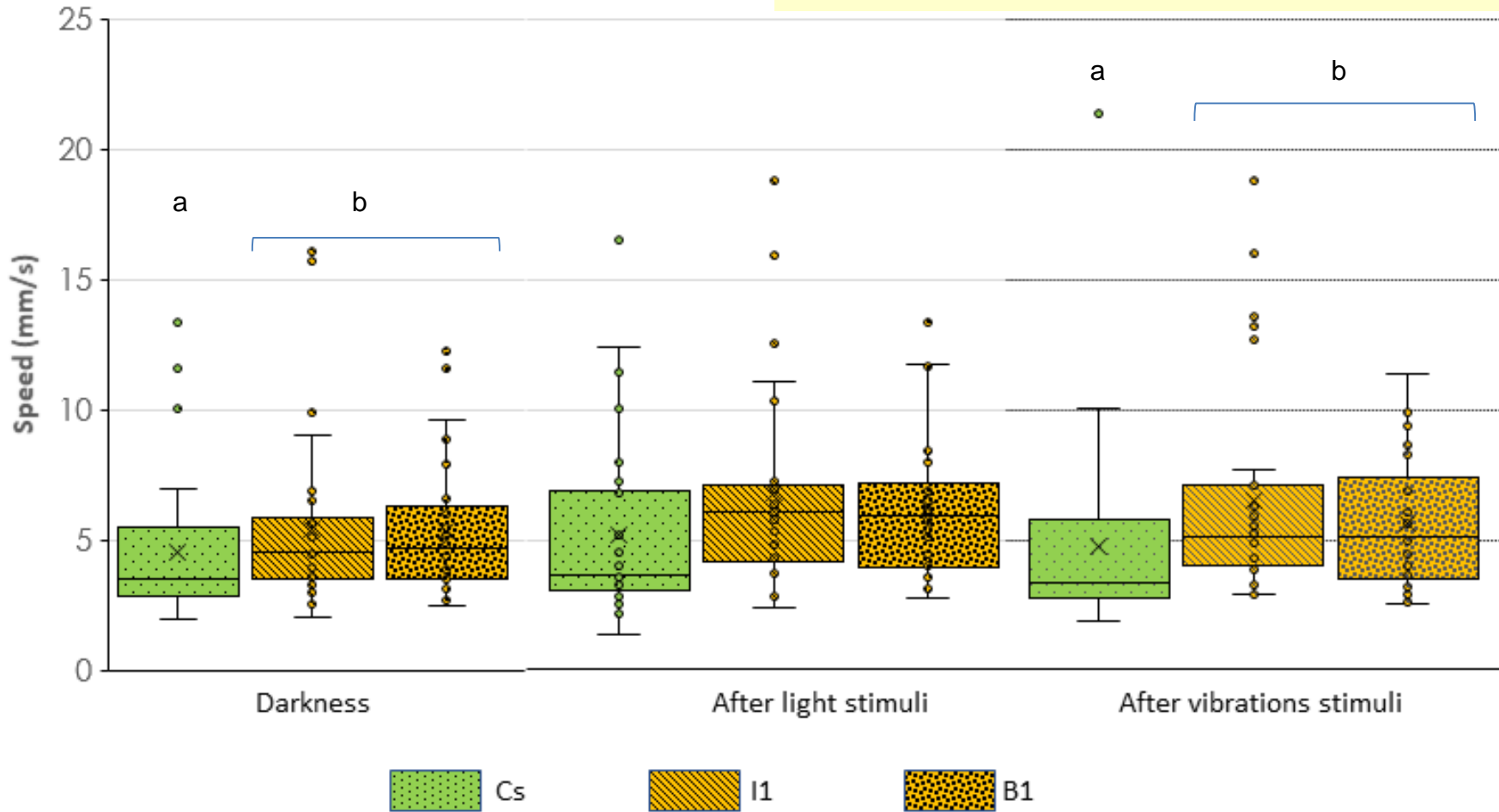
- High embryonic mortalities
- No difference between conditions in Exp A.
- **Imazalil₂_B is the most drastic condition**
- Few abnormal individuals
- Very little variations between replicates within each condition





Swimming behaviour (Exp. A)

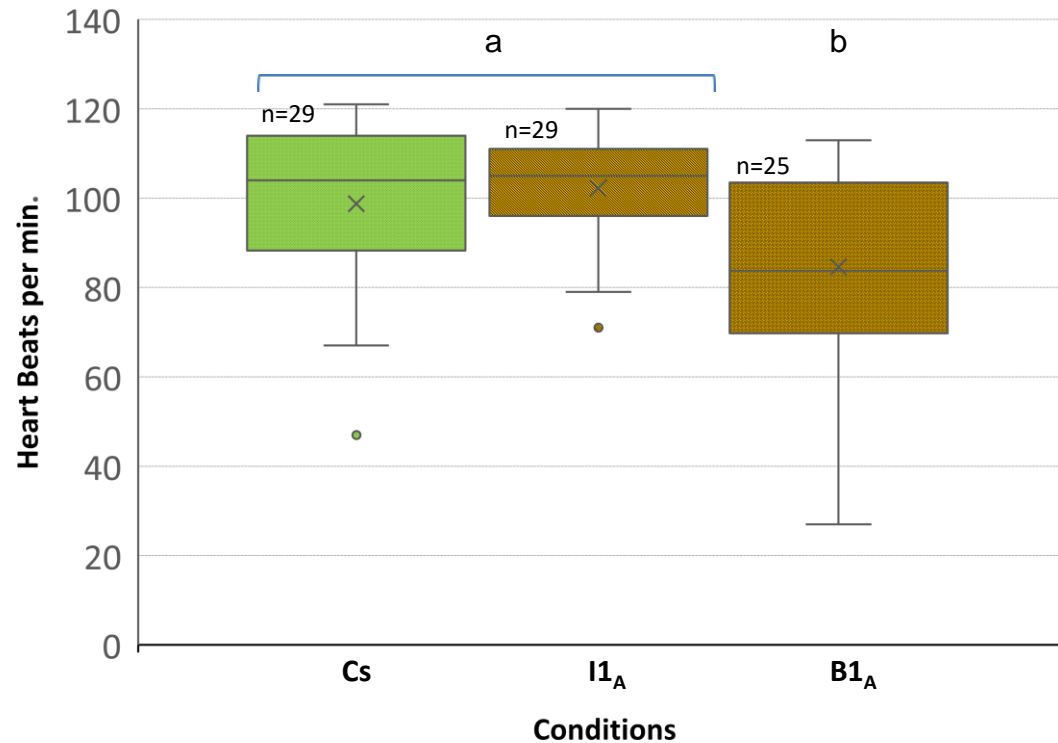
- In darkness (before stimuli) and after vibrations stimuli, exposed individuals are significant faster than control ones





Heart rate (Exp. A)

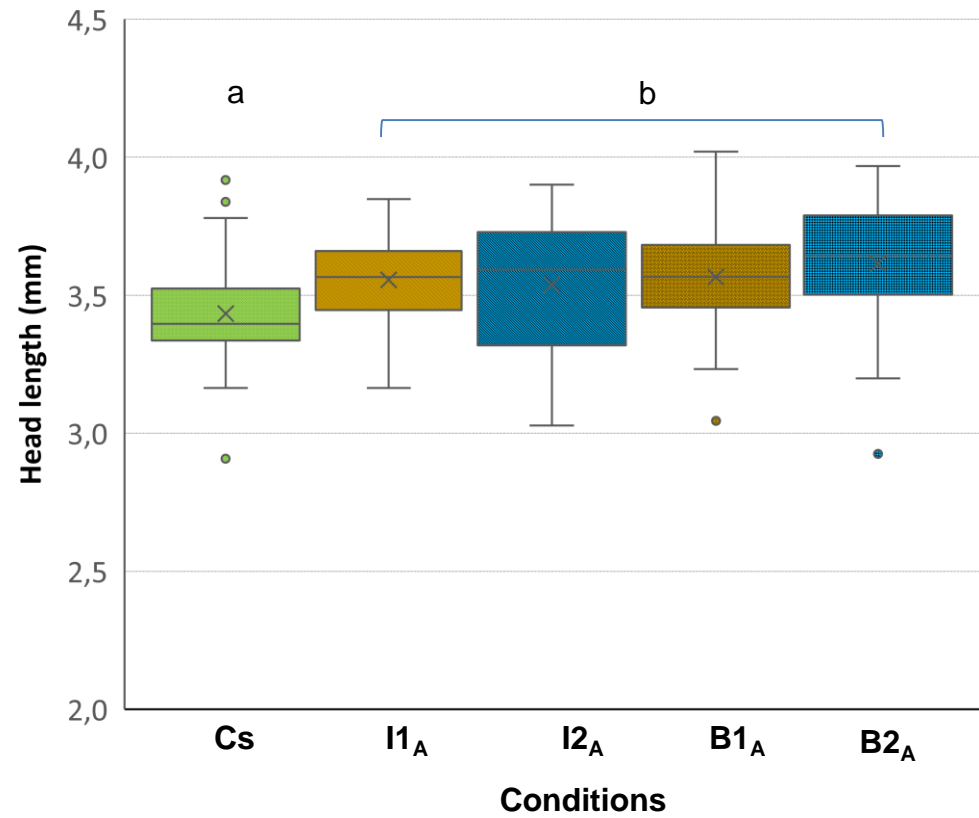
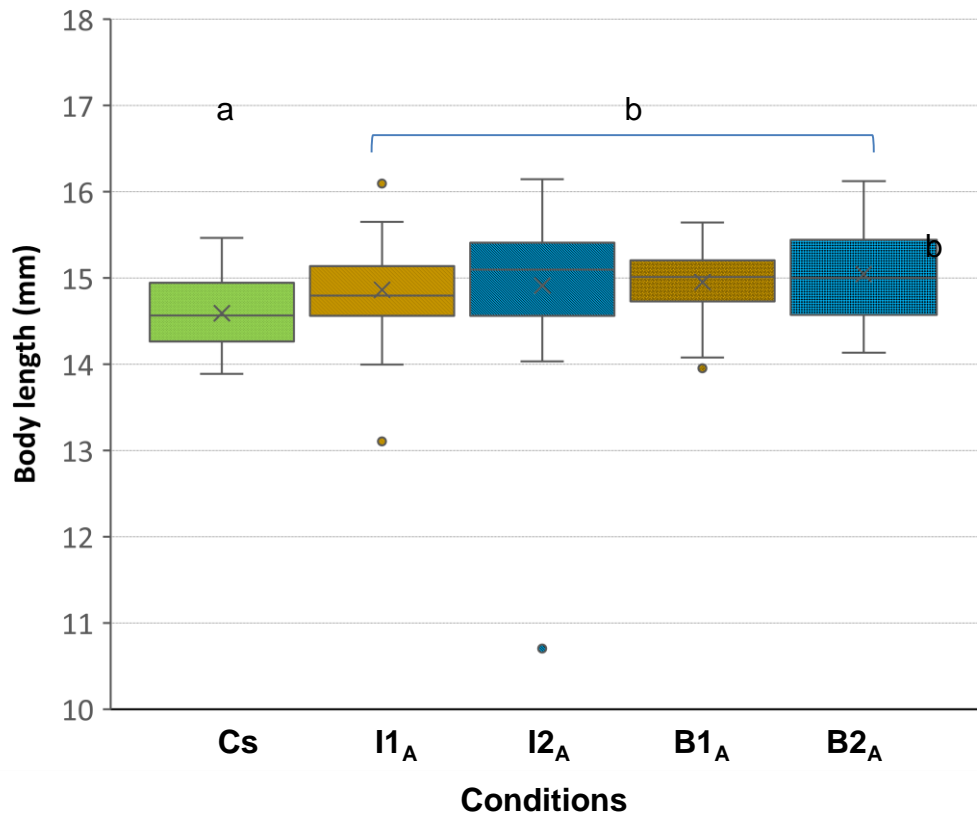
- Significant difference between the heart rates of the control and B1_A larvae.
Exposure to boscalid (B1_A) significantly reduces heart rate of larvae
- No difference between the imazalil condition and the control





Biometry (Exp. A)

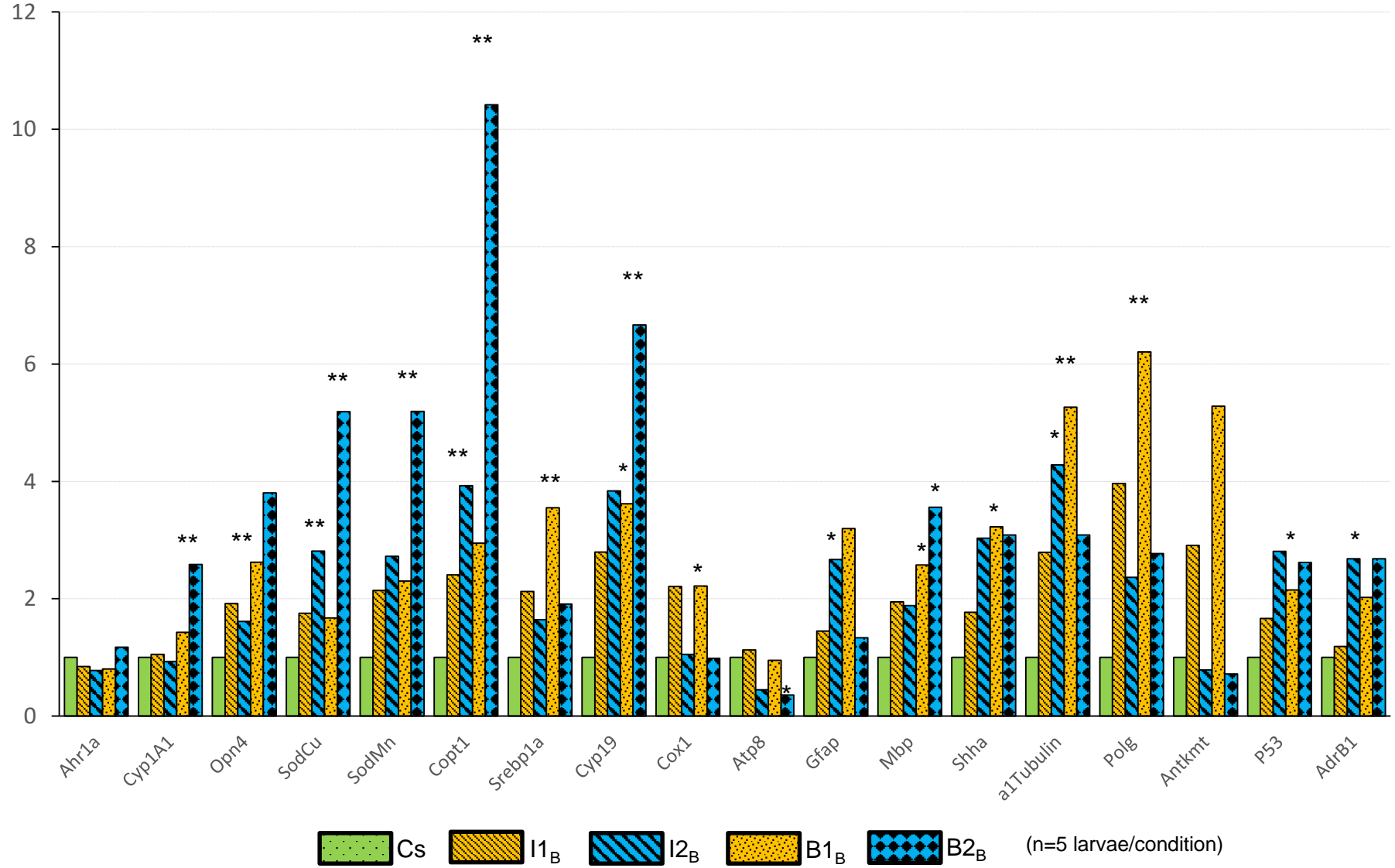
- At the same age, individuals from imazalil and boscalid conditions are **significantly larger** than those from the control
- No difference in ratio head/body length





Genetics Exp. B.

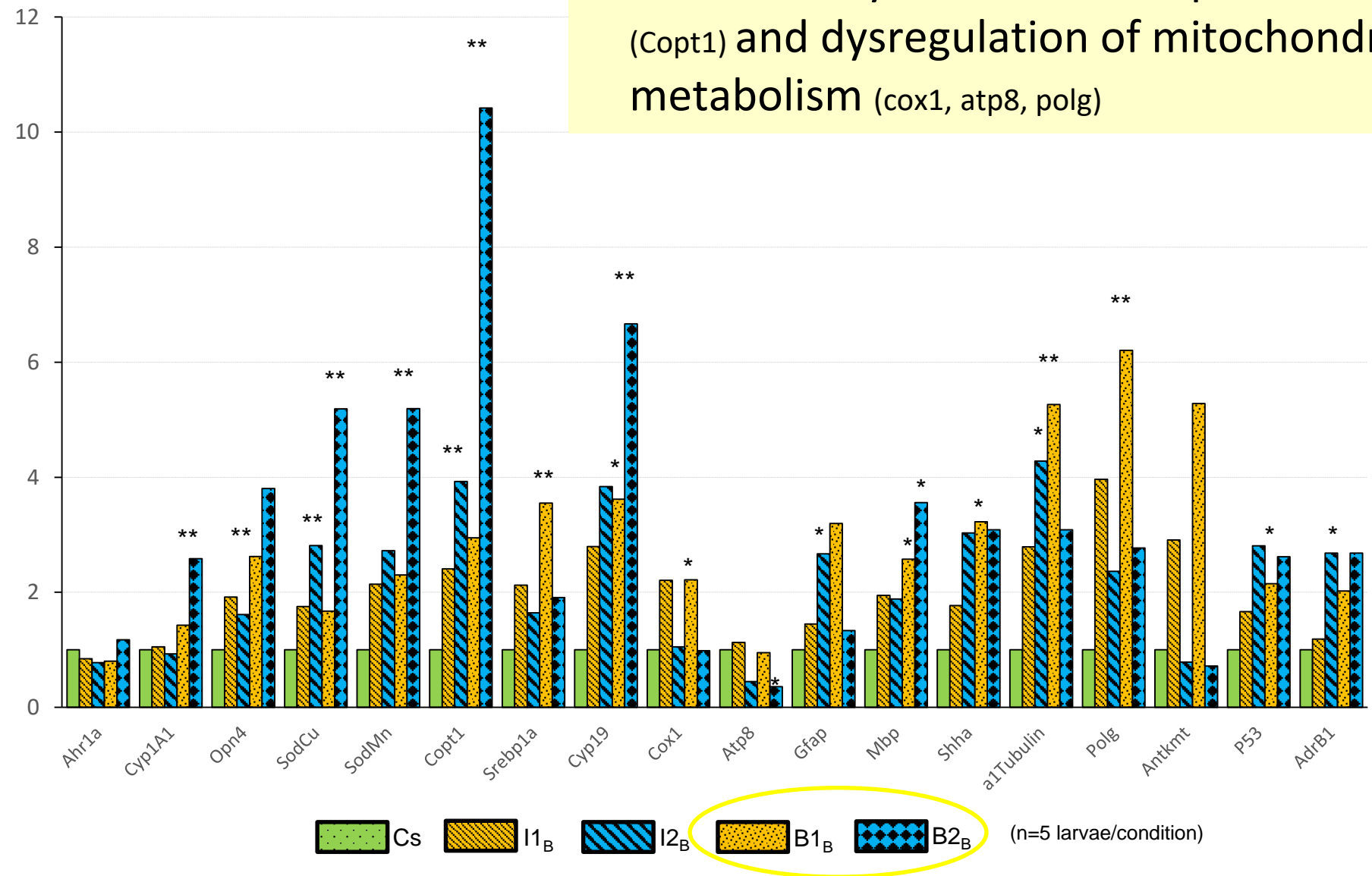
- Variations in gene expression levels were observed for practically all the 18 genes selected





Genetics Exp. B.

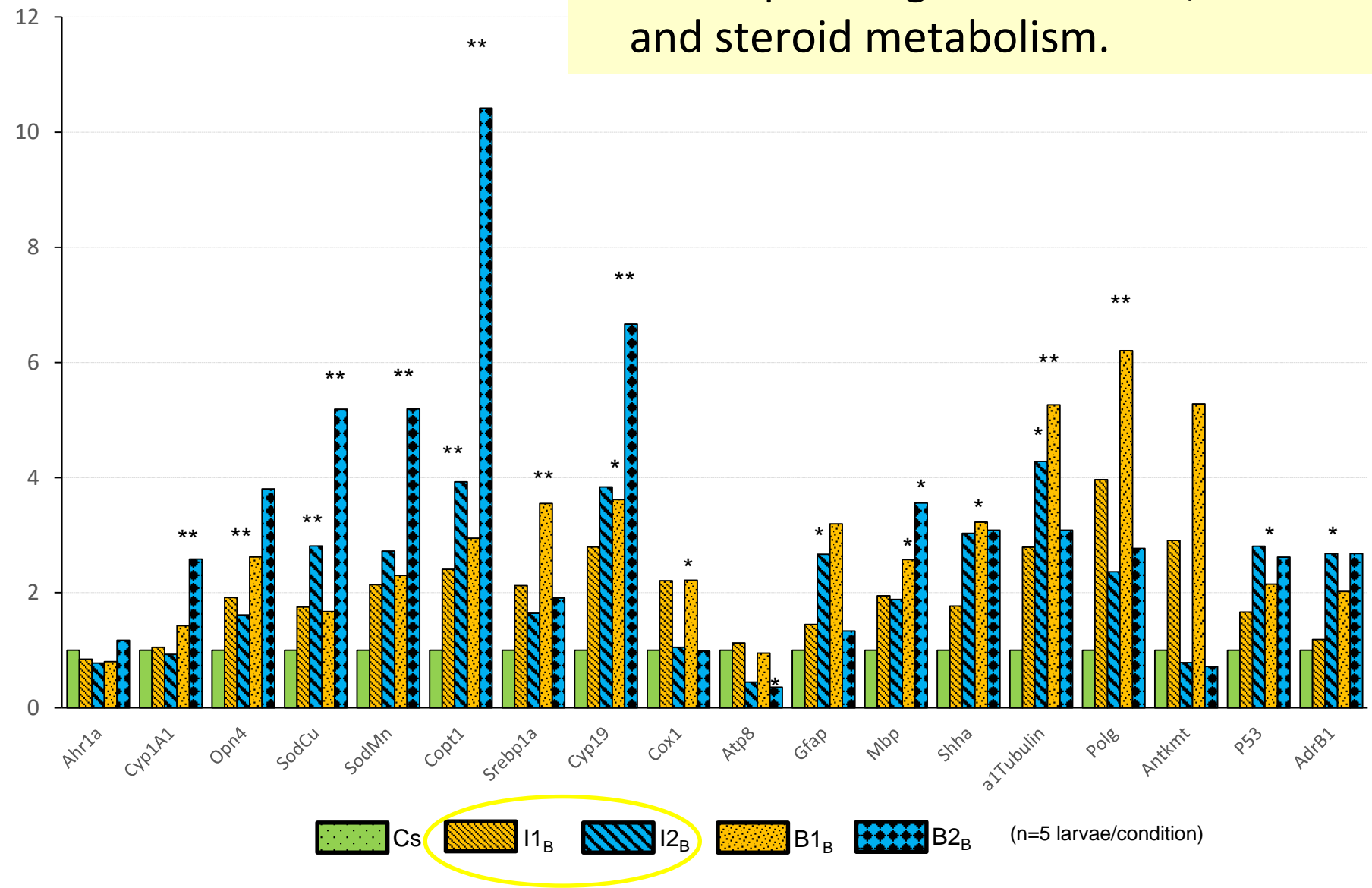
- Boscalid led to overexpression of genes involved in defence against oxidative stress (*sodCu*, *sodMn*), xenobiotic synthesis (*cyp1a1*), lipid metabolism (*Copt1*) and dysregulation of mitochondrial metabolism (*cox1*, *atp8*, *polg*)





Genetics Exp. B.

- Imazalil altered genes involved in defence against oxidative stress (*sodCu* and *sodMn*), overexpressing *copt1* and *cyp19*, involved in lipid and steroid metabolism.





Discussion, conclusion

		Imazalil			
		I1 _A	I1 _B	I2 _A	I2 _B
Lethal effects	/Control solvent				
	[] µg/l	0.22	0.62	1.47	8.05
	Hatching	=	=	=	-
	Embryonic mortality	=	=	=	+
Sublethal effects	Larval mortality	=	=	=	=
	Heat rate	=	na	na	na
	Biometry	+	na	+	na
	Swimming speed, mobility	+	na	=	na
	Over expression of genes	na	+	na	+



Discussion, conclusion

	Imazalil				Boscalid			
	I1 _A	I1 _B	I2 _A	I2 _B	B1 _A	B1 _B	B2 _A	B2 _B
/Control solvent								
[] µg/l	0.22	0.62	1.47	8.05	0.56	1.40	5.28	24.20
Lethal effects	Hatching	=	=	=	-	=	=	=
	Embryonic mortality	=	=	=	+	=	=	=
	Larval mortality	=	=	=	=	=	=	=
Sublethal effects	Heat rate	=	na	na	na	-	na	na
	Biometry	+	na	+	na	+	na	+
	Swimming speed, mobility	+	na	=	na	+	na	=
	Over expression of genes	na	+	na	+	na	+	na



Conclusion

- Operational experimental device for exposing embryos to contaminants
 - Controlled environment (temperature, oxygen, water flow...)
 - Interesting approach for getting an initial idea of the effects, but not very realistic (fungicides alone)
 - Difficulty in achieving and maintaining expected concentrations (absorption, chemical changes)
- New information about the effects of these fungicides, imazalil and boscalid
 - No evidence of any protection offered by these fungicides at environmental concentrations
 - Both have harmful consequences
 - At the maximum concentration of imazalil tested we observed a lethal effect



Thank you very much for your attention