Long-term exposure to polybrominated diphenyl ethers (PBDEs) or polychlorinated biphenyls (PCBs) through diet affects embryonic development of Common Sole (*Solea solea*)

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Background

How environmental conditions may influence fish survival and eventually affect the recruitment dynamics of fish populations?

❖ **Previous studies**

- *in situ:* levels and patterns of contamination of three French coastal fish nurseries [1]
- experimental controlled studies of dietary POPs exposure in common sole juveniles under long-term exposure: toxicokinetics [2]

❖ **Common Sole** *Solea solea,*

selected as a model that incorporates the physiological potential impact of organic contaminants

- flatfish of economical relevance in France and Europe; benthic habitat; spawning grounds not far from the nursery areas located in coastal environments or estuaries, that make this fish particularly vulnerable to environmental toxicants
- spawners: batches of eggs, with translucent pelagic eggs; reared fish





Introduction

In the natural environment, persistent organic pollutants (POPs) do not reach high concentrations in surface waters due to their lipophilicity so the main route of exposure is food. This property causes them to be retained in lipid-rich organism tissues. Organic contaminants have been shown to bioaccumulate with increases in trophic levels. For juveniles and adult fishes, the main exposure route is via consumption of contaminated prey.

At the earliest stages of development, maternal-transfer through lipid reserves of the oocytes can be a significant pathway for embryonic exposure to contaminants such as polychlorinated biphenyls **(PCBs)** [3] and polybrominated diphenyl ethers **(PBDEs)**.

A study was designed to evaluate toxicological responses at an environmental exposure concentration, to mixtures of these persistent organic pollutants that have potential biological effects.

Objectives

We present a method used to incubate common sole egg batches to assess the survival of early stages, the developing embryos and the hatching success: these are preliminary results. This is an additional study to that of the physiological function impact in adult fishes.

Materials & Methods

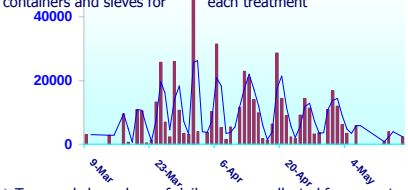
❖ Fish and experimental conditions

-3 Sole broodstocks (replicate x2; ♀: 267g ♂: 207g)
-seawater open system, wastes treated with active carbon column
-ambient 9.3 to 15°C temperature, 35‰ salinity, 12L/12D photoperiod

-3 diets of commercial pellets (CP 40%; CF 23%) coated with mixtures of POPs congeners diluted in an evaporated isooctane solvent
-**PBDEs** (BDE 47, 100, 153, BDE 209) diet
-**PCBs** (CB 149, 118, 153, 105) diet or
-with solvent alone, **Control** diet
-thirty-six month period of experimental dietary exposure
-ad libitum feeding, at 0.2 to 0.6% daily biomass

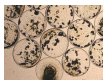
❖ Spawns

-spawning naturally overnight, eggs collected in a mesh collector fitted to the tank, transferred using individual labelled stainless steel containers and sieves for each treatment

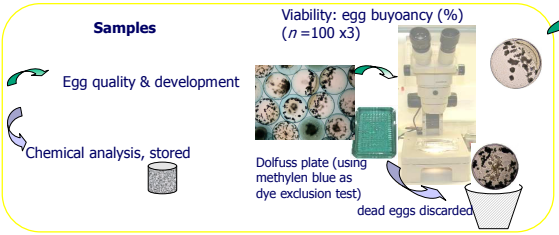


➢Temporal chronology of daily spawns collected from one tank: March to May 2011

❖ Egg viability assessment

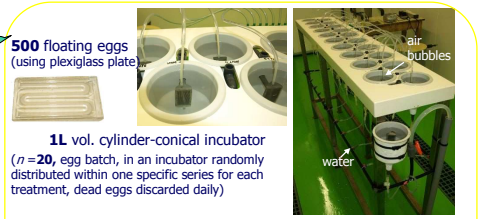


Sole egg characteristics:
moisture 92.7% fresh weight (FW)
dry matter (DM) 7.3% FW
lipid content 11.7% DM
size Ø 1.27 mm, oil goblets



❖ Egg incubation

using an open seawater (desaturated, filtered & UV sterilised) incubation sytem (water flow rate per incubator: 67.5 ml.min^{-1}, CV: 2.5%) at 12 to 15° C temperature



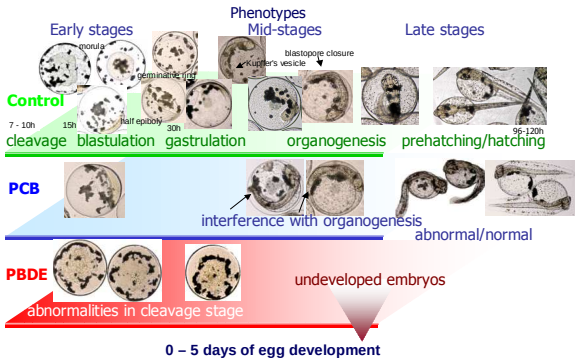
1L vol. cylinder-conical incubator
(*n* =**20,** egg batch, in an incubator randomly distributed within one specific series for each treatment, dead eggs discarded daily)

Incubators set in a fibreglass table (designed for embryonic development in fishery biology studies for farmed fish species)

Results

❖ Embryonic development

(at 12-15.5°C checked under light microscope, magnification x40)

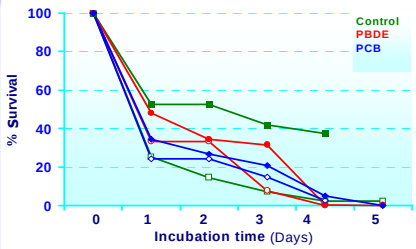


➢ Common sole embryonic development: snapshot of
- normal phenotypes in the **Control** group
- cases of abnormalities in the **PCBs** group
- apparent alterations of cell cleavages in the **PBDEs** group

➢ Hatching success (per replicate group) was observed in the **Control** and **PCBs** egg batches. No hatch was observed in the **PBDEs** group, that suggested that the organic contaminants must have altered egg constituents prior to spawning time.

❖ Survival

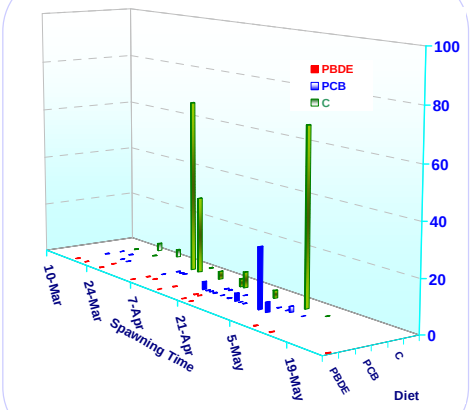
(floating eggs around 7-10h post-spawn incubated)



➢ Common sole embryo mortality following parental exposure to non **(Control)** or contaminated diets of different organic contaminants (**PBDEs** – **PCBs**). Each survival curve is the mean of two to four incubations of two phases throughout the spawning period. Variable patterns of egg survival were observed over the incubation period.

❖ Hatching success

(hatching rate (%) per egg batch incubated, batch characterised by mean egg buoyancy of **PBDE:** 55.3% (*n* =14), **PCB:** 48.0% (*n* =26), **C:** 65.3% (*n* =13))



❖ Features

Advantages	Disavantages
Common sole embryos	
~large number of offspring	~lack of molecular markers (recently performed, *Cousin et al.*)
~translucent small marine pelagic embryos	
~well characterised developmental stages (*Ramos, 1986*)	
~previous ELS study (*Foekama et al, 2008*)	
Incubation conditions	
~mimicking natural environment of temperate & cold marine flatfishes	~space requirement
~controlled & reproducibility of experimental conditions	~number of bioassays limited

Conclusion

Incubation is a valid tool for ecotoxicological studies on marine fish. Contaminants may accumulate in the gonads of broodstocks and affect the gametes and their quality. Reproductive traits were modified or reduced: exposure to PBDES in females produced non developed eggs. The cause of decreasing capacity leading to the absence of the fertilization process, should be elucidated. Sole eggs appeared to be sensitive to pollutants and can be used to evaluate the possible toxicity of parental contaminanted feeding. This is a preliminary study. Further studies will later describe the mechanisms involved in the deregulation observed.

Perspectives

Future work will include chemical as well as biochemical analysis to assess any possible hormonal disruption in broodstocks. Measures of concentrations of PCBs or PBDEs (congeners or hydroxylated forms), in the whole-egg are required to explain the induction of non developed embryos, as well as in different tissues (muscle, liver and female gonads). Morphological alterations or mortality rates are often described as non-specific criteria for eggs. Future studies should be conducted on the molecular effects for this species to confirm the impact of toxicants.

References

(1) Munschy et al., 2011. Science of the Total Environment 409, 4618-4627.
(2) Eichinger et al., 2010. Journal of Sea Research 64, 373-385.
(3) Daouk et al., 2011. Aquatic Toxicology 105, 270-278.

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