



ICMGP 2024  
CAPE TOWN • SOUTH AFRICA • 21 - 26 JULY

**Jabi Zabala**

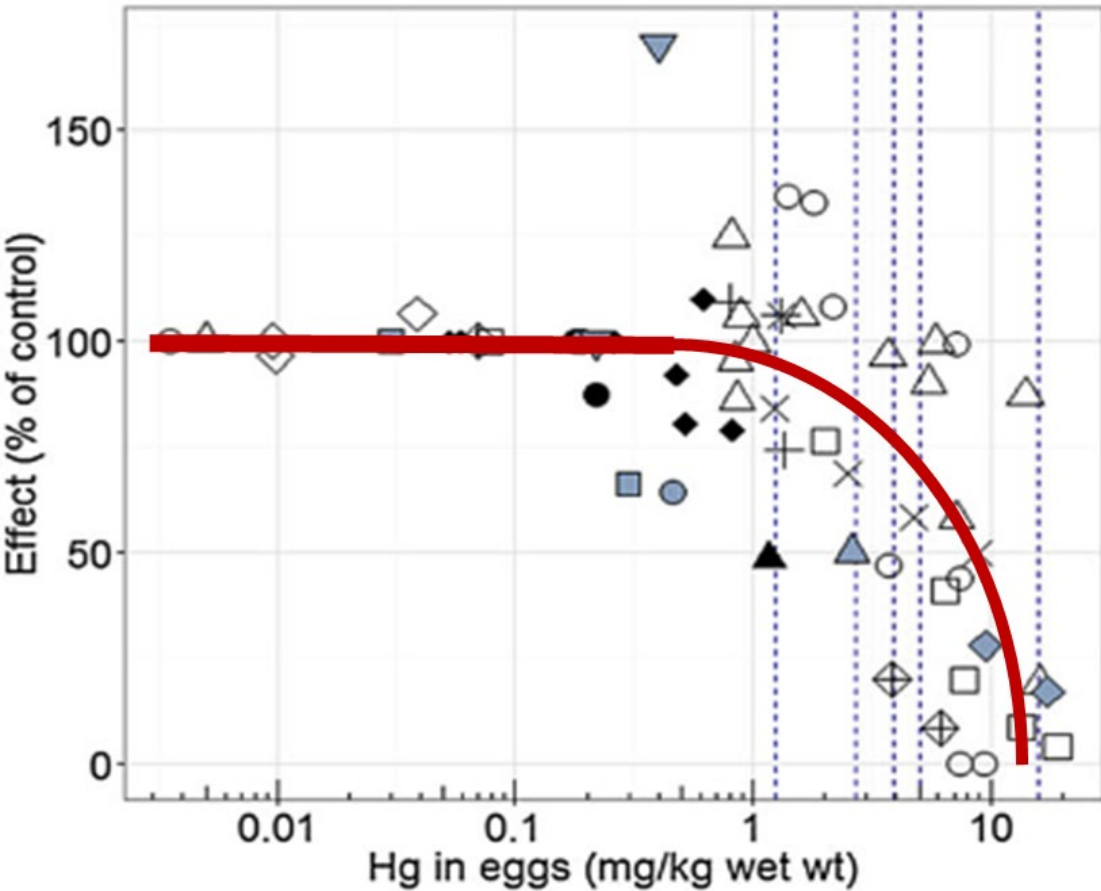
**Portugal-Baranda T. Ortiz-Zarragoitia, M**

Experimental exposure of a model fish species to methylmercury and food restriction reveals interactive effects of stressors and complex responses at multiple reproductive endpoints.



# Introduction

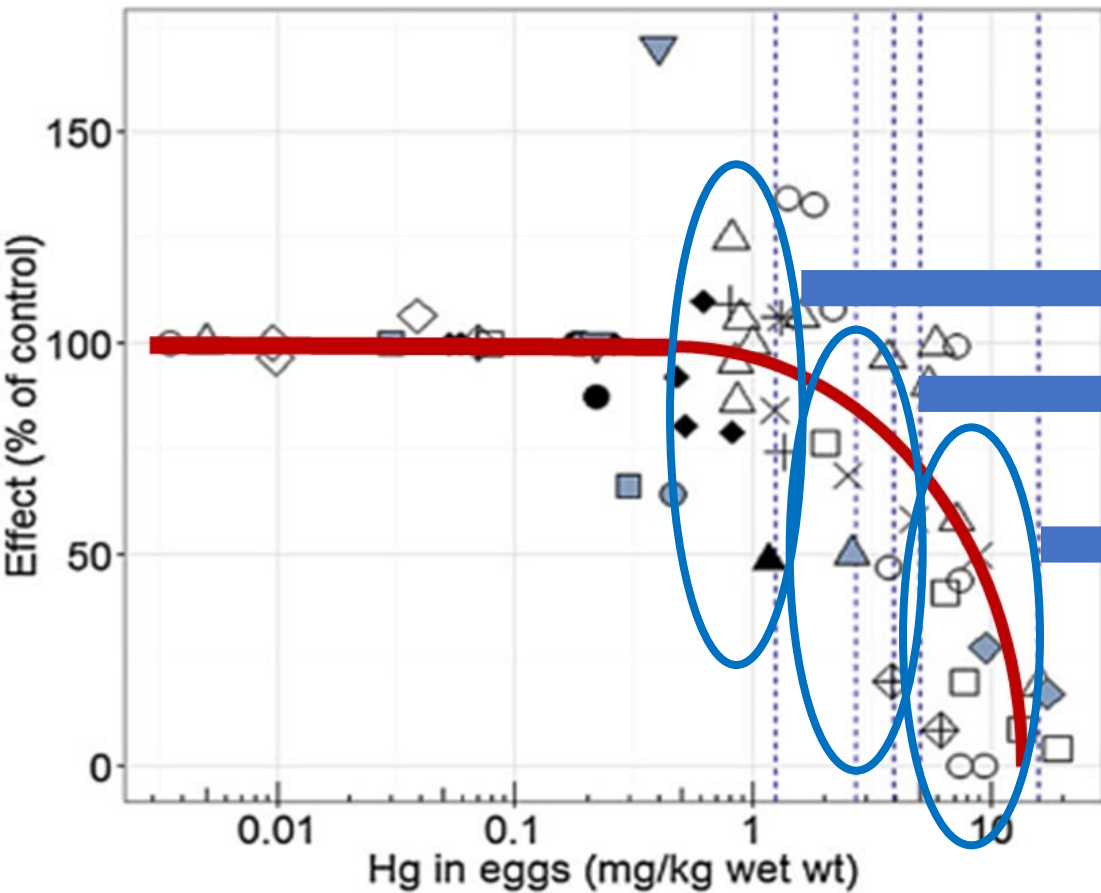
In general clear negative effects beyond a [Hg] threshold, but...



Fuchsman et al. *Environ. Tox. & Chem.* 2017.



# Introduction



Fuchsman et al. *Environ. Tox. & Chem.* 2017.

In general clear negative effects beyond a [Hg] threshold, but...

Strong variation in effects among studied-populations

Inter-specific variability

+

**Interaction with other stressors?**



# Introduction

Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*)

Rick A. Relyea<sup>\*†</sup> and Nathan Mills<sup>†</sup>

PNAS | February 27, 2001 | vol. 98 | no. 5 | 2491–2496

**Anti-parasite treatment removes negative effects of environmental pollutants on reproduction in an Arctic seabird**

Jan O. Bustnes<sup>1,\*</sup>, Kjell E. Erikstad<sup>1</sup>, Sveinn A. Hanssen<sup>1,2</sup>,  
Torkild Tveraa<sup>1</sup>, Ivar Folstad<sup>2</sup> and Janncehe U. Skaare<sup>3</sup>

## Trends in Ecology & Evolution

Volume 19, Issue 6, June 2004, Pages 274–276

ARTICLES

Two stressors are far deadlier than one

Andrew Sih<sup>1</sup>✉, Alison M Bell<sup>2</sup>, Jacob L Kerby<sup>1</sup>



Contents lists available at [ScienceDirect](#)

Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)



Interactions between toxic chemicals and natural environmental factors — A meta-analysis and case studies

Ryszard Laskowski<sup>a,\*</sup>, Agnieszka J. Bednarska<sup>a</sup>, Paulina E. Kramarz<sup>a</sup>, Susana Loureiro<sup>b</sup>, Volker Scheil<sup>c</sup>,  
Joanna Kudłek<sup>a</sup>, Martin Holmstrup<sup>d</sup>

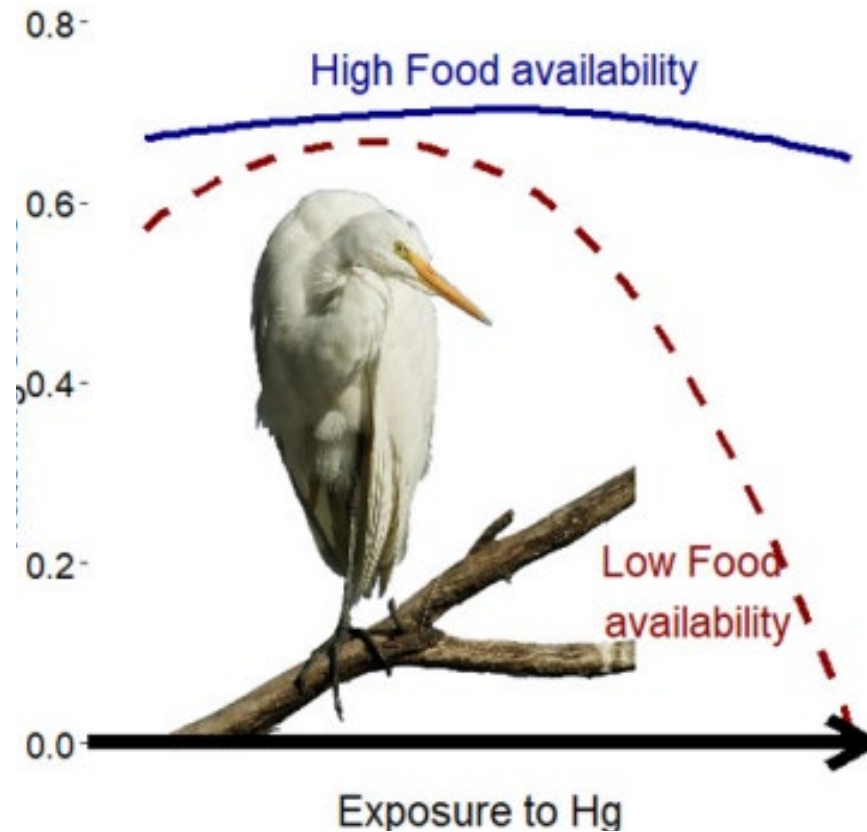


# Introduction

Accounting for food availability reveals contaminant-induced breeding impairment, food-modulated contaminant effects, and endpoint-specificity of exposure indicators in free ranging avian populations



Jabi Zabala <sup>a,b,\*</sup>, Ignacio Rodríguez-Jorquera <sup>a,c</sup>, Joel C. Trexler <sup>d,e</sup>, Sophie Orzechowski <sup>a</sup>, Lindsey Garner <sup>a</sup>, Peter Frederick <sup>a</sup>



# Aim and hypothesis

## AIM

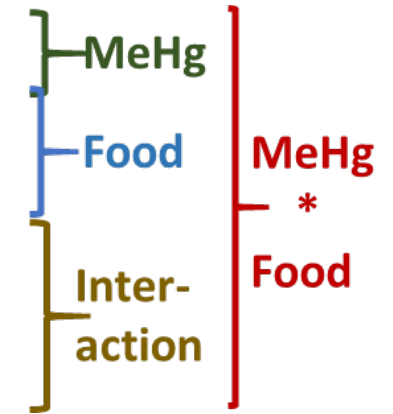
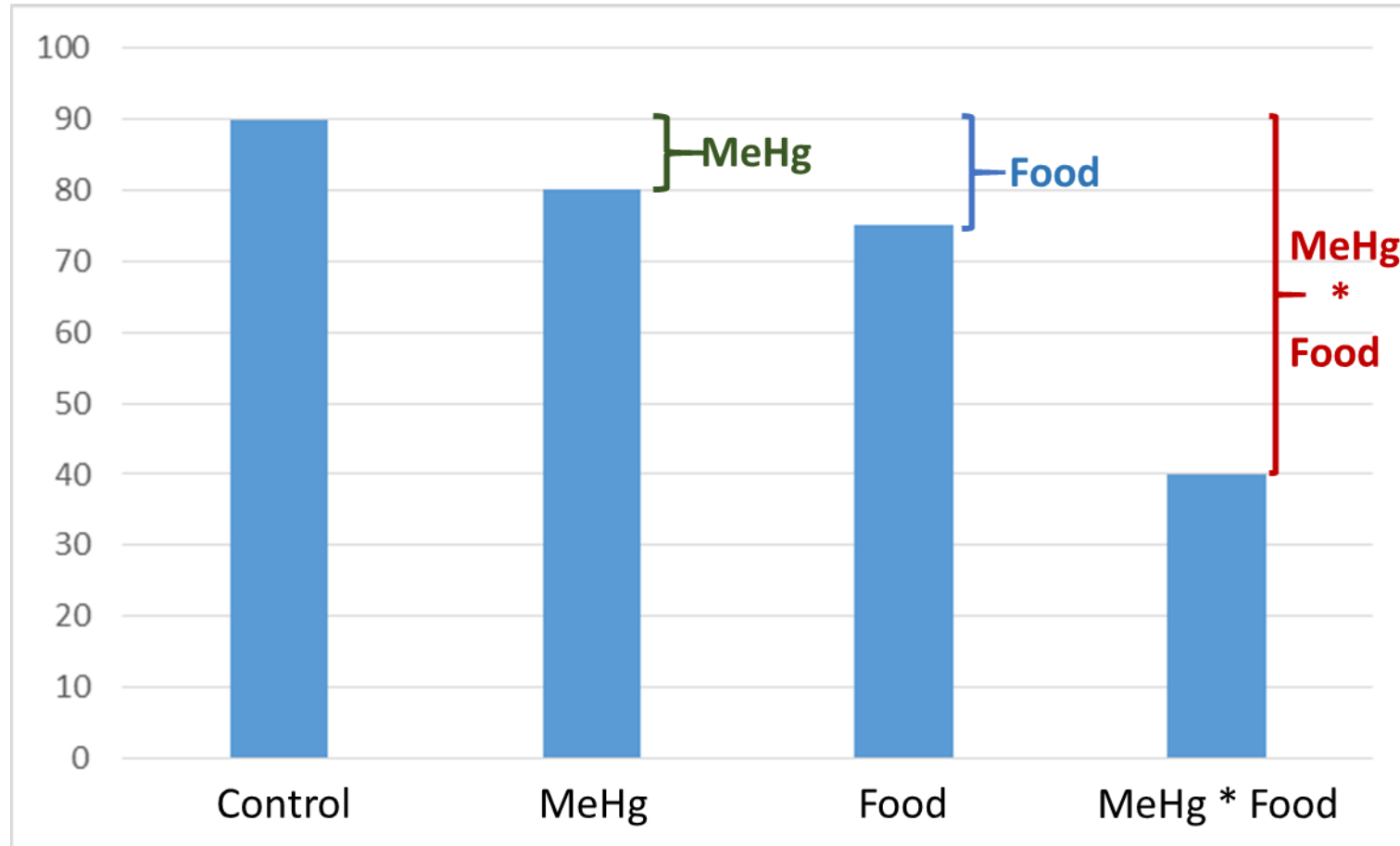
Experimentally expose zebra fish (*Danio rerio*) to combined effects of MeHg and food restriction.

## Hypotheses

- Combined effects will be synergistic. This is, the effect of combined exposure to MeHg and food restriction will differ from the addition of individual effects.
- Effects of combined exposure will be more severe.




# Aim and hypothesis

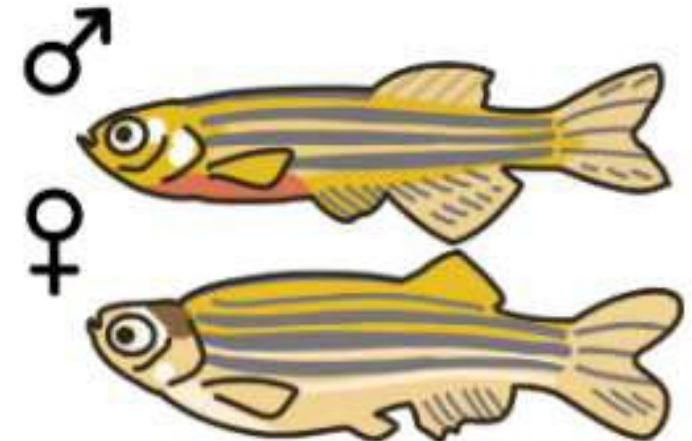
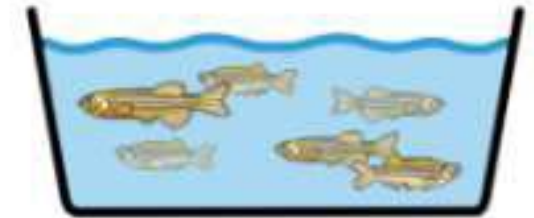


- Effect of combined exposure will differ from the addition of individual effects
- Effects of combined exposure will be more severe.



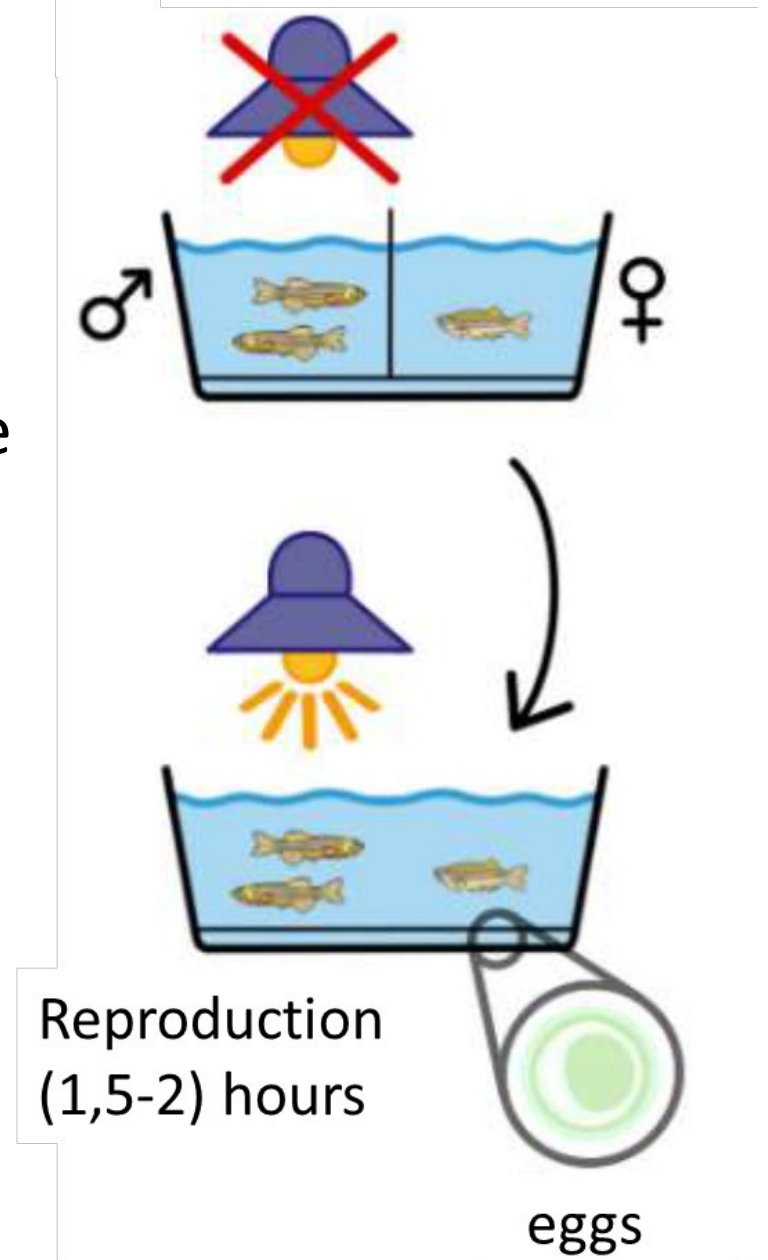
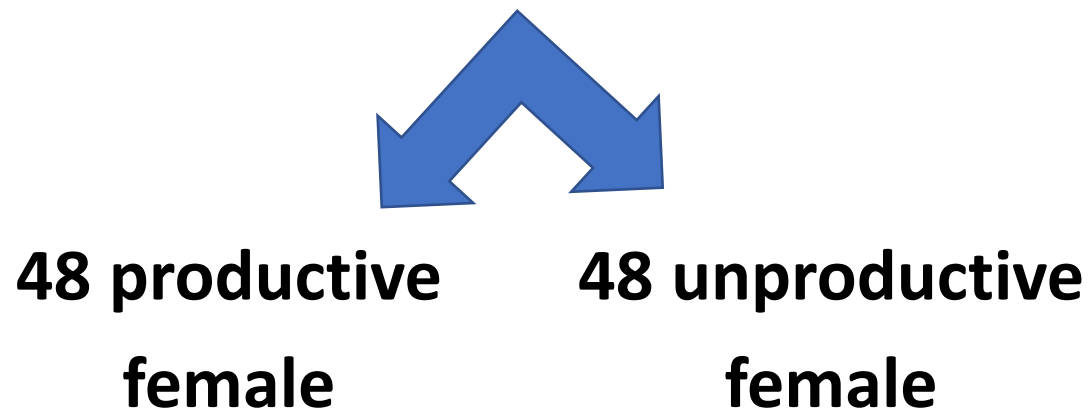
# Experimental design: Pre trial

- Zebra fish: Model species
  - Small  easy to manage large amounts of fish
  - High reproductive capacity (up to every 4-7 days)
- 240 adult fish (8-14 months old) from local registered purveyor.
- 14 hours dark, 10 hours light, constant 28°C.
- 7 days acclimation (sex segregated), fed *Artemia salina* larvae



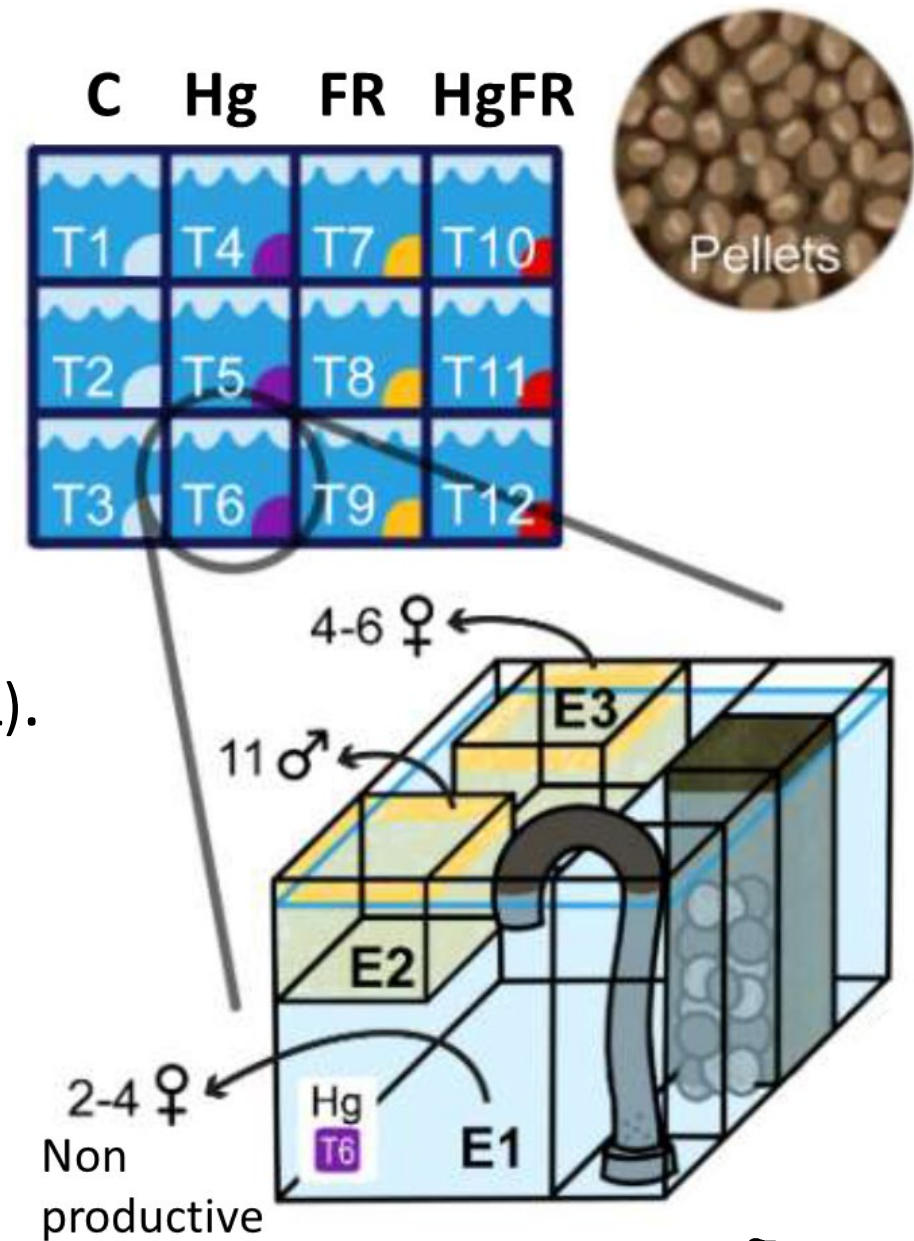
# Experimental design: Pre trial

- 3 reproductive trials → discard unproductive fish + homogenization of conditions.
- 1 female + 2 males overnight in breeding tanks
- Bottom mesh to exclude fish from eggs.
- Turn on light and allow reproduction for 1,5-2 hours



# Experimental design: Trial

- FOOD: pellets
- C: 4% weight/day
- **FR: 1% weight/day**
- **MeHg: + 5ppm MeHg**
- 3 tanks per treatment. 12 tanks (48x30x28 cm, 40L).
- Zebra fish randomly assigned.
- 14 days of exposure.
- **3 reproductive attempts (7 days apart)**
- Reproductive attempt all productive females +2 unproductive female



# Experimental design: Endpoints

1. Reproduction probability (lay at least 1 egg).
2. Clutch size (number of eggs laid).
3. Clutch fertility: probability a laid egg being fertile
4. Probability of alive larva at 120 hpf (from fertile egg)

} Process



5. Viable larvae per breeding occasion
6. Probability of alive larvae (120 hpf) from egg laid

} Integrative



# Experimental design: Analysis

Each endpoint 5 models + AICc

1. Null
2. Hg
3. Food Restriction
4. Hg + Food
5. Hg \* Food

Our expectation



- All models included clutch number (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) as factor.
- GLMs binomial; proportional or Poisson.



# Results



203 reproductive occasions

| Group                 |            | Clutches  | Eggs          | Fertile                | Alive larvae 120 hpf  |
|-----------------------|------------|-----------|---------------|------------------------|-----------------------|
| Control               | 51         | 16        | 2 953         | 2 216 (75,0 %)         | 599 (27,0 %)          |
| MeHg                  | 51         | 23        | 5 602         | 3 908 (69,8 %)         | 568 (14,5 %)          |
| Food Restriction      | 51         | 20        | 2 756         | 2 201 (79,9 %)         | 978 (44,4 %)          |
| MeHg Food Restriction | 50         | 18        | 2 163         | 1 873 (85,6 %)         | 1 204 (64,3 %)        |
| <b>Overall</b>        | <b>203</b> | <b>77</b> | <b>13 474</b> | <b>10 198 (75,7 %)</b> | <b>3 349 (32,8 %)</b> |



# Results

## Model selection ( $\Delta AICc$ )

| <b>Group</b> | <b>Rep. prob.</b> | <b>Clutch size</b> | <b>Clutch fertility</b> | <b>Alive larvae</b> | <b>Viable larvae</b> | <b>Larvae per fertile egg</b> |
|--------------|-------------------|--------------------|-------------------------|---------------------|----------------------|-------------------------------|
| Null         | 0                 | 2054               | 516                     | 1258                | 426                  | 1743                          |
| MeHg         | 1,44              | 1733               | 482                     | 2111                | 427                  | 1694                          |
| FR           | 1,99              | 897                | 76                      | 238                 | 82                   | 296                           |
| MeHg + FR    | 3,45              | 578                | 72                      | 235                 | 84                   | 298                           |
| MeHg * FR    | 3,57              | 0                  | 0                       | 0                   | 0                    | 0                             |



# Results: Combined effects were synergistic

Model selection ( $\Delta AICc$ )

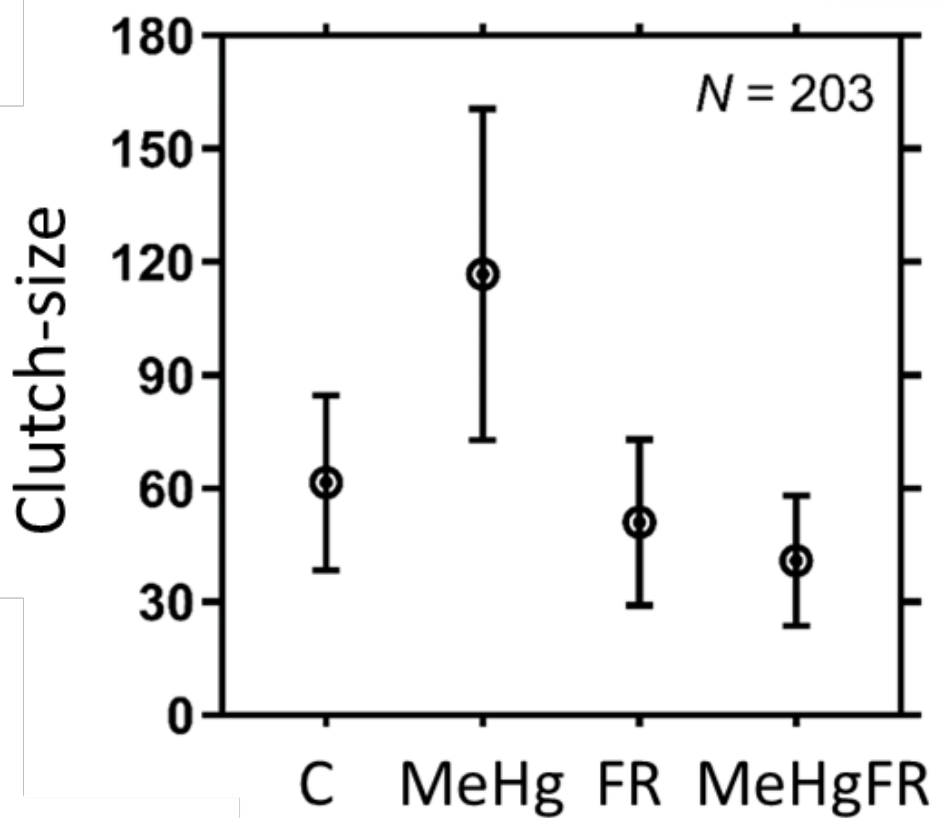
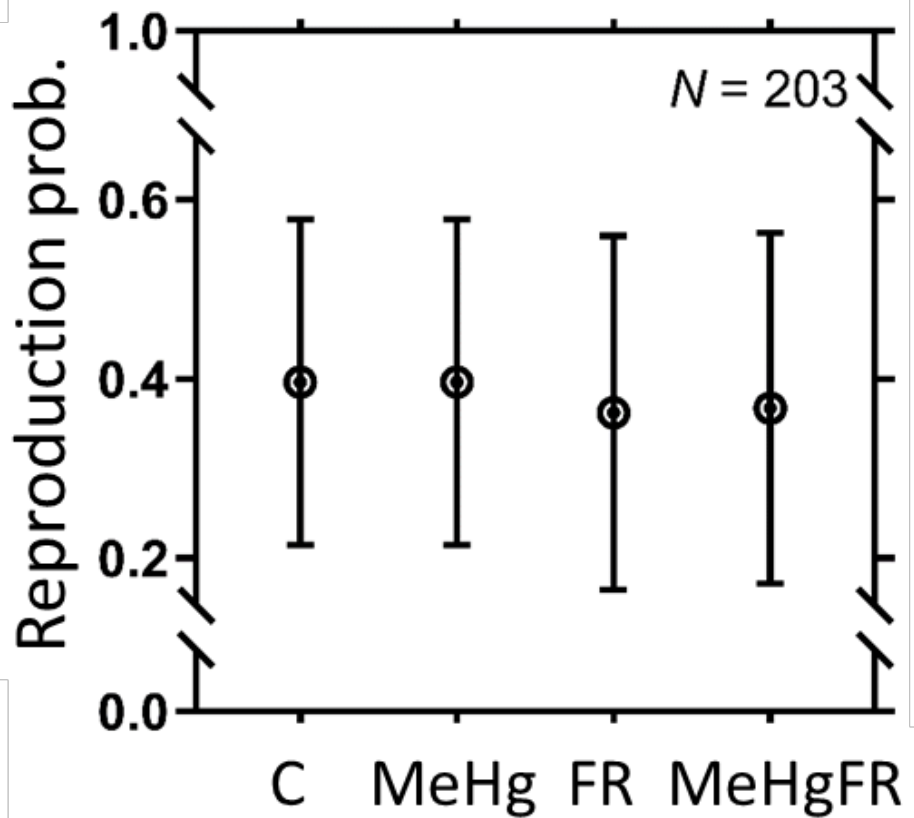
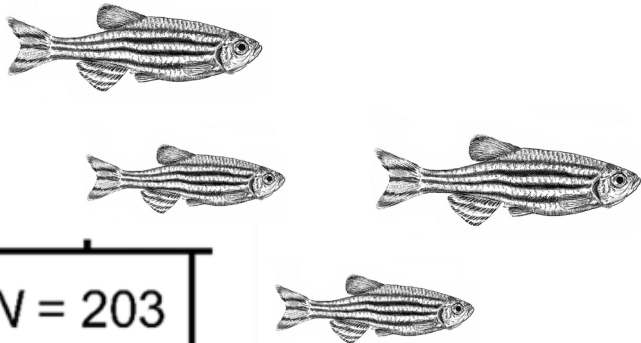
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| <b>MeHg * FR</b> | 3,57       | 0           | 0                | 0            | 0             | 0                      |

**All other models well beyond 10 AICc units.**



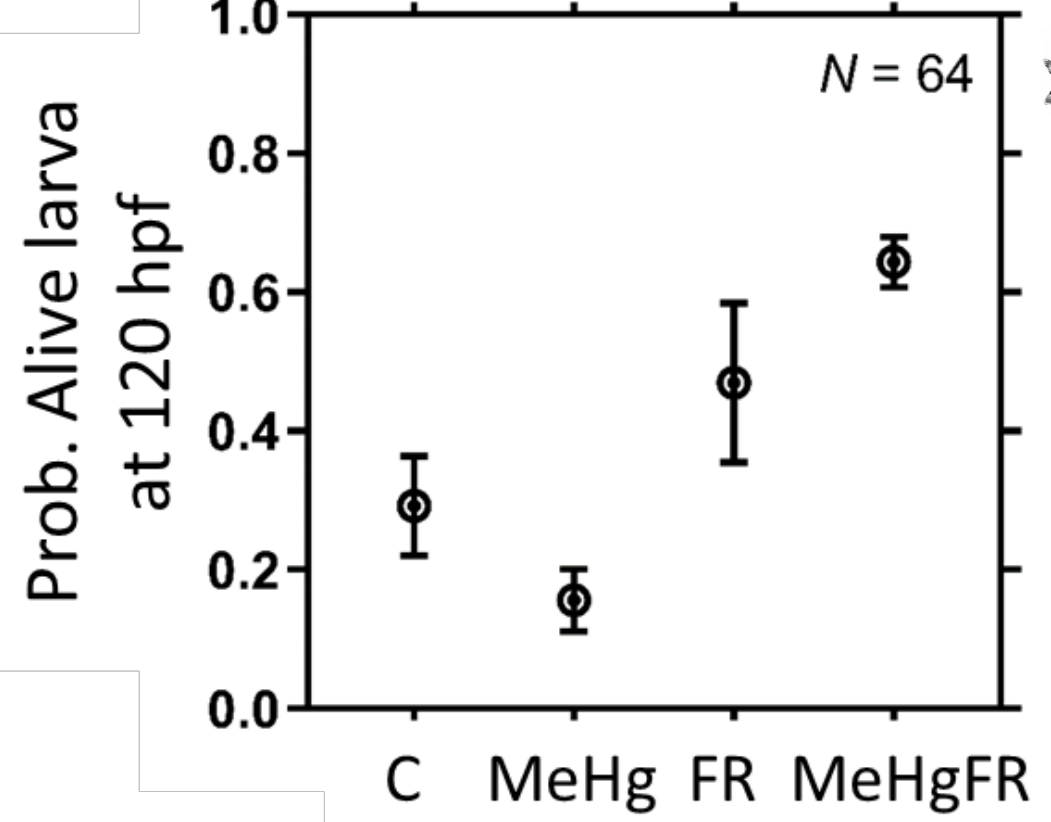
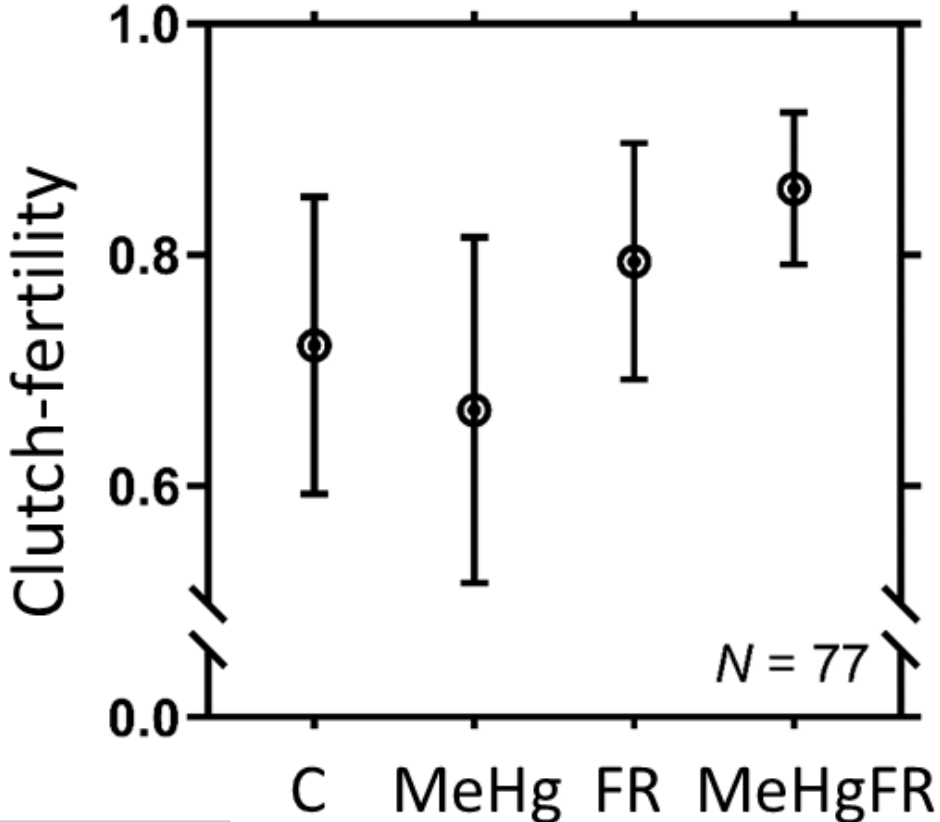
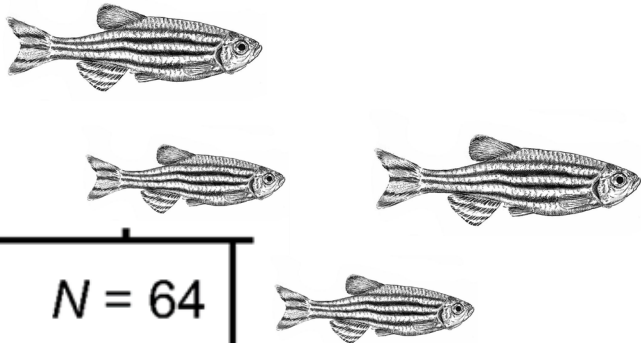
# Results: Combined effects more severe?

Model predicted averages: process endpoints



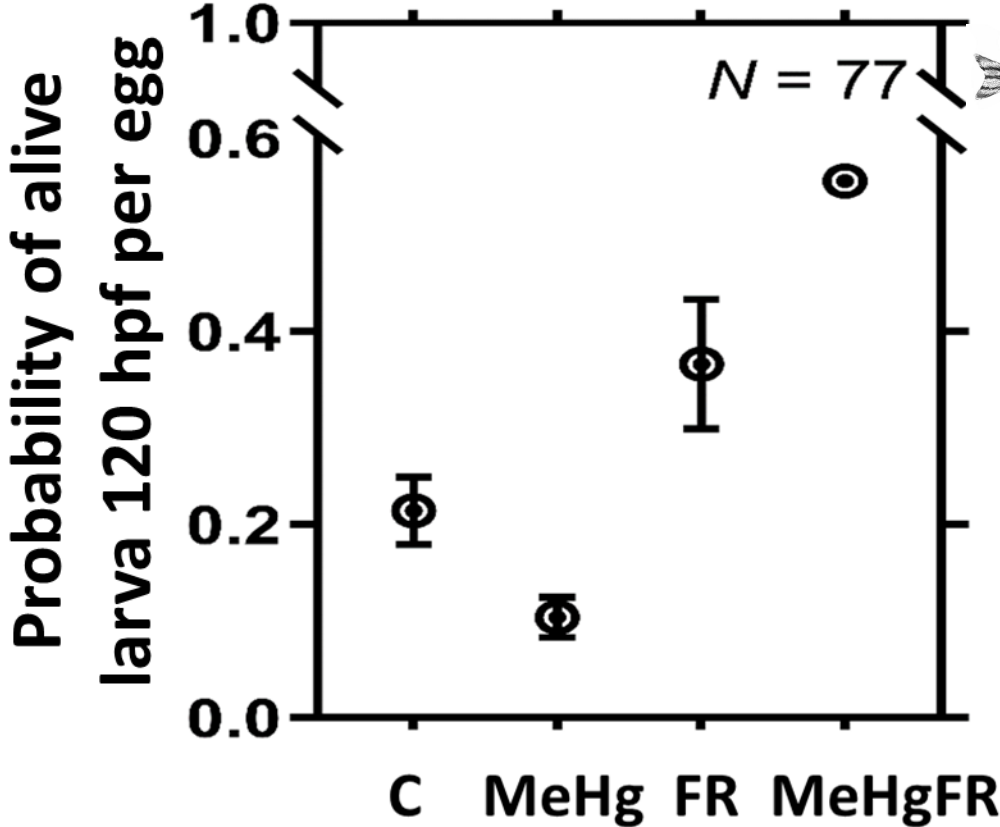
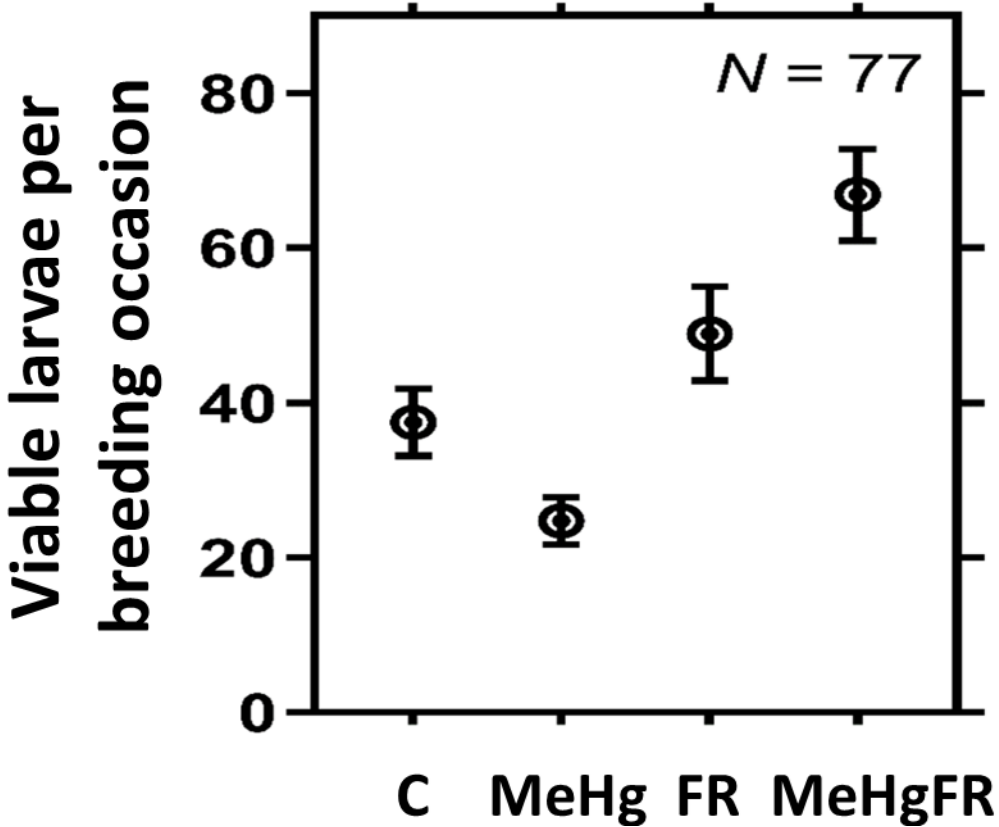
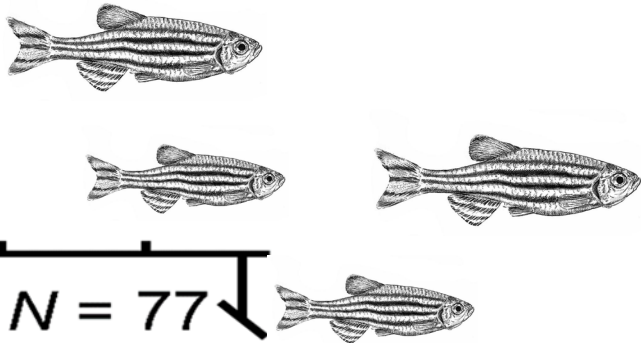
# Results: Combined effects more severe?

Model predicted averages: process endpoints



# Results: Combined effects more severe?

Model predicted averages: integrative endpoints



# Aim and hypothesis

- Hypothesis
  - Combined effects will be synergistic. This is, the effect of combined exposure to MeHg and food restriction will differ from the addition of individual effects.

**YES**

- Effects of combined exposure will be more severe.

**NO**

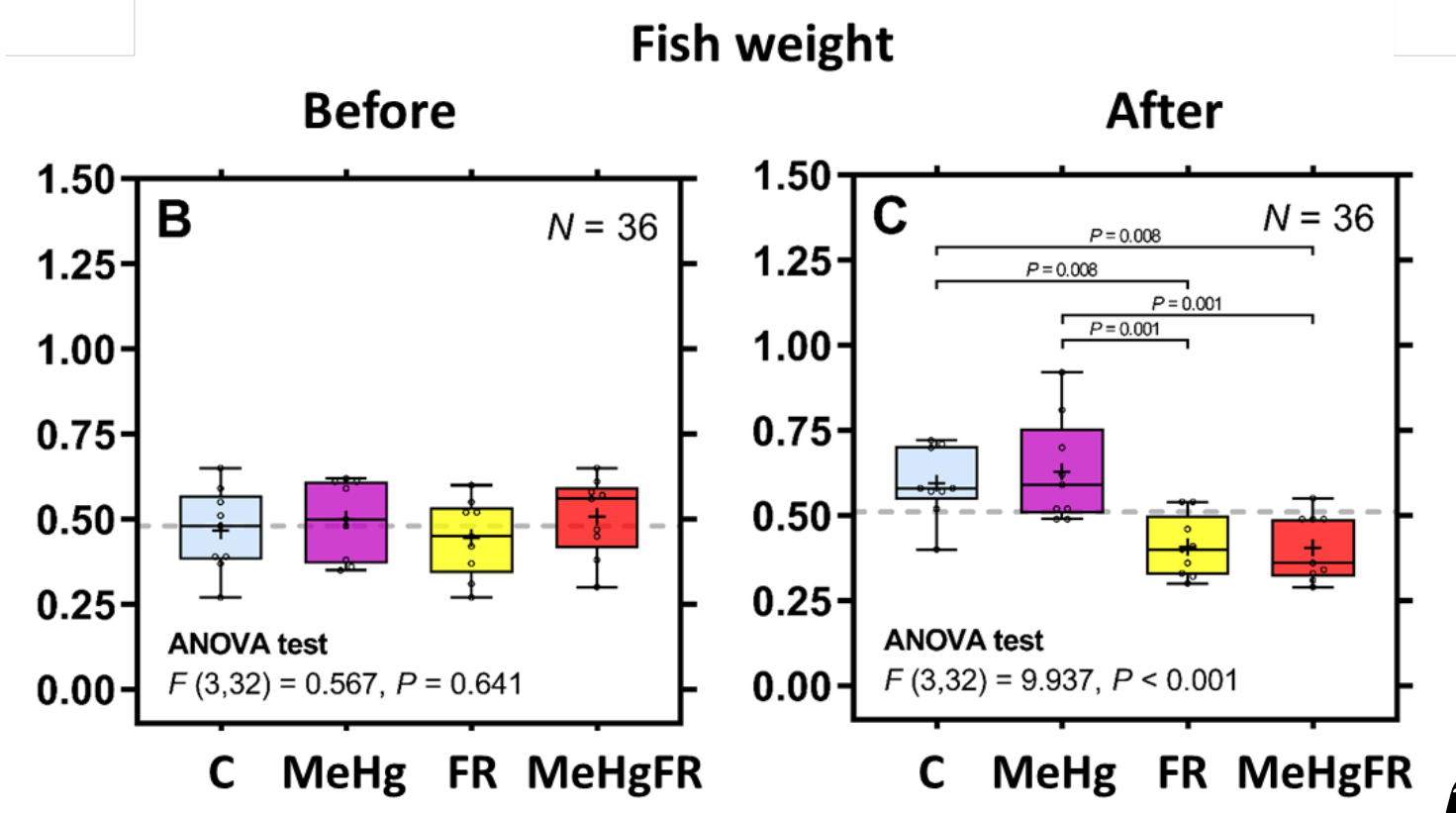


# Discussion

## 1. Not long/toxic enough?

## 2. Hormesis

## 3. Trade-offs

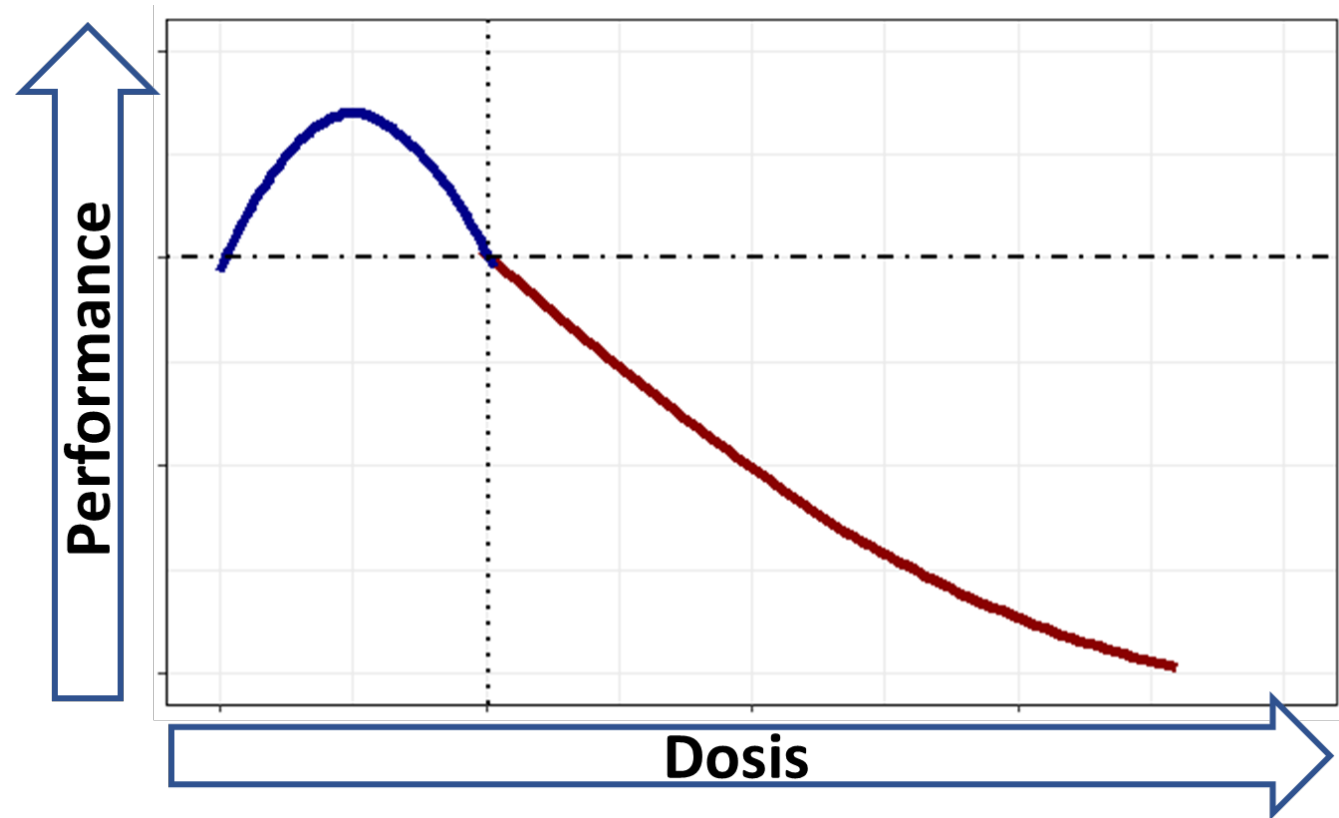


# Discussion

1. Not long/toxic enough?

2. Hormesis

3. Trade-offs



# Discussion

1. Not long/toxic enough?

2. Hormesis

3. Trade-offs

Reproduce

Self maintenance



Survival

Reproduction

Offspring quality



**GRACIAS**  
**ARIGATO**  
**SHUKURIA**  
**GOZAIMASHITA**  
**EFCHARISTO**  
**JUSPAXAR**  
**DANKSCHEEN**  
**TASHAKKUR ATU**  
**YAQHANYELAY**  
**SUKSAMA**  
**EKHMET**  
**MEHRBANI**  
**PALDIES**  
**GRAZIE**  
**MEHRBANI**  
**MAAKE**  
**LAH**  
**KOMAPSUMNIDA**  
**MAKETAI**  
**YOUSAGARATAM**  
**HUI**  
**UNALCHEESH**  
**HATUR GU**  
**EKOJU**  
**SIKOMO**  
**TINGKI**  
**BIYAN**  
**SHUKRIA**  
**THANK**  
**YOU**  
**BOLZIN**  
**MERCI**  
**MIMMONCHAR**

