

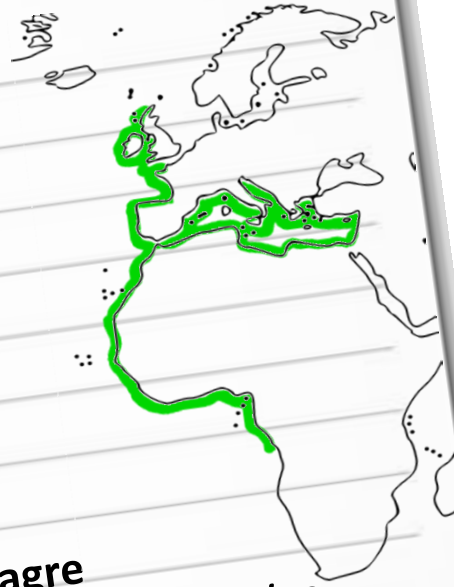


Gamete quality and management for in vitro fertilisation in meagre (*Argyrosomus regius*) to facilitate the implementation of genetic breeding programs

Presenter: Sandra Ramos

Wendy Gonzalez (IRTA), Gilbert Dutto (IFREMER), Constantinos, C. Mylonas (HCMR), Christian Fauvel (IFREMER) Neil Duncan (IRTA).

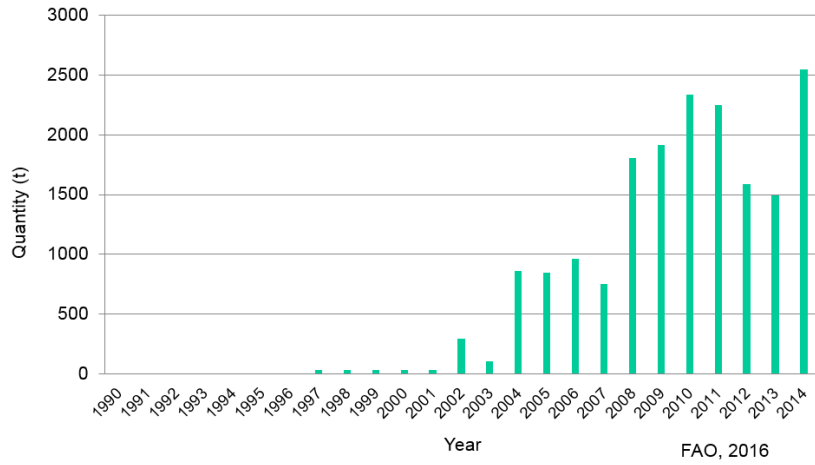
INTRODUCTION



- Common name: **Meagre**
Scientific name: *Argyrosomus regius*
Family: Sciaenidae
Great potential for aquaculture
- Fast grow (1kg/year)
 - Fast acclimation to captivity
 - Low FCR (0,9 – 1,2)...

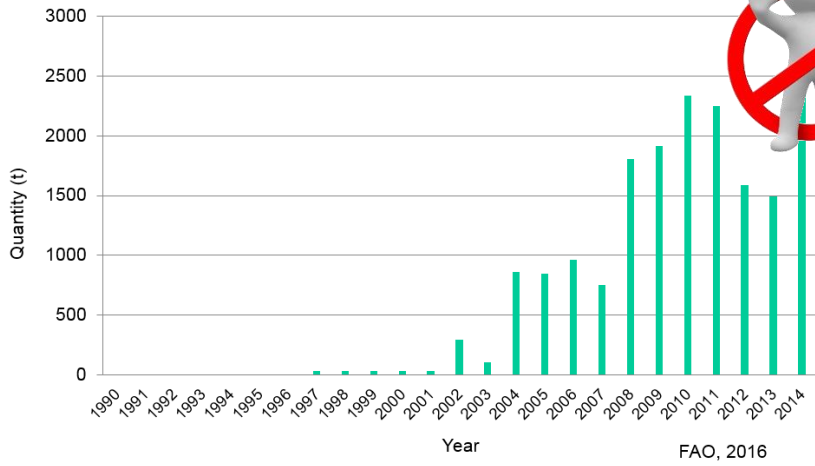
INTRODUCTION

European aquaculture production (t)



INTRODUCTION

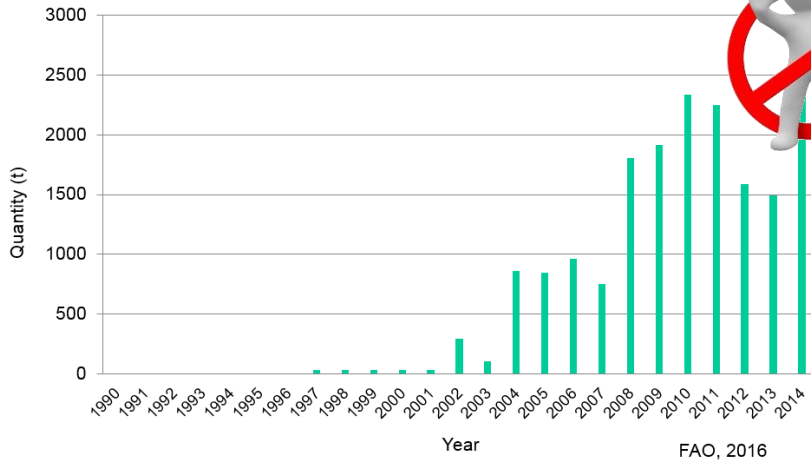
European aquaculture production (t)



Low variety of families and populations

INTRODUCTION

European aquaculture production (t)

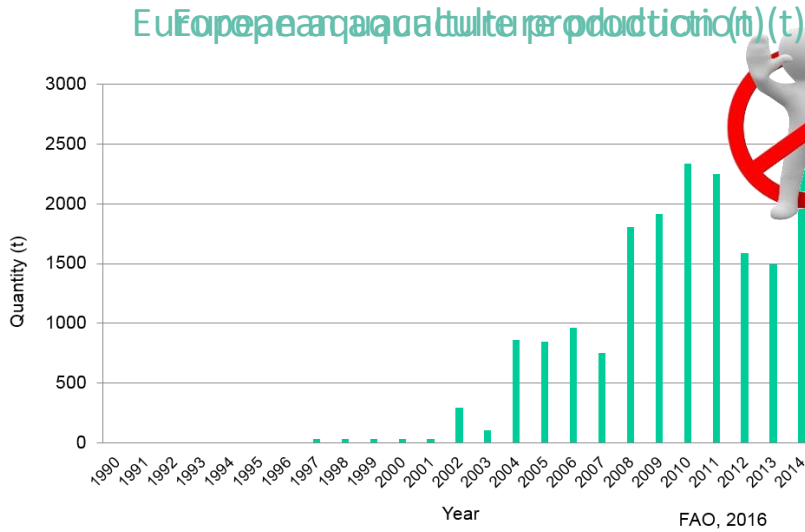


Low variety of families and populations



Breeding programs to avoid inbreeding

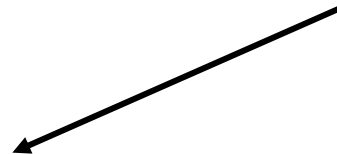
INTRODUCCIÓN



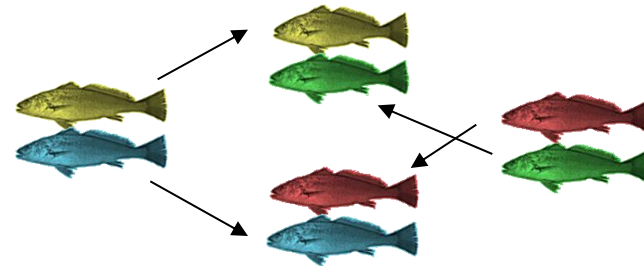
Low variety of families and populations



Breeding programs to avoid inbreeding

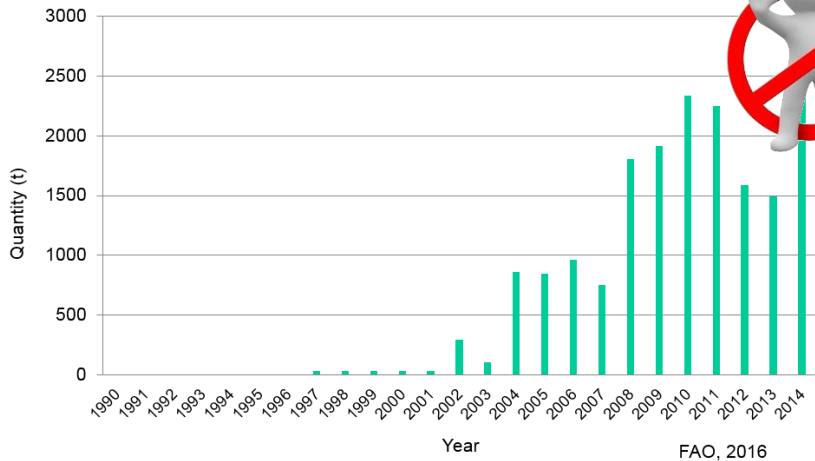


**PAIRED BREEDING WITH A
CROSS MATING DESIGN**



INTRODUCTION

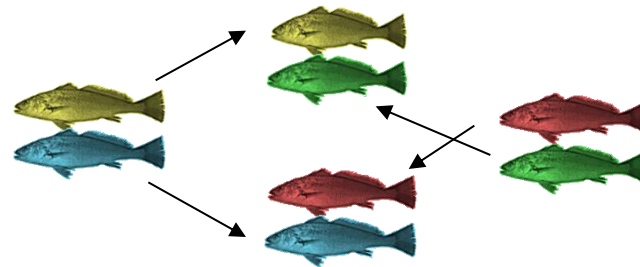
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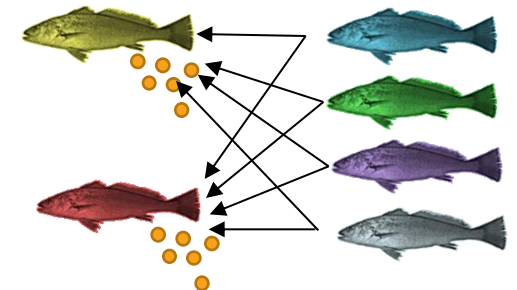
Breeding programs to avoid inbreeding

PAIRED BREEDING WITH A CROSS MATING DESIGN



ARTIFICIAL FERTILISATION

- Higher number of families.
- Gametes control.
- Less stress.



OBJECTIVES

- **To develop a protocol for artificial fertilization.**

- Determining the optimum time at which the egg is ready to be fertilized, establishing the time of ovulation after hormonal treatment.

- Establishing the optimal sperm:egg ratio.

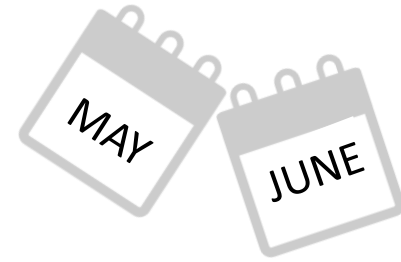
MATERIAL AND METHODS

- 1 Selection of breeders by their stage of maturity.



♀

♂



MATERIAL AND METHODS



1 Selection of breeders by their stage of maturity.

Anaesthesia
(70.6 mg/L MS-222)



♀

♂

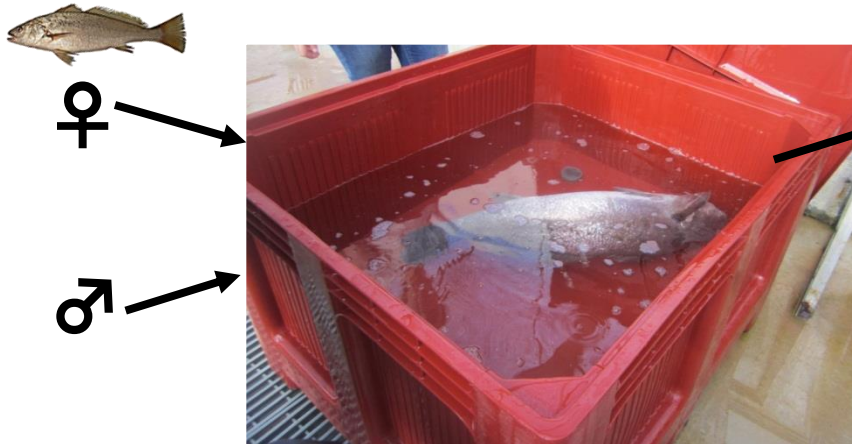


- Total: 13 females (20.45 ± 6.22 kg)
- Total: 5 males (15.94 ± 2.75 kg)

MATERIAL AND METHODS



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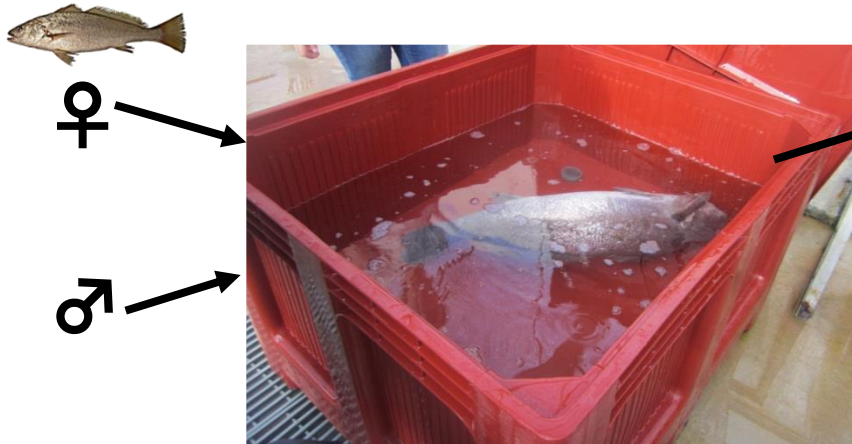
- Ovarian biopsies by cannulation



MATERIAL AND METHODS

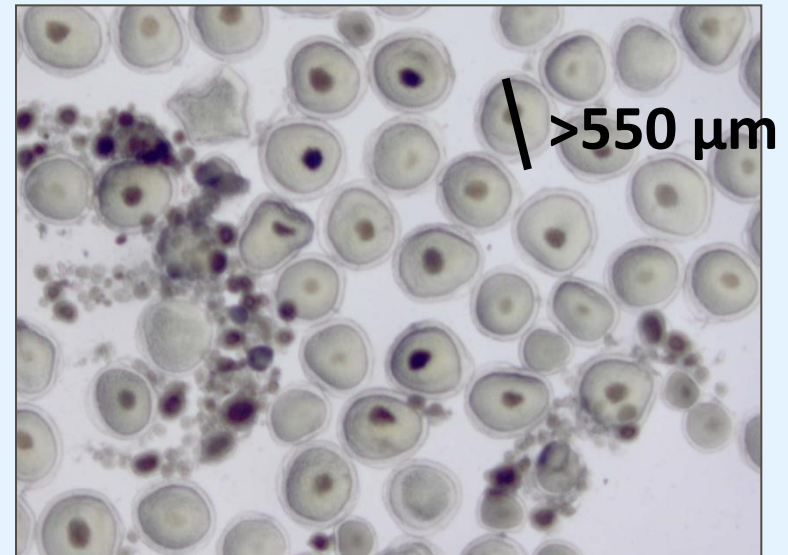


1 Selection of breeders by their stage of maturity.



- Total: 13 females (20.45 ± 6.22 kg)
- Total: 5 males (15.94 ± 2.75 kg)

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- **Selected females:** Oocytes in full vitellogenesis (diameter $>550 \mu\text{m}$)



MATERIAL AND METHODS



1 Selection of breeders by their stage of maturity.



- Ovarian biopsies by cannulation
- **Selected females:** Oocytes in full vitellogenesis (diameter >550 μm)
- Release of sperm by abdominal pressure.
- **Selected males:** in spermiation stage of 2 and 3.

0 = not fluent

1 = fluent but no sample can be obtained

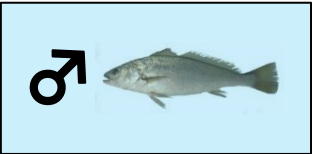
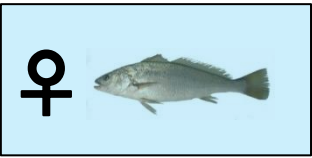
2 = fluent

3 = very fluent



MATERIAL AND METHODS

2 Protocol of hormonal induction.

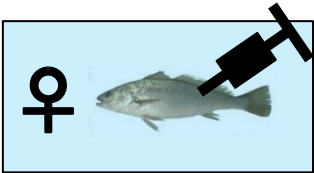


MATERIAL AND METHODS

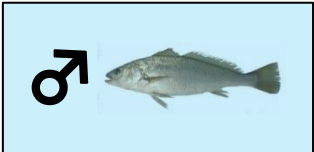
2 Protocol of hormonal induction.

Intramuscular injection of

GnRH α Dose = 15 μ g/kg [1]



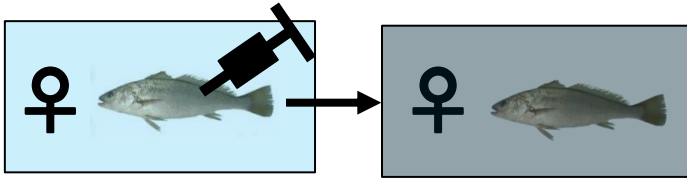
Total: 24 inductions with
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MATERIAL AND METHODS

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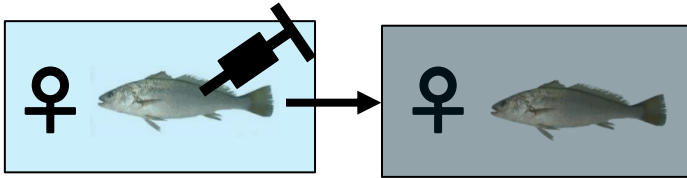
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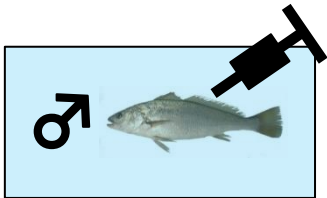
MATERIAL AND METHODS

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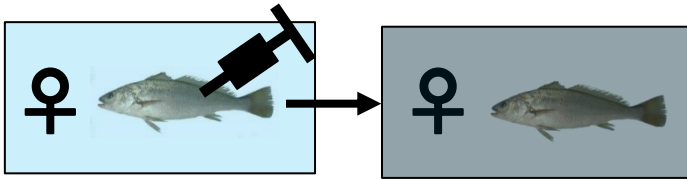


⌚ 9-10 h after

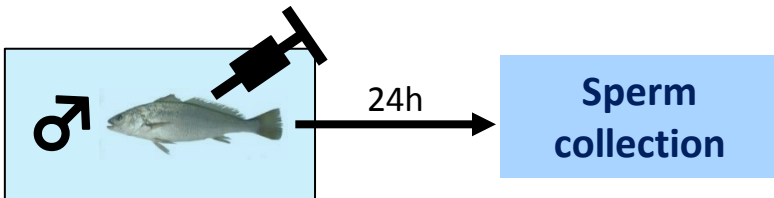
MATERIAL AND METHODS

3 Masculine gametes collection.

Intramuscular injection of
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Total: 24 inductions with
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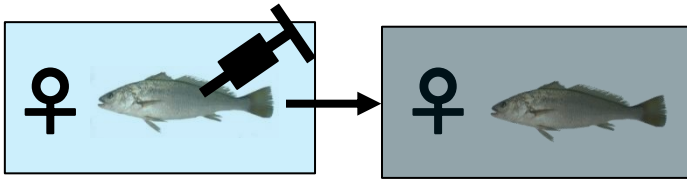


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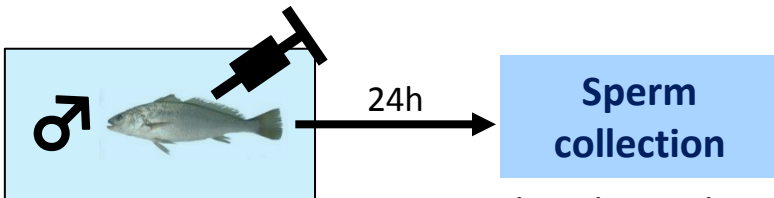
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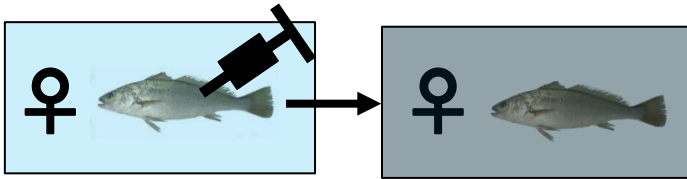
⌚ 9-10 h after

- Diluted in Leibovitz 1:4.
- Analysis (Image J – CASA plugin)
 - Motility (%)
 - Velocity (μ m/s)
 - Duration (min).
- Stored above ice until required.

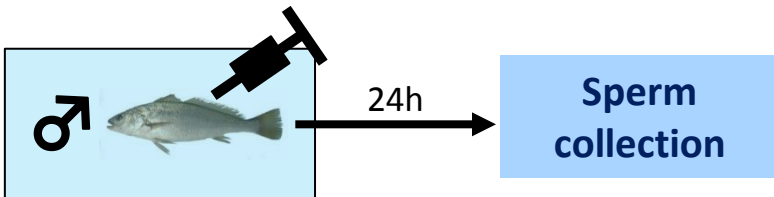
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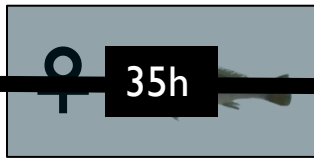
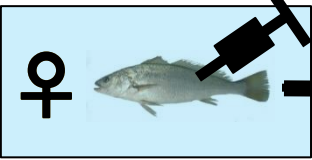
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 - Duration (min)
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MATERIAL AND METHODS

4 Femenine gametes collection.

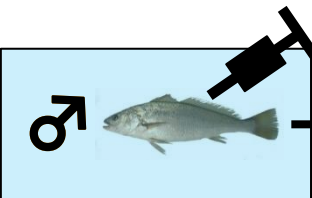
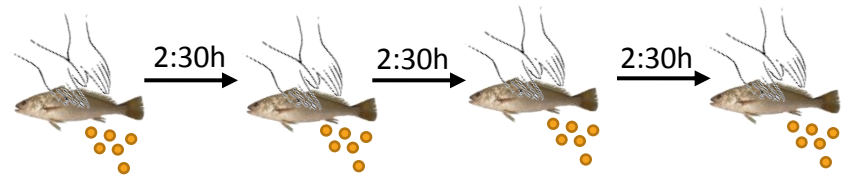
Intramuscular injection of
 GnRH α Dose = 15 μ g/kg

DETERMINATION OF OVULATION TIME AFTER HORMONAL INDUCTION



Strippings
 ⌚ Every 2 ½ hours.

Total: 24 inductions with
 GnRH α (in different weeks and
 rotating females).



24h

**Sperm
 collection**

Ovulation Time:
 Time elapsed between the hormonal induction and the
 first moment in which ovulated eggs are easily stripped.

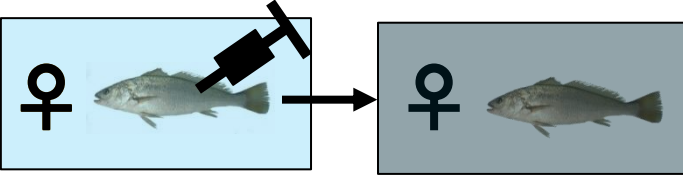
⌚ 9-10 h after



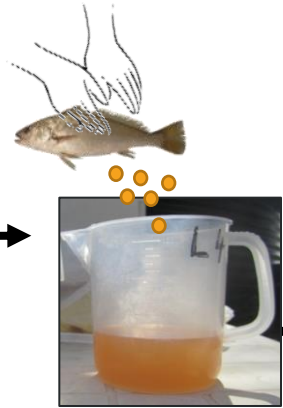
MATERIAL AND METHODS

5 Fertilización artificial.

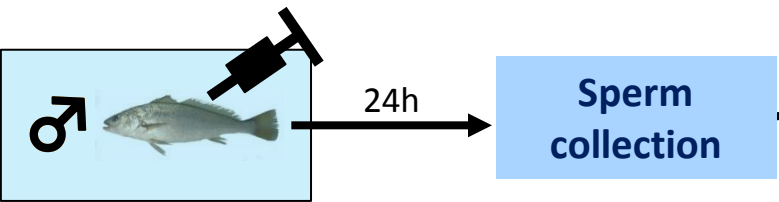
Intramuscular injection of
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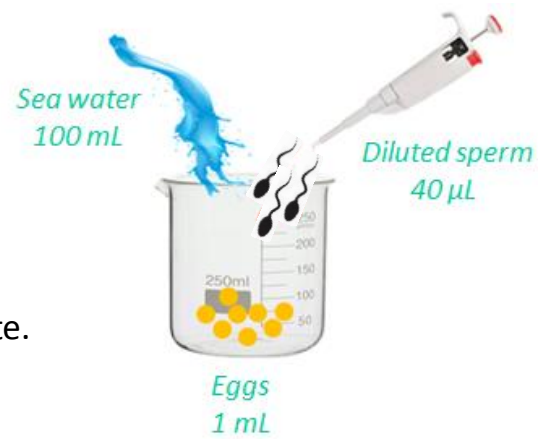
Total: 24 inductions with
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ARTIFICIAL FERTILIZATION



⌚ 9-10 h after

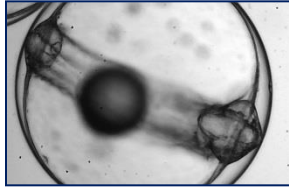


Incubations in triplicate.

🌡 17,8 °C to 18,4 °C

⌚ 30 h

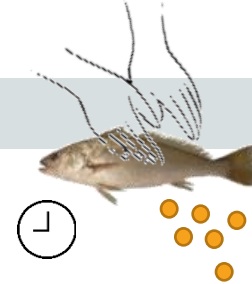
Fertilization rate assessed (% of
number of eggs with embryos) in
400 eggs / incubator.



MATERIAL AND METHODS

PART 1: DETERMINATION OF THE TIME OF OVULATION

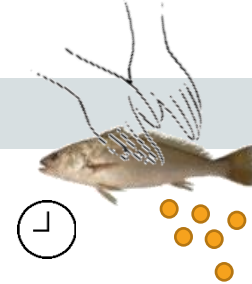
WHEN SHOULD WE MAKE ABDOMINAL MASSAGES TO OBTAIN GAMETES WITH THE BEST QUALITY?



MATERIAL AND METHODS

PART 1: DETERMINATION OF THE TIME OF OVULATION

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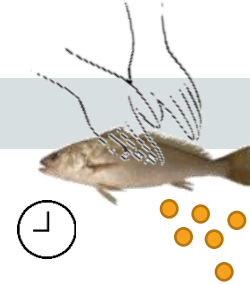


PART 2: DETERMINATION OF OPTIMAL SPERM: EGG RATIO

MATERIAL AND METHODS

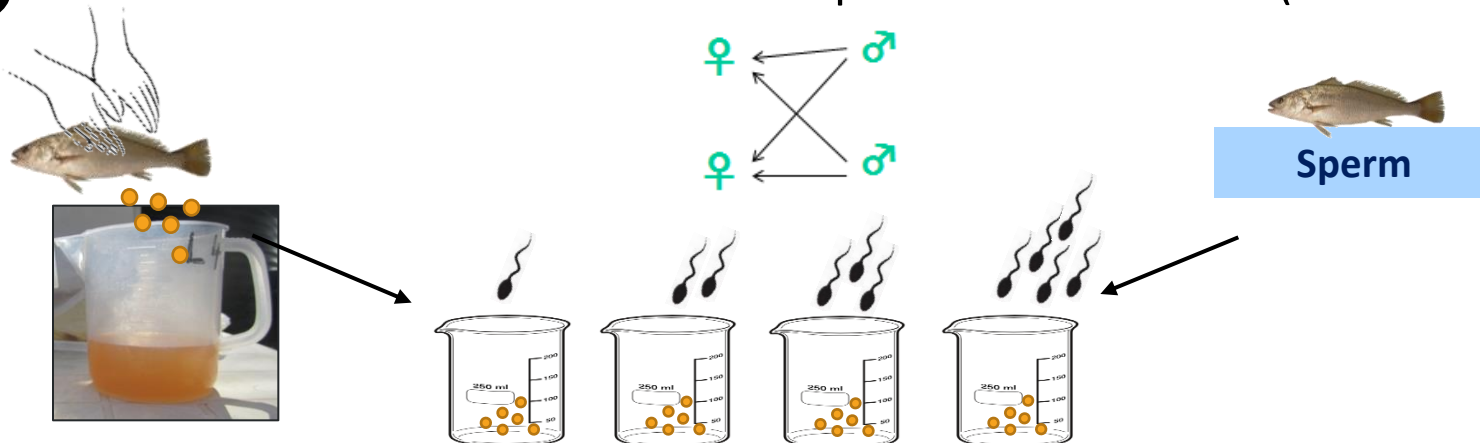
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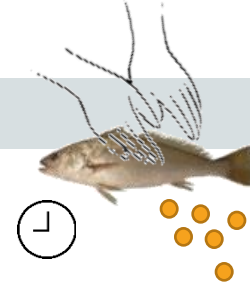
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MATERIAL AND METHODS

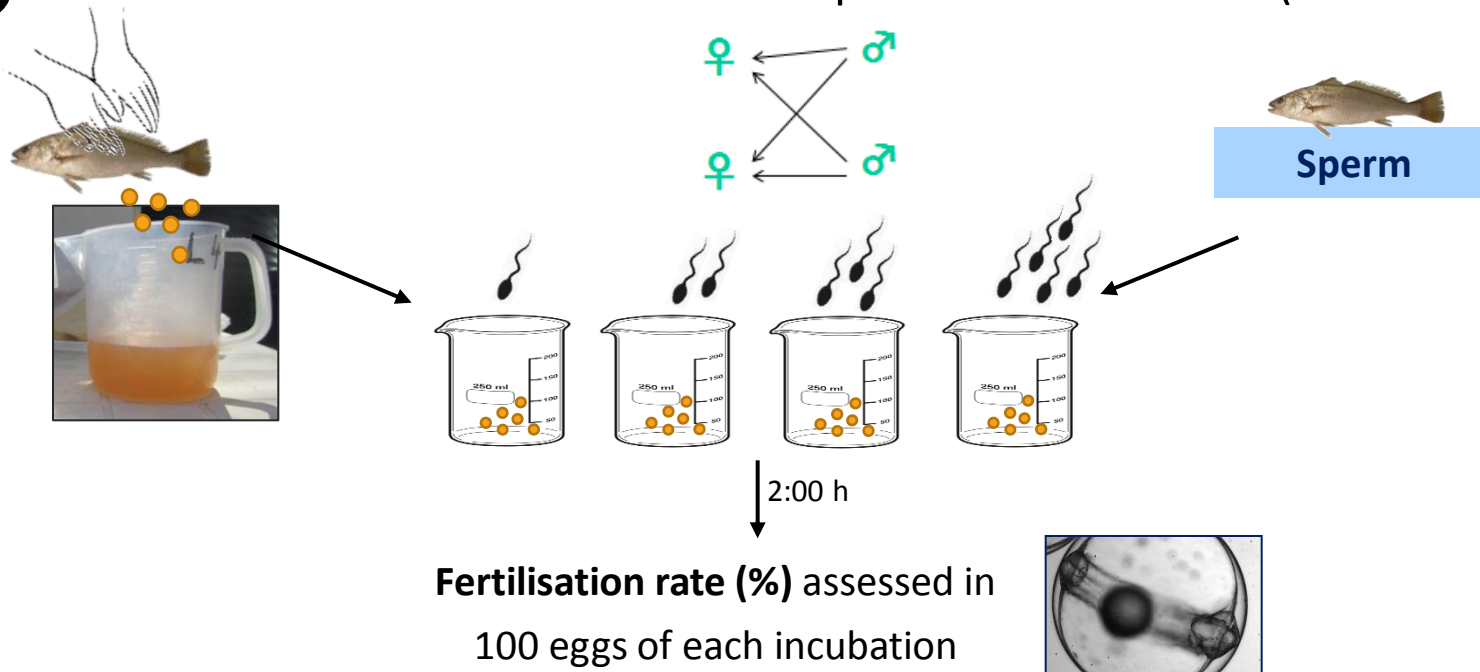
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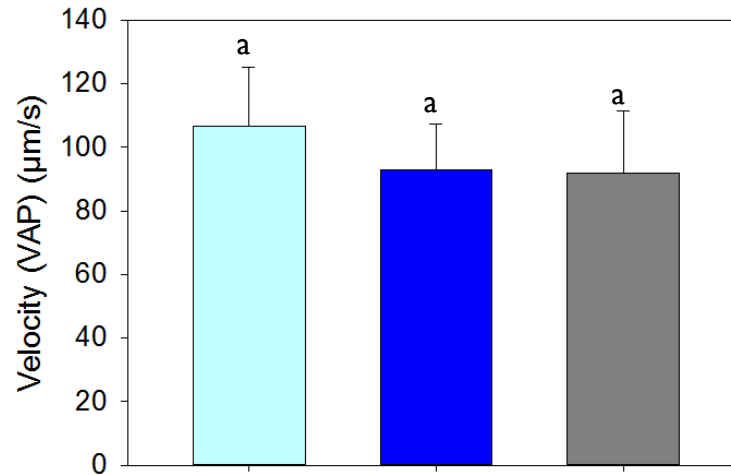
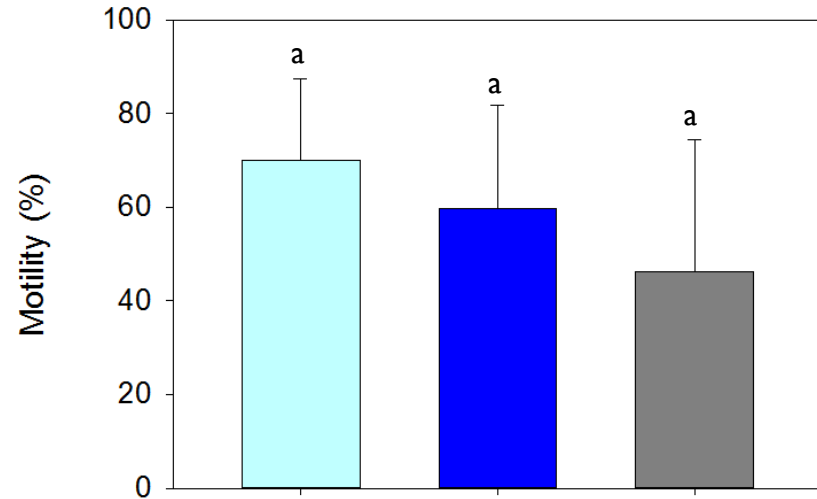
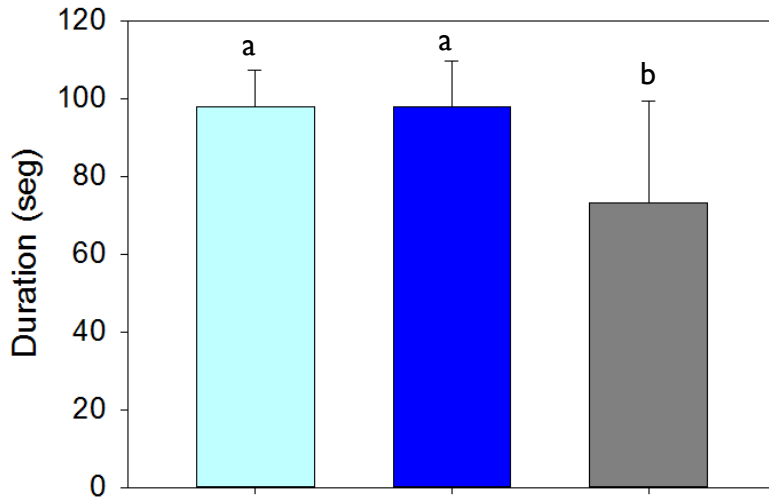


RESULTS

SPERM STORAGE

RESULTS

SPERM STORAGE



Analysis time (h)

- 10:30 h (fresh sperm)
- 13:30 h
- 17:30 h

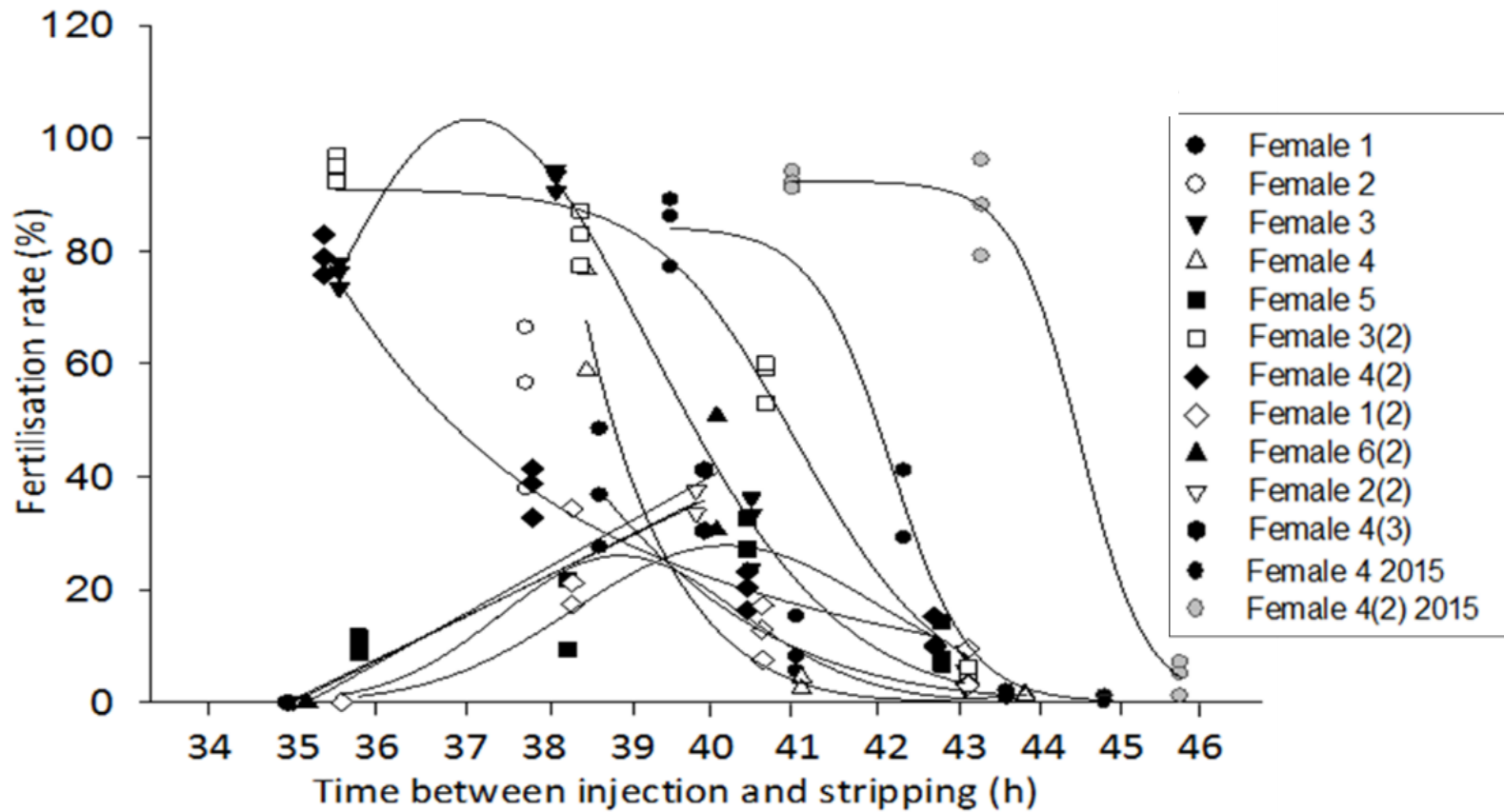
Sperm was successfully stored in Leibovitz culture medium for 7 h with no loss of fertilisation ability compared to fresh sperm.

RESULTS

PART 1: DETERMINATION OF THE TIME OF OVULATION AFTER HORMONAL INDUCTION

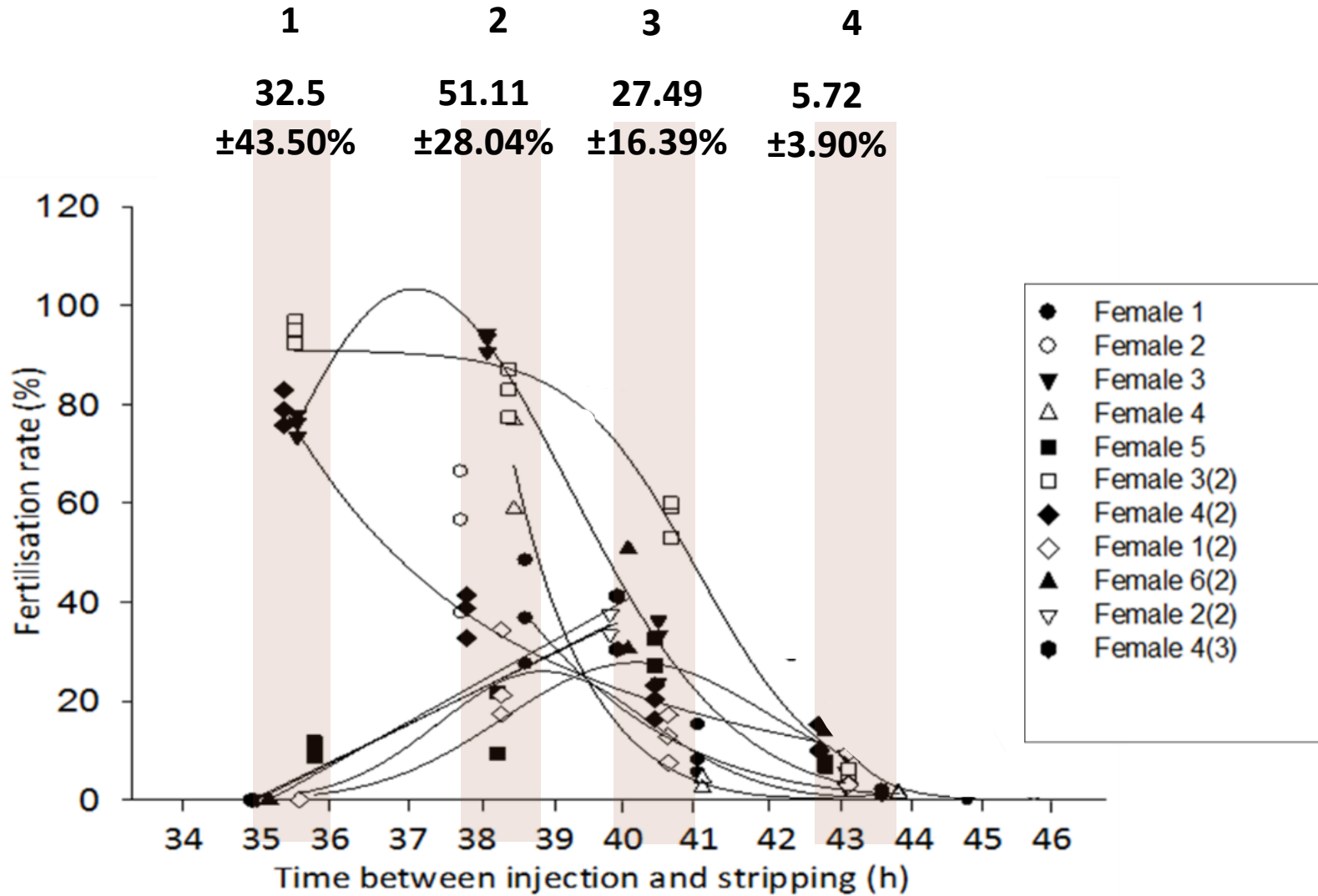
RESULTS

PART 1: TIME OF OVULATION



RESULTS

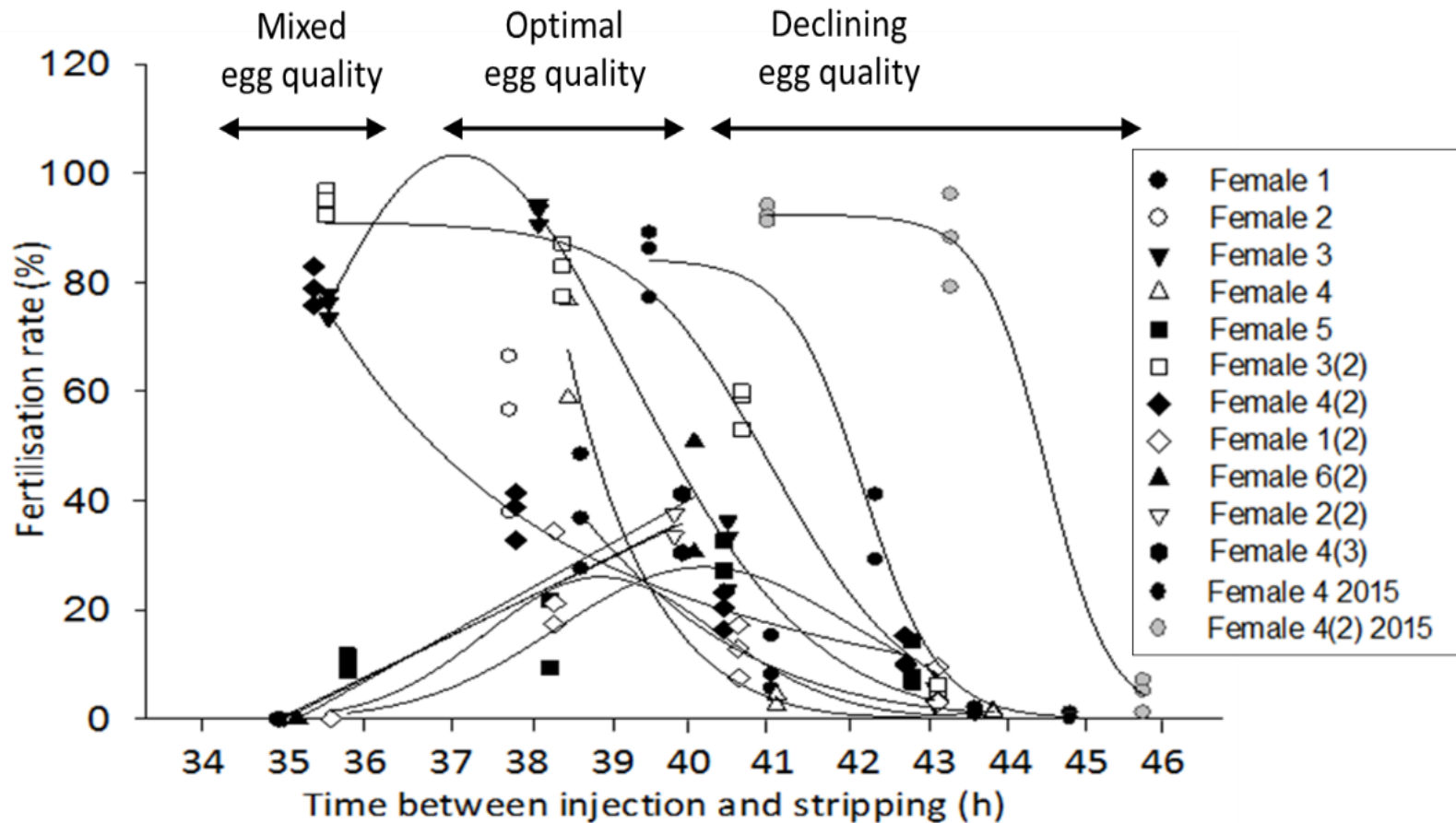
PART 1: TIME OF OVULATION



RESULTS

PART 1: TIME OF OVULATION

- The best stripping time was estimated to lie within 38-39 h following GnRH α injection at 18°C, and corresponds to the completion of ovulation.



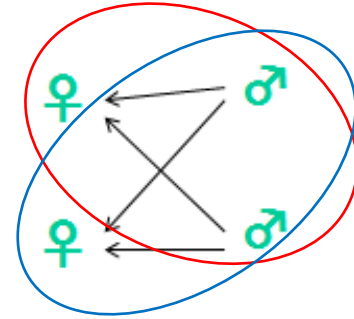
RESULTS

PART 2: DETERMINATION OF OPTIMAL SPERM: EGG RATIO

RESULTS

SPERM:EGG RATIO

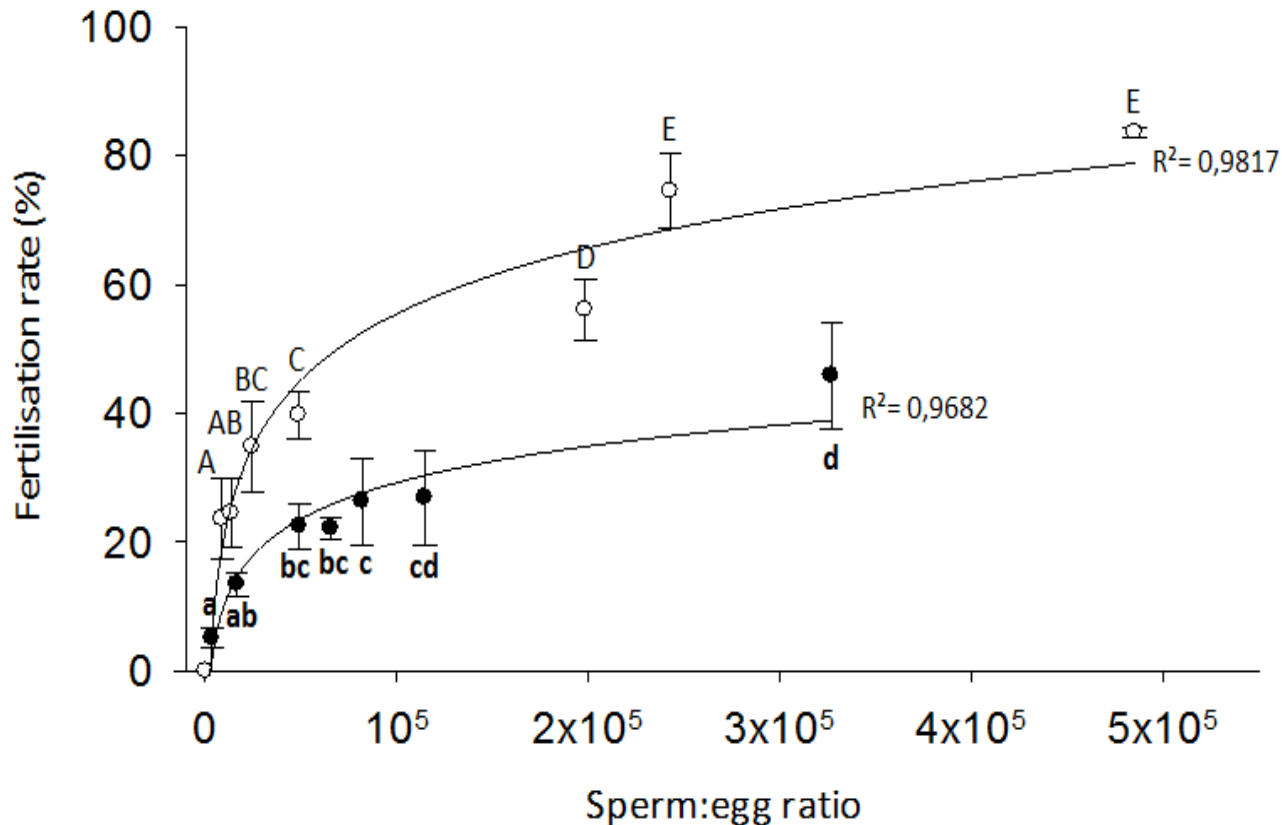
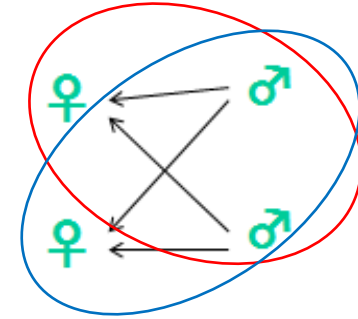
- ♂ No significant differences ($P > 0.05$) in the F.R. between males.
- ♀ Significant differences ($P < 0.05$) between females (different egg quality).
- Combined data in each female to obtain regressions.



RESULTS

SPERM:EGG RATIO

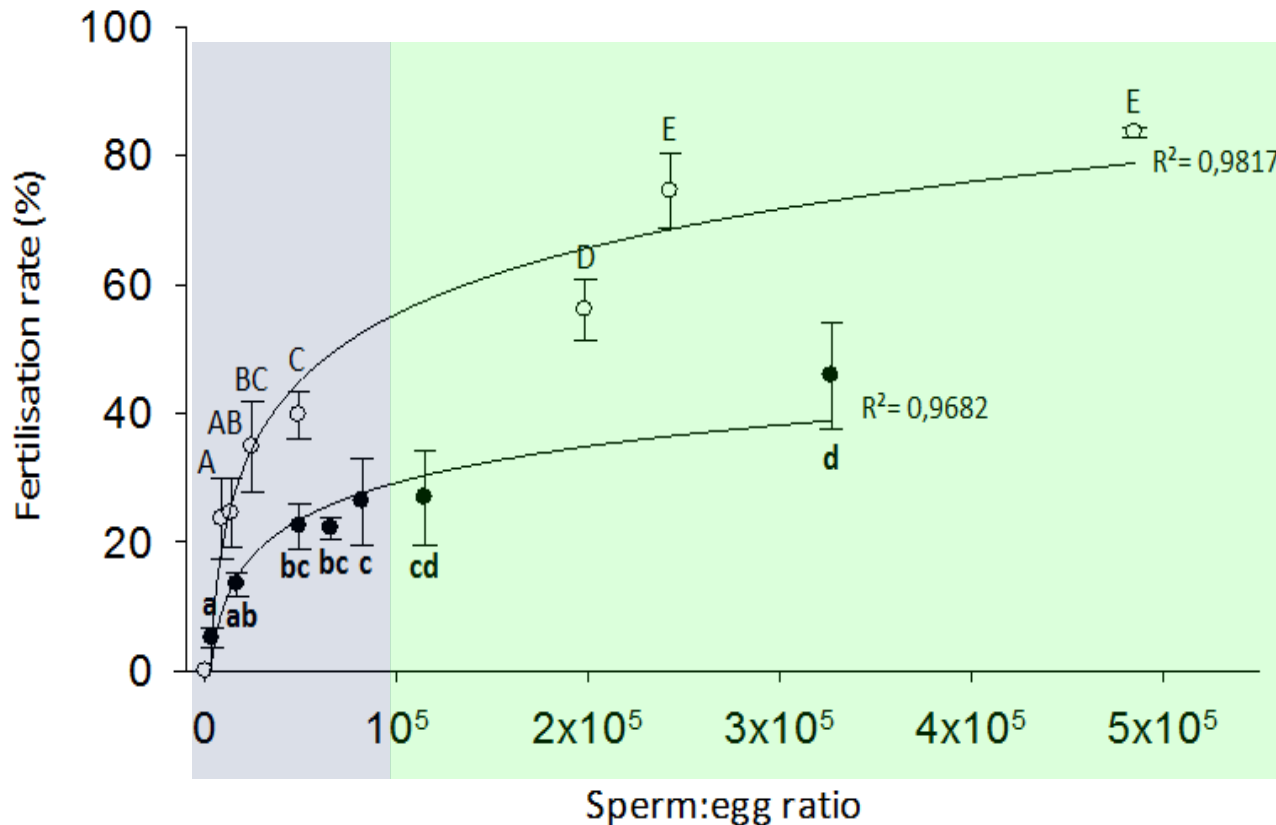
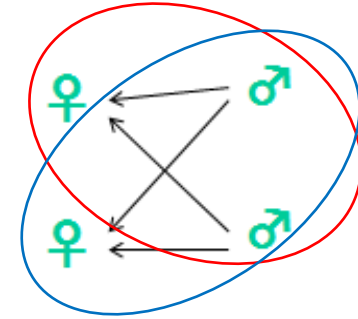
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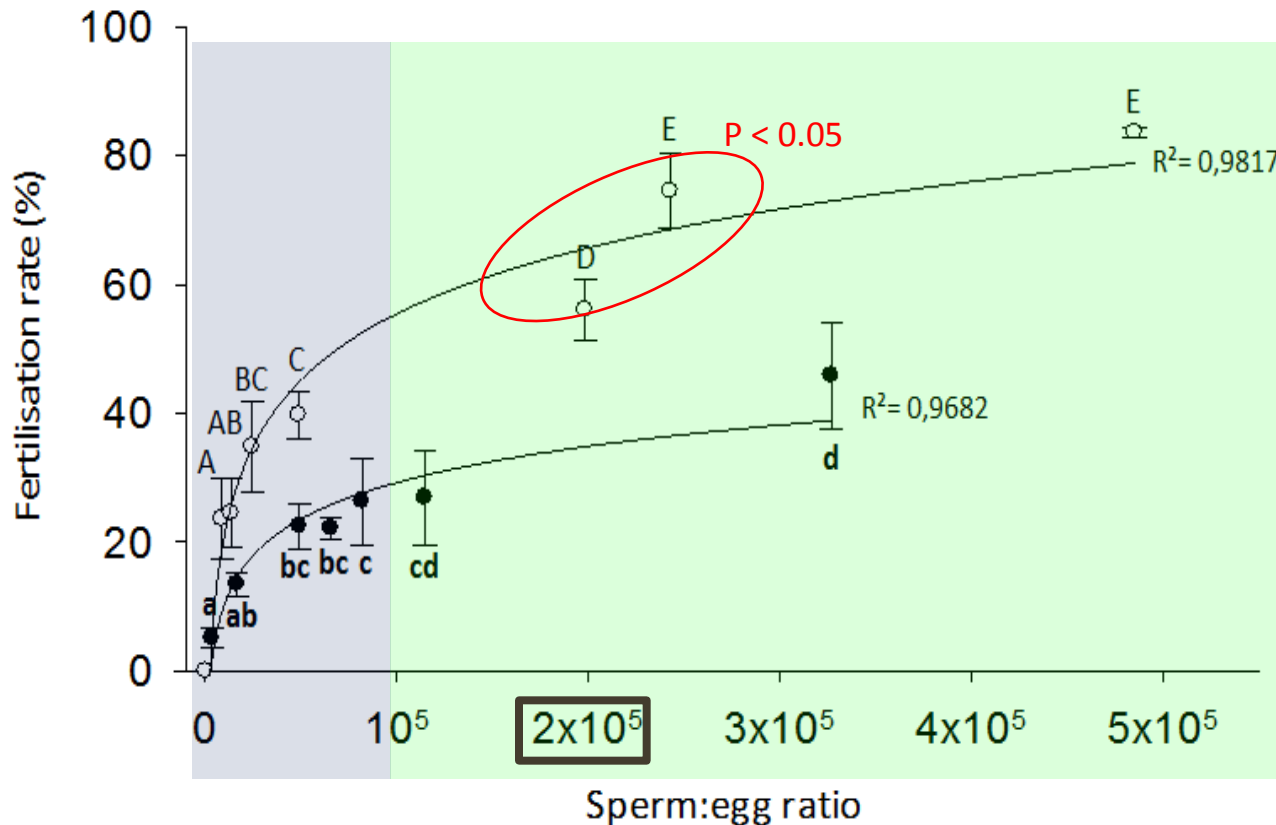
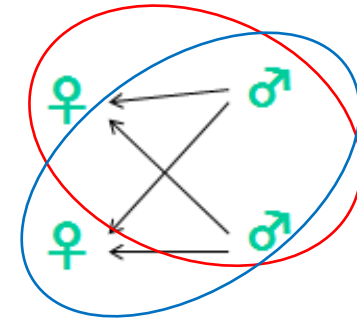
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CONCLUSIONS

The broodstock should be examined at 38 h post-injection at 18°C to obtain optimum egg quality.

Protocol for artificial fertilisation:

Mix:

Activation with a minimum
of 0.5 mL of sea water/mL
of eggs



Eggs

1 mL

(~ 1.700 eggs) stripped
between 2-3 hours after the
ovulation time

A large fish, possibly a salmon, is swimming in clear, shallow water. The water is a light greenish-blue color, and the fish is dark brown with a lighter belly. The fish is positioned in the center of the frame, swimming towards the right. The text "THANK YOU FOR YOUR ATTENTION" is overlaid in the center of the image in a bold, yellow, sans-serif font.

**THANK YOU FOR YOUR
ATTENTION**