DIVERSIFY
New species for EU aquaculture

Reproduction and rearing of the grey mullet, *Mugil cephalus* (Linnaeus, 1758) for restocking purposes

D. Vallainc, G. Brundu, G. Sanna, F. Antognarelli, N. Fois, S. Guerzoni and M. Baroli

IMC International Marine Centre

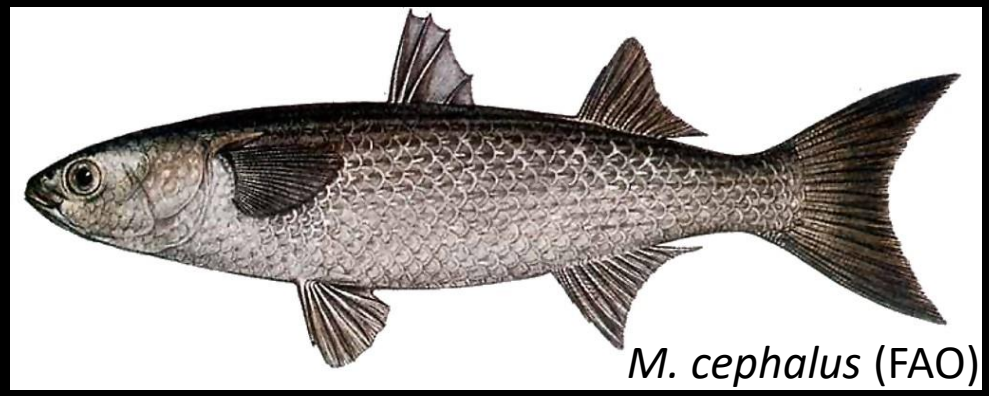
Dario Vallainc, PhD

d.vallainc@fondazioneimc.it

GREY MULLET KNOW-HOW TRANSFER WORKSHOP
14TH MAY 2018, BARI, ITALY

Grey mullet, *Mugil cephalus*

- Euryhaline
- Eurytherm
- Omnivorous detritivore
- Catadromouse



M. cephalus (FAO)

- **Growning demand of grey mullet roe**

Bottarga: salted and dried grey mullet roe

- **Traditional product since 3000 years**
- **Price: 230 €/kg**
- **More than 20 producers**
- **400 tons produced (Producer Association, 2007)**

- Aquaculture is based on wild fry collection (Crosetti & Blaber, 2015)
- High fishing pressure during the spawning season

Only 2 % of the bottarga produced in Sardegna is made from local grey mullet

- Depauperation of natural stock (Withfield et al., 2012)
- Unpredictable fluctuations of lagoon productivity (Oren, 1971; Ravagnan, 1978)

Artificial propagation in hatcheries could be the solution for obtaining a steady supply of fry (Liao et al., 2015)

Propagation techniques should be updated (Crosetti & Blaber, 2015)

Bottlenecks:

- High hormone doses to induce spawning (Crosetti & Blaber, 2015)
- High mortality of the larvae (Saleh, 2008)
- Slow growing species (Espino-Barr et al., 2015)

Objective:

Concur to re-define hatchery and rearing techniques to maximize the production of grey mullet juveniles for restocking purposes

Funded by:
R.L. n°. 7 07/08/2007: Reproduction trials of *M. cephalus* (Linnaeus 1758) for restocking purposes (2015-2017)

Agris

IMC International Marine Centre

Università di Cagliari

uniss UNIVERSITÀ DEGLI STUDI DI SASSARI

Production process

Reproduction and rearing

Broodstock capture

Transport

Selection

Hormone treatment

Spawning

Eggs incubation

3 densities

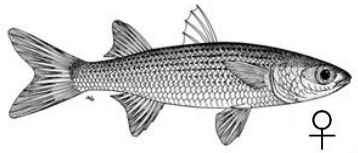
Larvae seeding

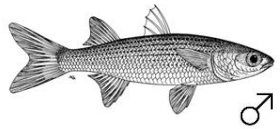
2 methods

Larval rearing

Juvenile rearing

3 densities





Live feed

Phytoplankton

Rotifers

Artemia nauplii

Indoor on-growing

C 1

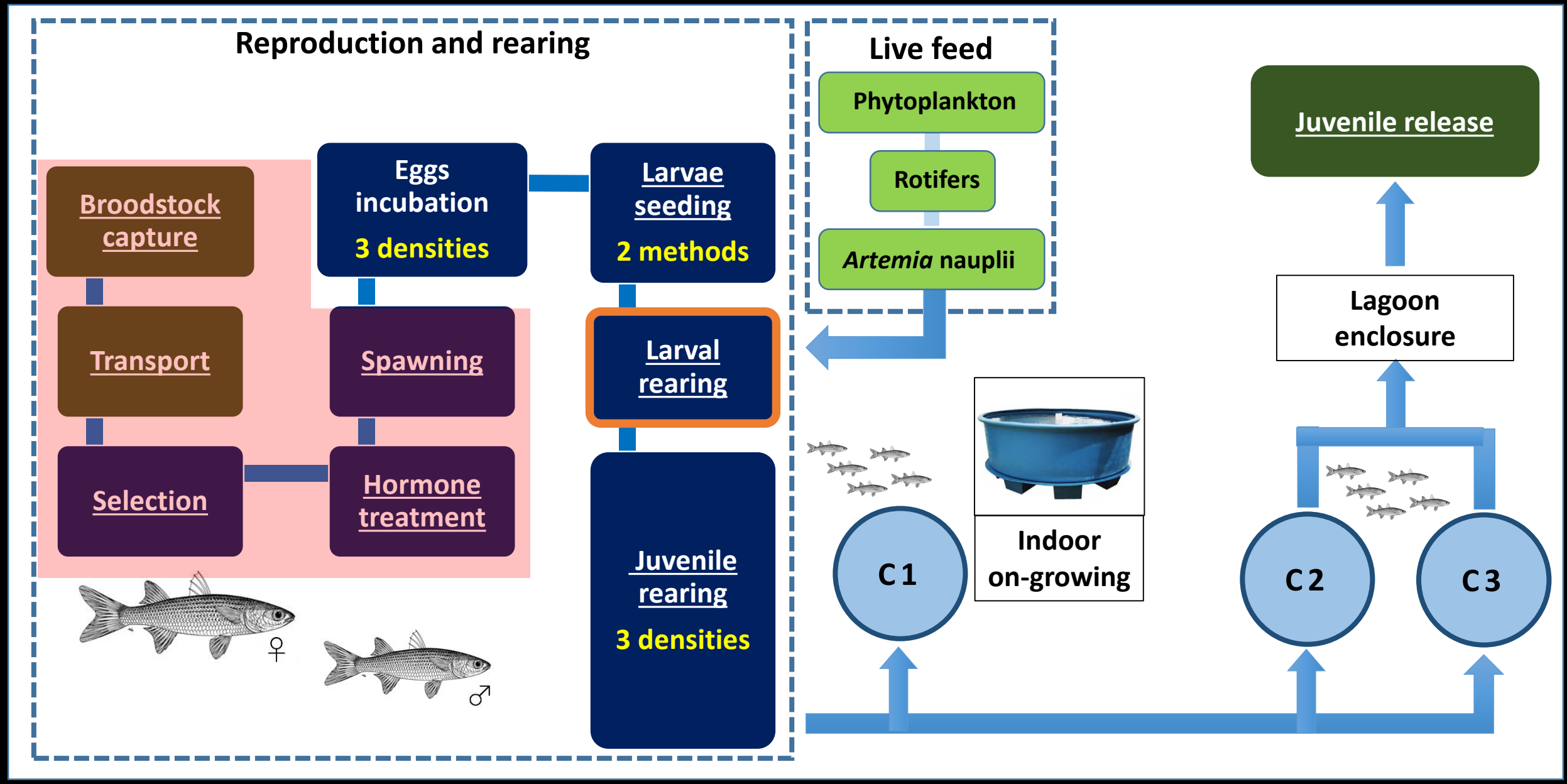
C 2

C 3

Lagoon enclosure

Juvenile release

Phase 1: Spawning induction



Sampling sites

<u>Cabras lagoon</u>: 2.200 ha

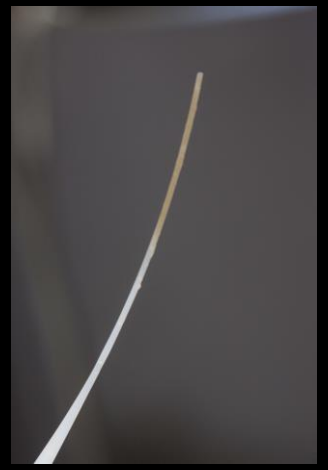
<u>Mistras lagoon</u>: 450 ha

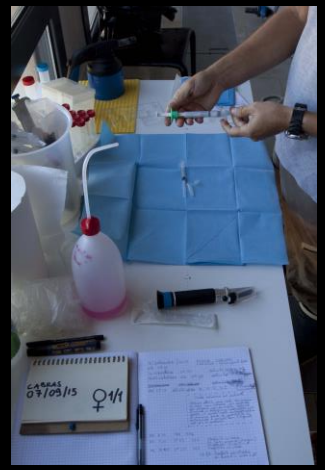
Broodstock collection

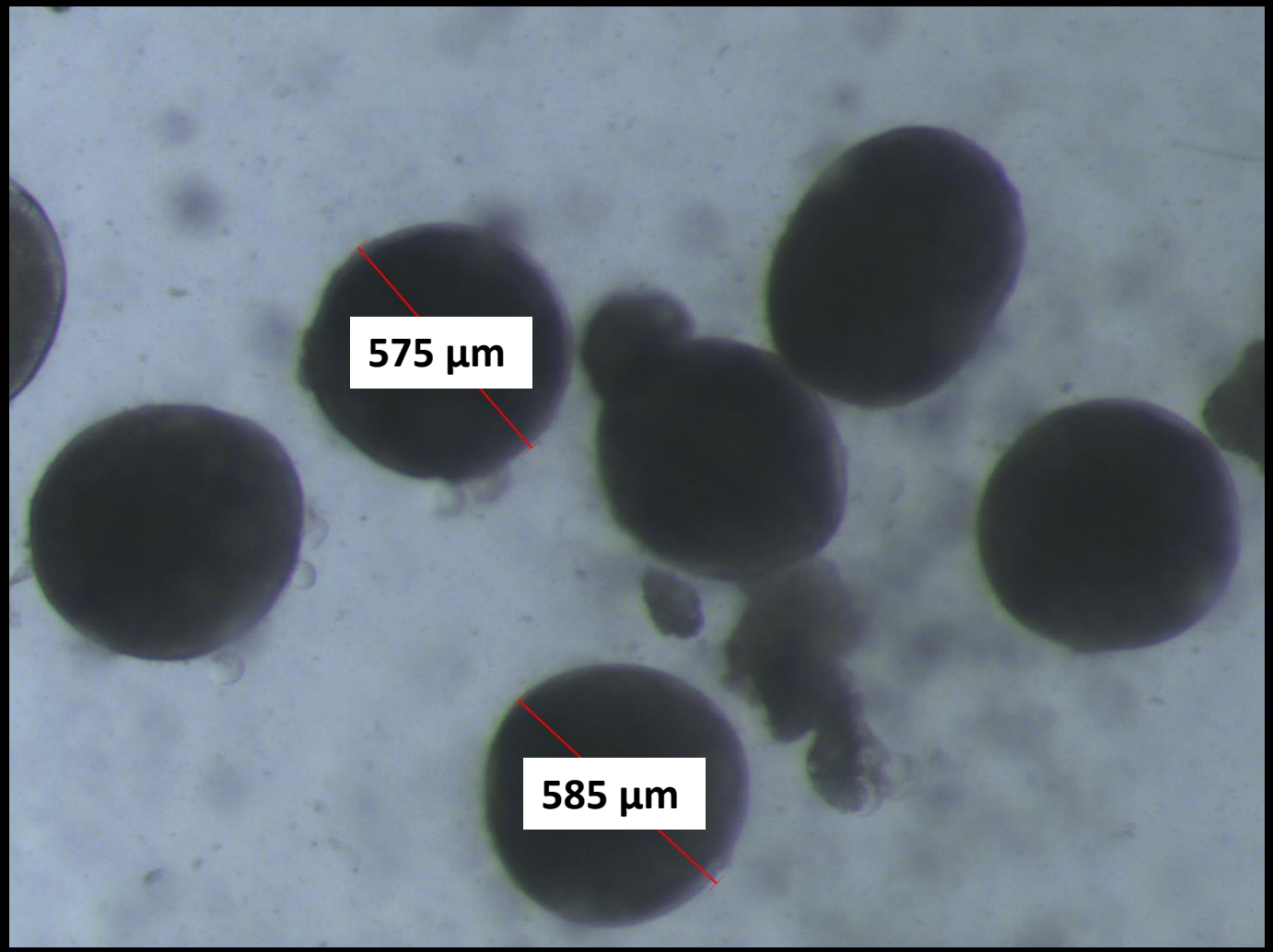
Cabras lagoon

Mistras lagoon

Acclimation and hormone treatment







Reproduction

Standard protocol
mullet PG 7 or carp PG 3 + LHRH 100/200 µg + DOM 10 mg + leuprolin acetate 500/700 µg + leuprolin acetate 600/700 µg /kg BW (Crosetti, 2001); (Crosetti & Cordisco, 2001)

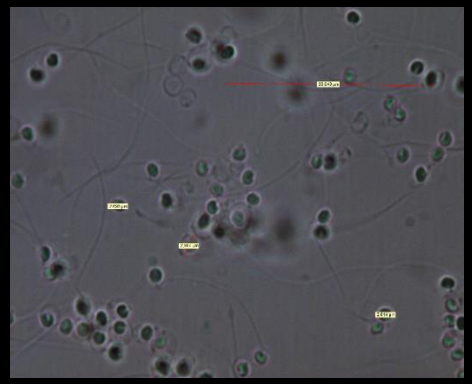
Protocol adopted in this study
Slow release LHRHa preparation (leuprorelin acetate) ENANTONE ® (Takeda Italia S.p.a.)
200 µg /kg BW

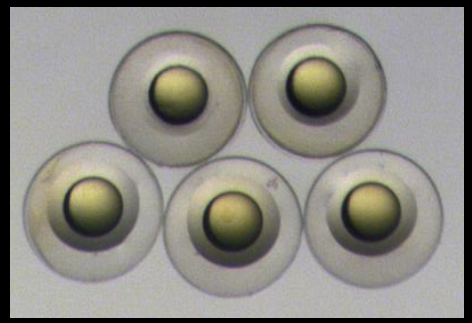
Reproduction results

Oocyte diameter	Spawning		Success %
	success	total	
<550 µm	0	8	0
>550 µm	7	11	64

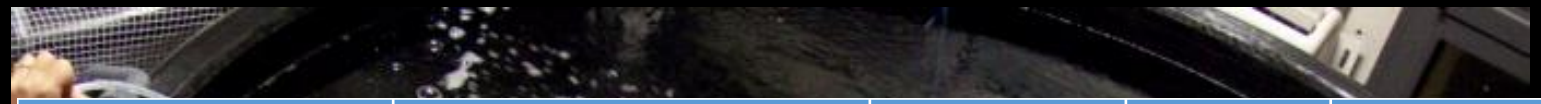
Fertilization		Success %
success	total	
3	7	43

Reproduction method	
Stripping	Natural
1	2



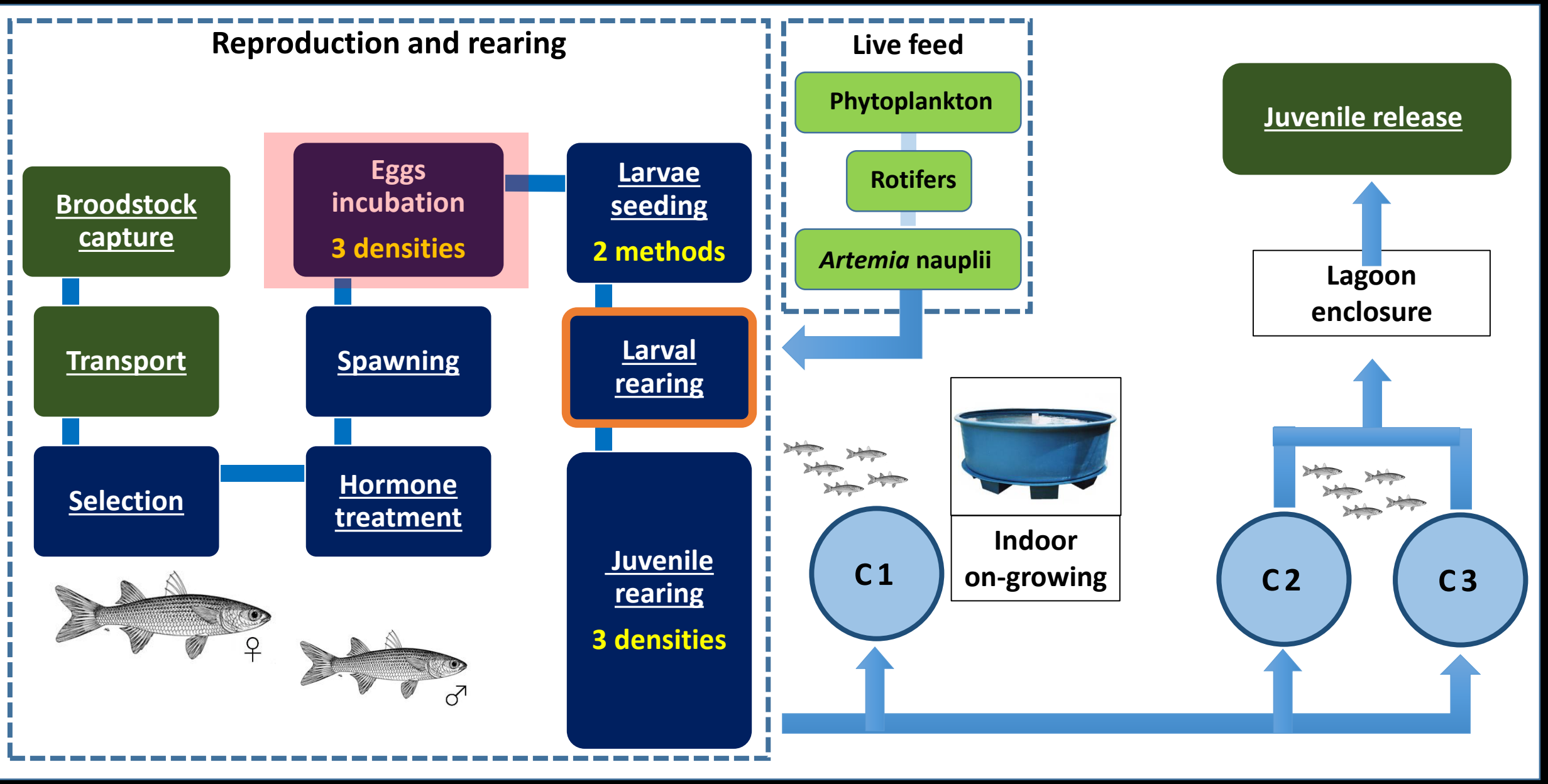


Reproduction results



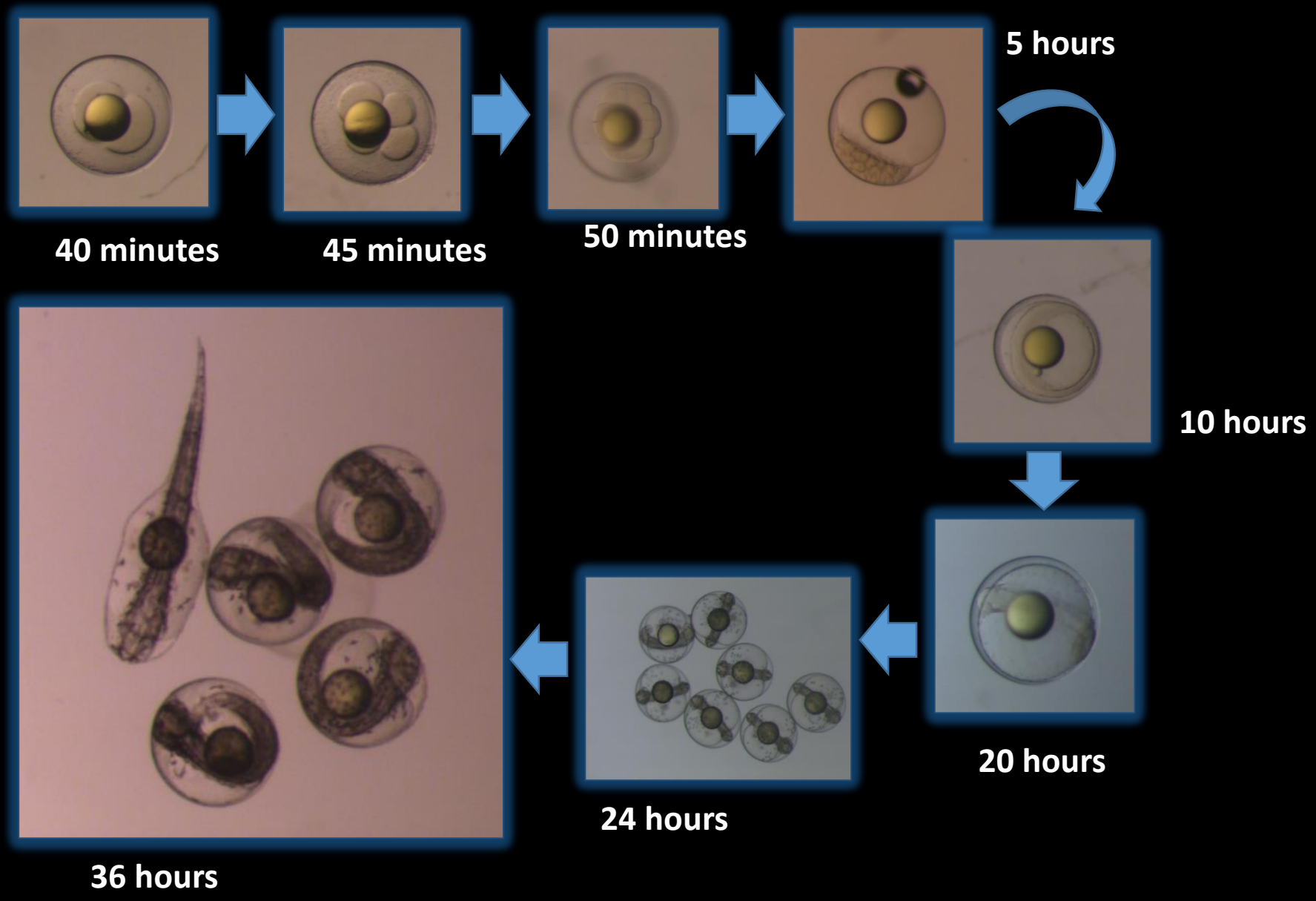
Reproductive Cycle	Sex ratio		Oocyte Ø (µm)	Egg N° (10^6)	Fecundity (eggs/g BW)	Fertilizzation %	Method
	F 2-4 kg BW	M 1-2 kg BW					
C1	1	3	562±6	3.1	704	84±6	Dry stripping
C2	1	3	599 ±5	1.6	842	88±7	Natural spawning
C3	1	4	593 ±6	1.8	818	92±6	Natural spawning

Phase 2: Incubation of the embryos



Hatching system

Embryo development

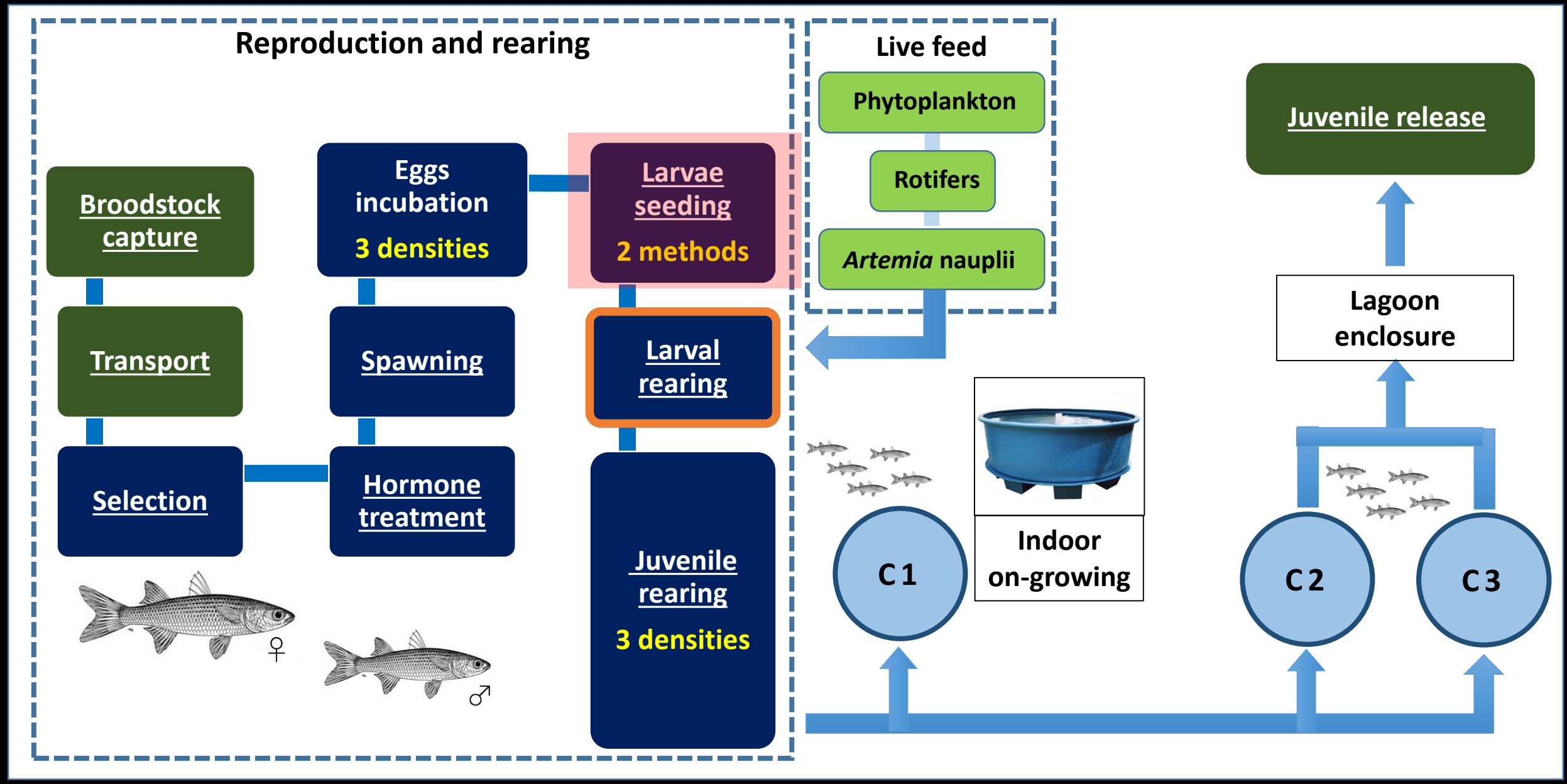


Hatching rate at different egg densities

	Density (eggs/L)	Hatching rate (%)
C1	200	87 ± 8
C2	400	84 ± 11
C3	**1000**	**72 ± 12**

Optimal density: 250/L (Nash et al., 1980)

Phase 3: Stocking of the larvae



Larval transfer from the hatching to the rearing system

- **Just hatched (JHL)**

M. cephalus **just hatched larva (JHL)**

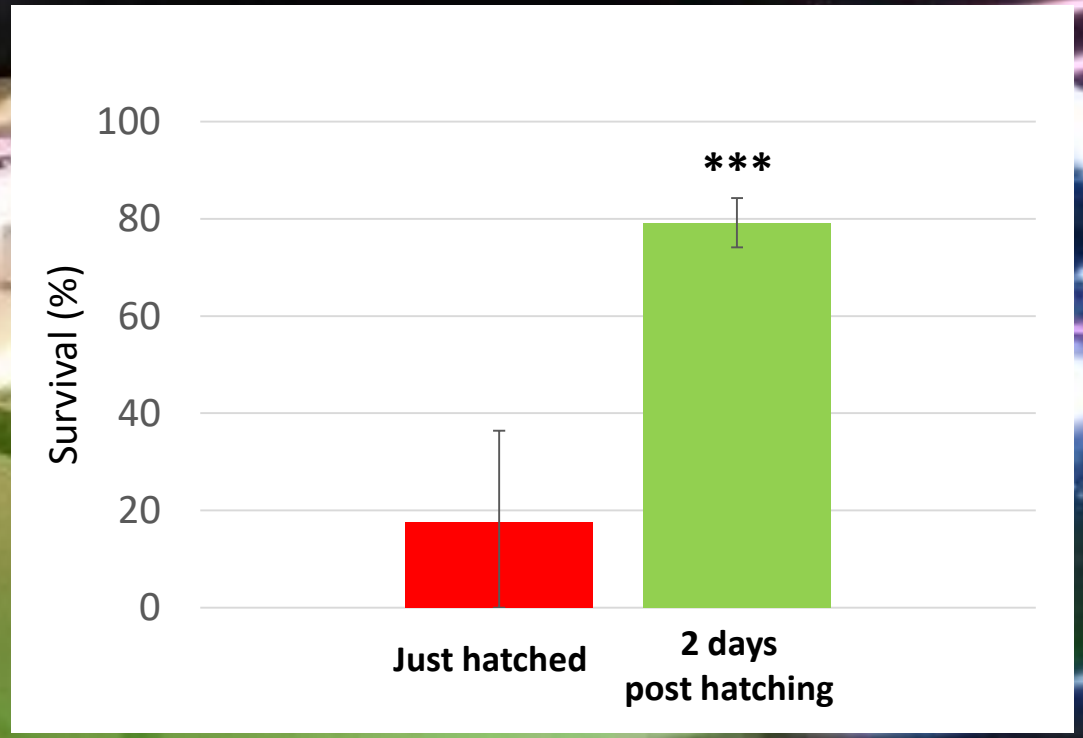
- **Two days post hatching (2 DPH)**

M. cephalus **larva at 2 days post hatching (2DPH)**

Larval survival at 3 days post hatching

C1: JHL

C2 & C3: 2 DPH



p-value < 0.0001

Phase 4: Larval and juvenile rearing

Reproduction and rearing

Broodstock capture

Transport

Selection

Hormone treatment

Spawning

Eggs incubation

3 densities

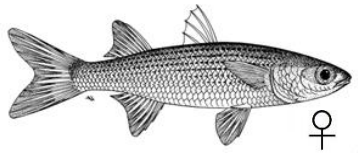
Larvae seeding

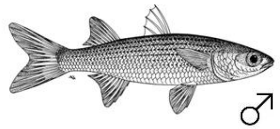
2 methods

Larval rearing

Juvenile rearing

3 densities





Live feed

Phytoplankton

Rotifers

Artemia nauplii

Indoor on-growing

C 1

Lagoon enclosure

C 2

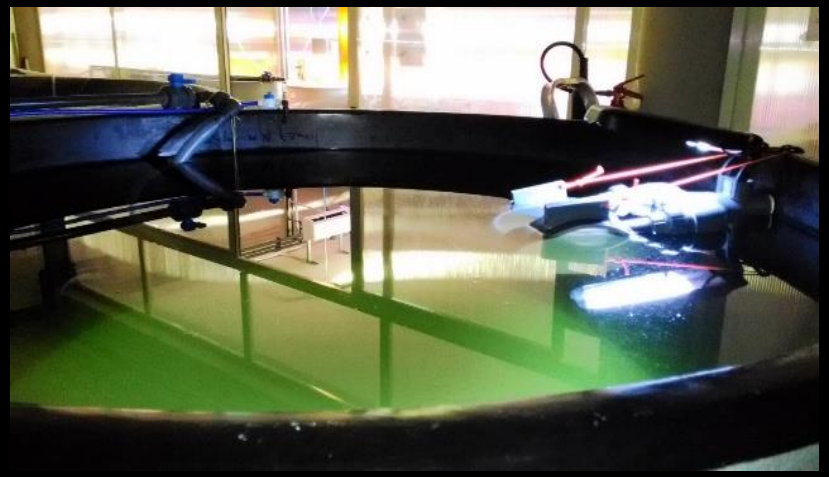
C 3

Juvenile release

Larval rearing

Larval rearing system

RAS

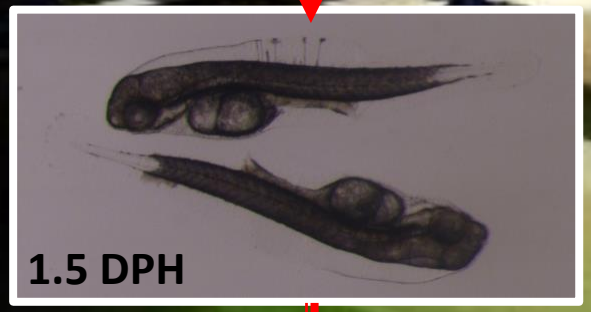


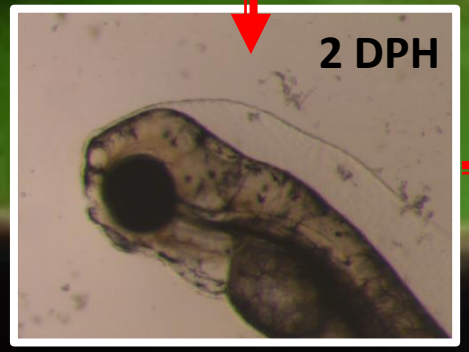
Green water

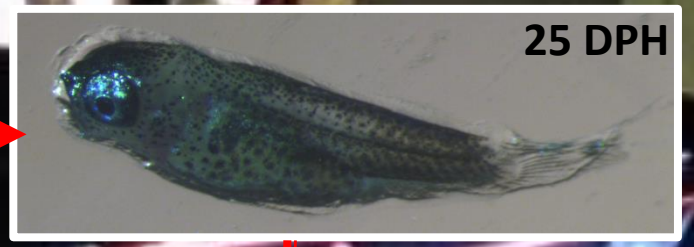
Phyto-zoo-plankton

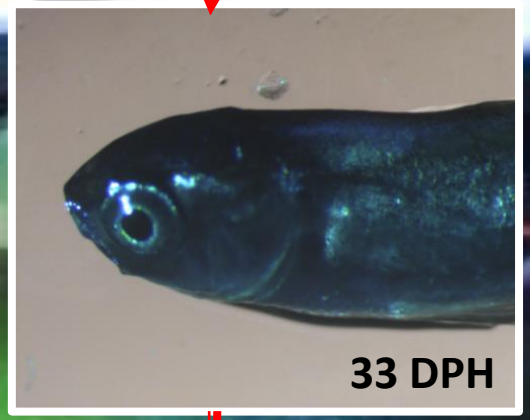
Feed	Density	Days
Phytoplancton *Isochrysis galbana* *Nannochloropsys oculata*	300, 000 cells/ml	2-35
Rotifers *B. plicatilis* L-type	4-5/ml	2-35
Artemia *Artemia* nauplii	3-4/ml	12-45
Gemma micro 50	*Ad libitum*	20-30
GEMMA Wean 0.1	*Ad libitum*	28-55
Perla Larva	*Ad libitum*	50-200
Larva plus	*Ad libitum*	195-365

Larval development



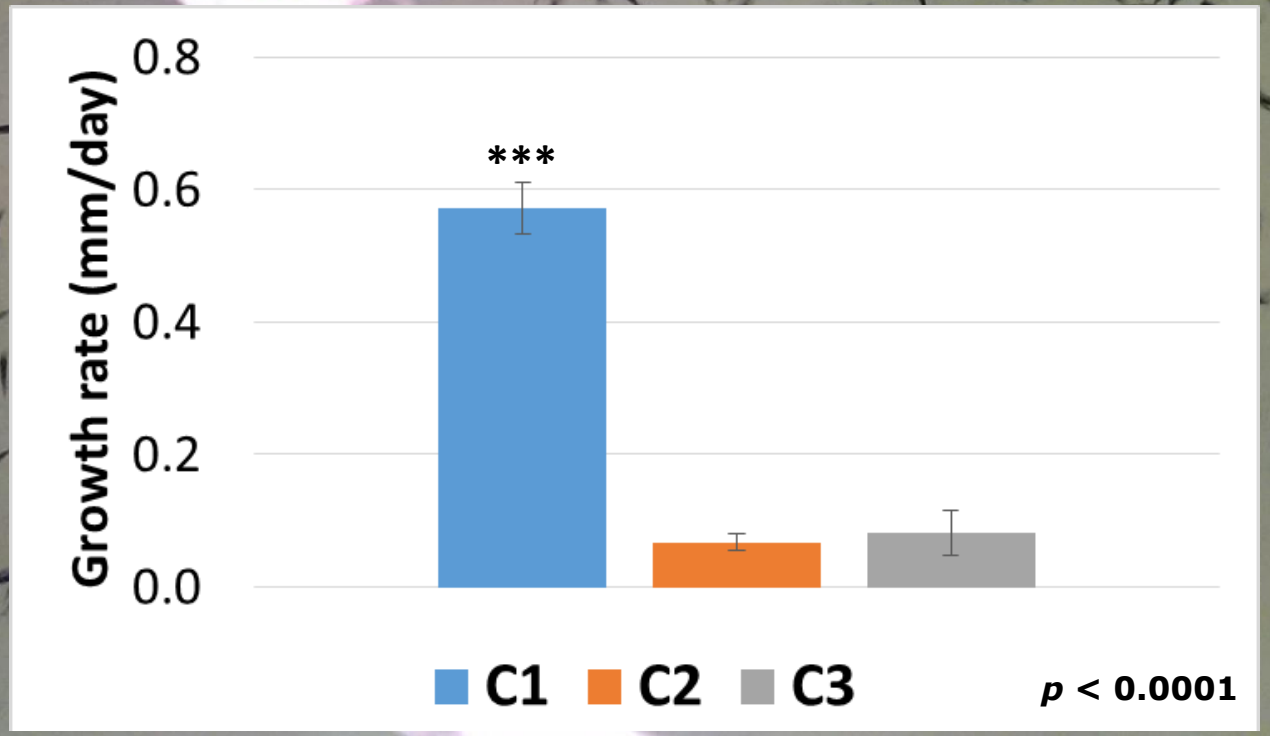






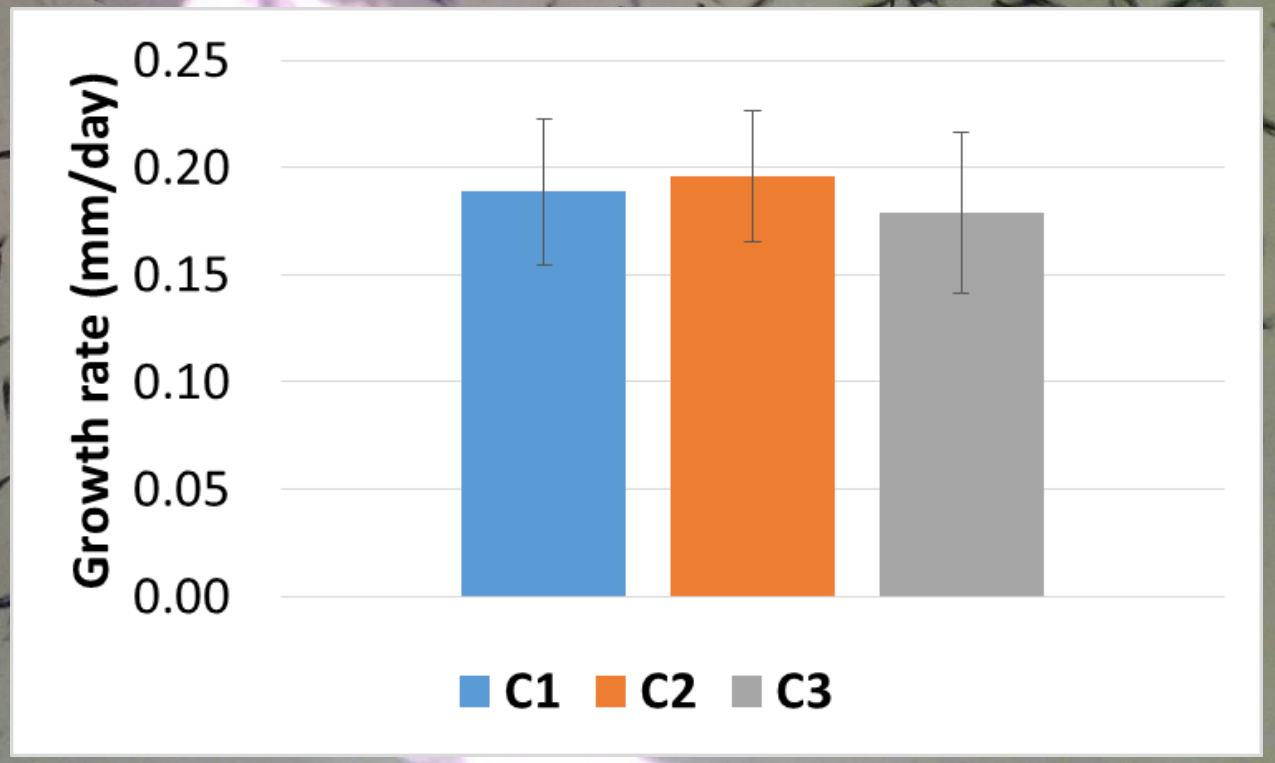
Larval survival and growth at different densities

	Initial density (3 DPH)	Survival (45 DPH)	N° (45 DPH)
C1	6.8/L	2.4 %	960
C2	30.0/L	18.2%	14,560
C3	33.2/L	35.8%	28,640

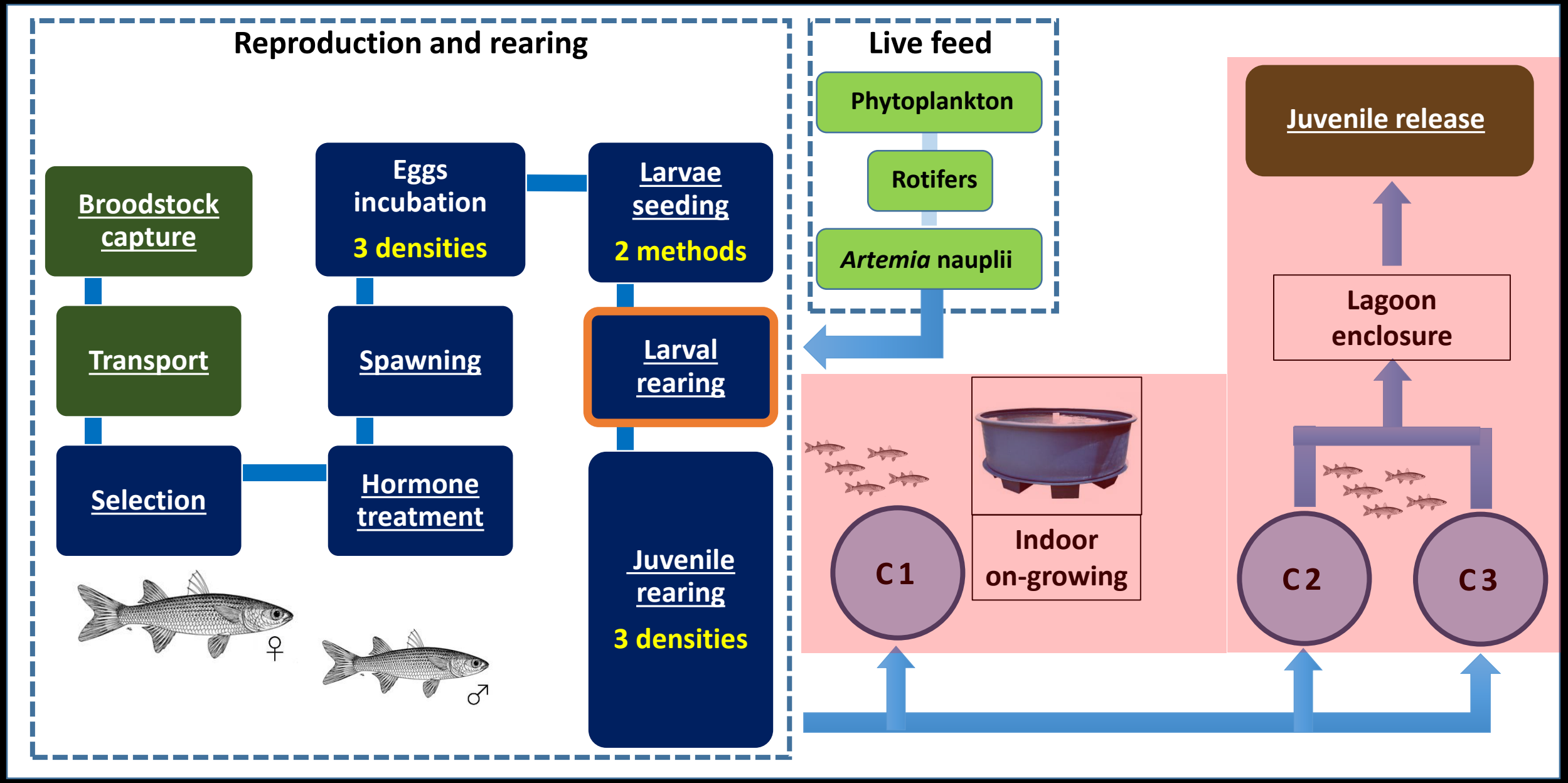


Juvenile survival and growth at different densities

	Initial density (45 DPH)	Juvenile survival (45-200 DPH)	Final survival (200 DPH)	N° (200 DPH)
C1	0.5/L LOW	36.5%	0.9%	350
C2	7. 3/L MEDIUM	32.6%	5.9%	4,746
C3	14.3/L HIGH	14.4%	5.2%	4,138



Phase 5: Juvenile outdoor rearing



Growth 100 % indoor

Indoor (N=350)
April

- TL= 59 ± 6 mm
- BW= 2.4 ± 0.7 g

Growth rates

	GR TL (mm/day)	GR BW (g/day)
April-August	0.2 ± 0.04	0.04 ± 0.01
August-November	0.1 ±0.1	0.04 ± 0.04
Total	0.2 ± 0.04	0.05 ± 0.01

November

- TL: 101±8 mm
- BW: 11.2±2.4 g

C1

Growth 50 % indoor

Lagoon enclosure (N=5000)
April
TL= 33 ± 6 mm
BW= 0.5 ± 0.3 g

Growth rates

	GR TL (mm/day)	GR BW (g/day)
April-August	0.3 ± 0.1	0.03 ± 0.02
August-November	0.1 ± 0.2	0.01 ± 0.05
Total	0.2 ± 0.1	0.02 ± 0.01

November
TL: 82±14 mm
BW: 5.7±2.9 g

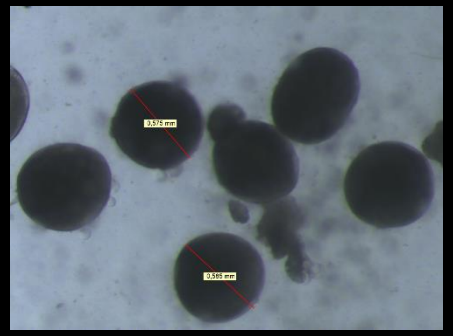
C2+C3

Size for release: 70-110 mm TL (Liu & Kelley 1994; Leber et al., 1996)

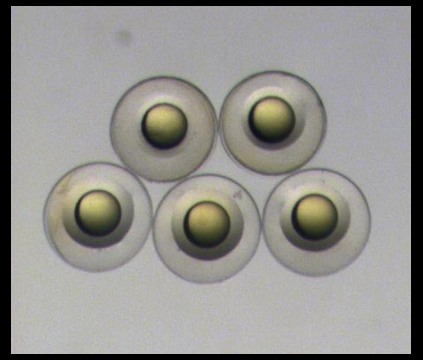
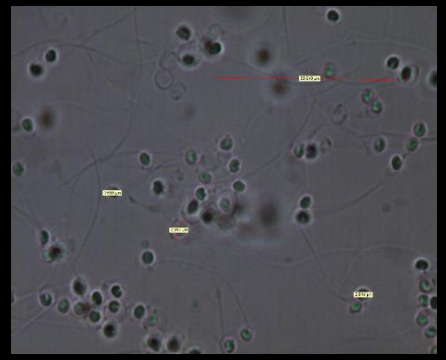
Conclusions 1

- Enantone is a valid treatment for inducing spawning in grey mullet

- Doses used, administered by a singular injection were ~ 3 times lower than those reported in literature and successfuly induced spawning in female with oocyte diameters larger than 550 µm

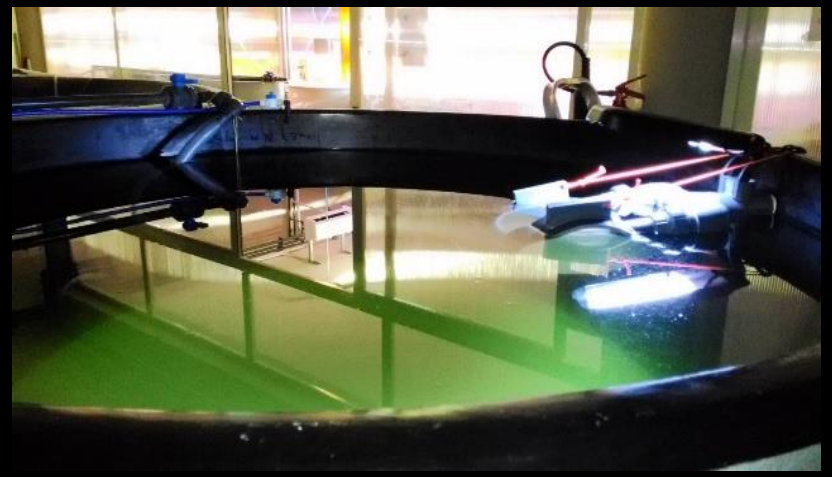


Conclusions 2

- Embryos can be incubated at much higher densities than those reported in literature as optimal (> 250 egg/L)

- Transfer the larvae from the incubators to the larval rearing tanks at 2 days post hatching increased survival rate compared to transfer the larvae immediately after hatching



Conclusions 3

- Medium density juveniles (7/L) performed better in terms of survival
- The indoor production process followed by the outdoor growing phase resulted in juvenile seeds at restocking size within 14 months



FURTHER STUDIES

TECNOMUGILAG (SARDEGNA RICERCHE, 2018-2020)

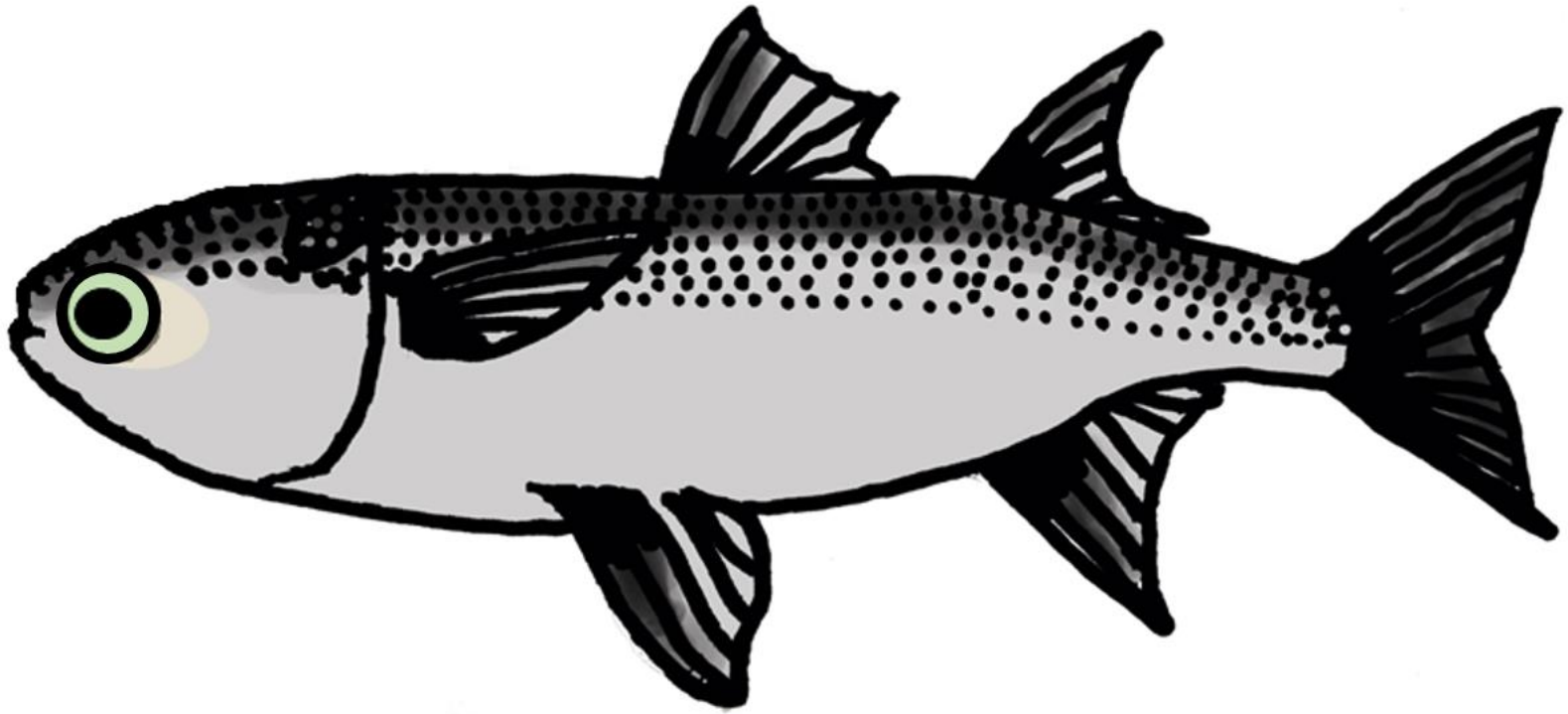
Transfer of *Mugil cephalus* rearing techniques to cooperatives of fisherman operating in lagoons

- **Individuate suitable diets**
- **Individuate suitable sites for extensive rearing**
- **Mark-release-recapture of juveniles (PIT tag)**
- **Transfer of rearing techniques**

12 SARDINIAN COOPERATIVES OF FISHERMEN

Thank you

EAT MORE



MULLET