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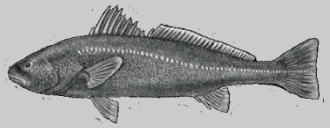
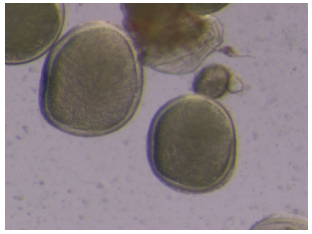
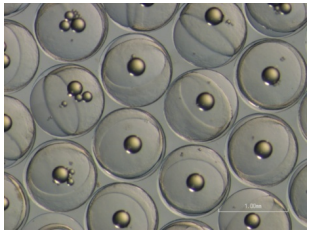
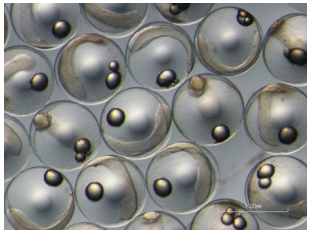
Meagre *Argyrosomus regius*



Natural spawnings and larval rearing

Pedro Pousão Ferreira
IPMA – Instituto Português do Mar e da Atmosfera
EPPO - Estação Piloto de Piscicultura de Olhão

*pedro.pousao@ipma.pt

TUESDAY 9TH OCTOBER 2018,
PALAU MACAYA, BARCELONA, SPAI

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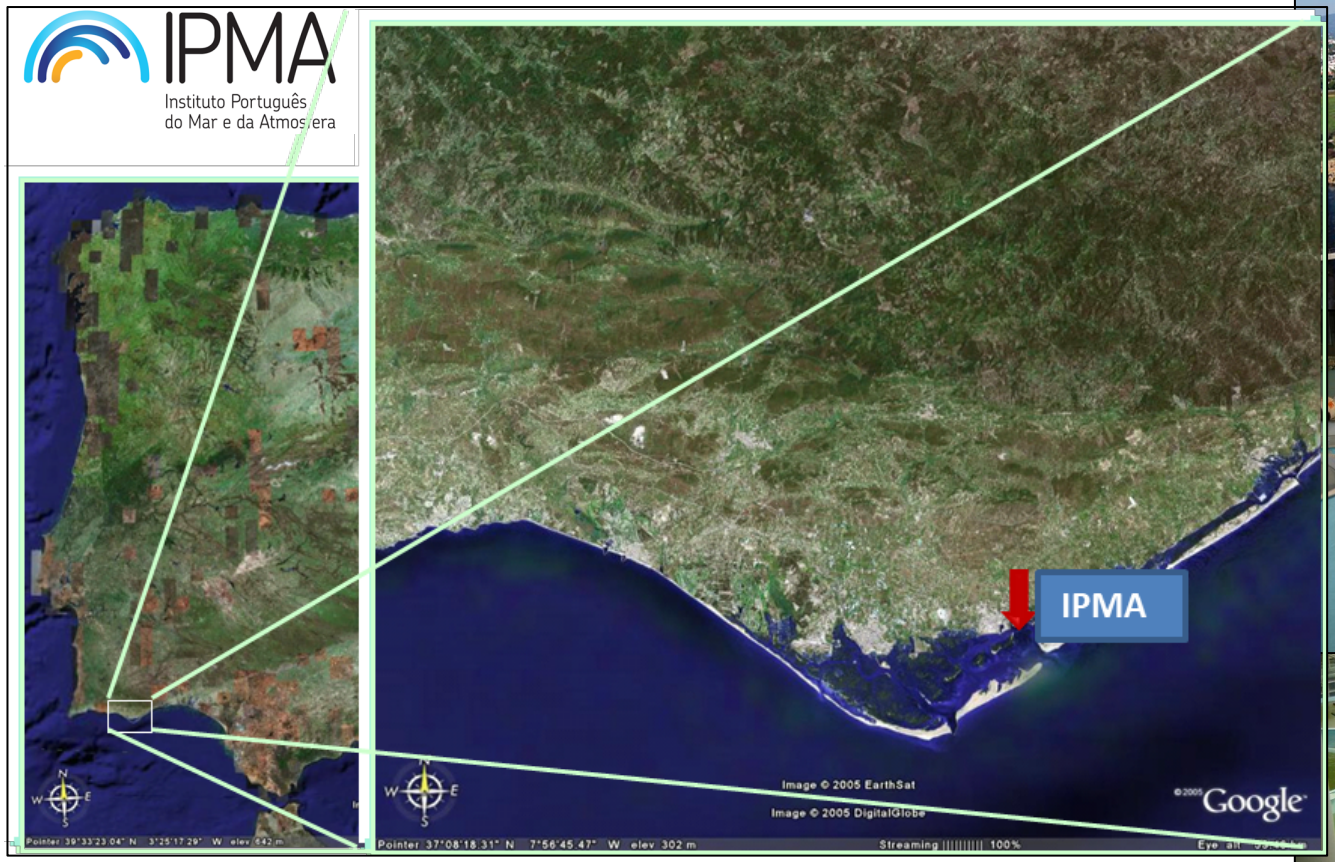


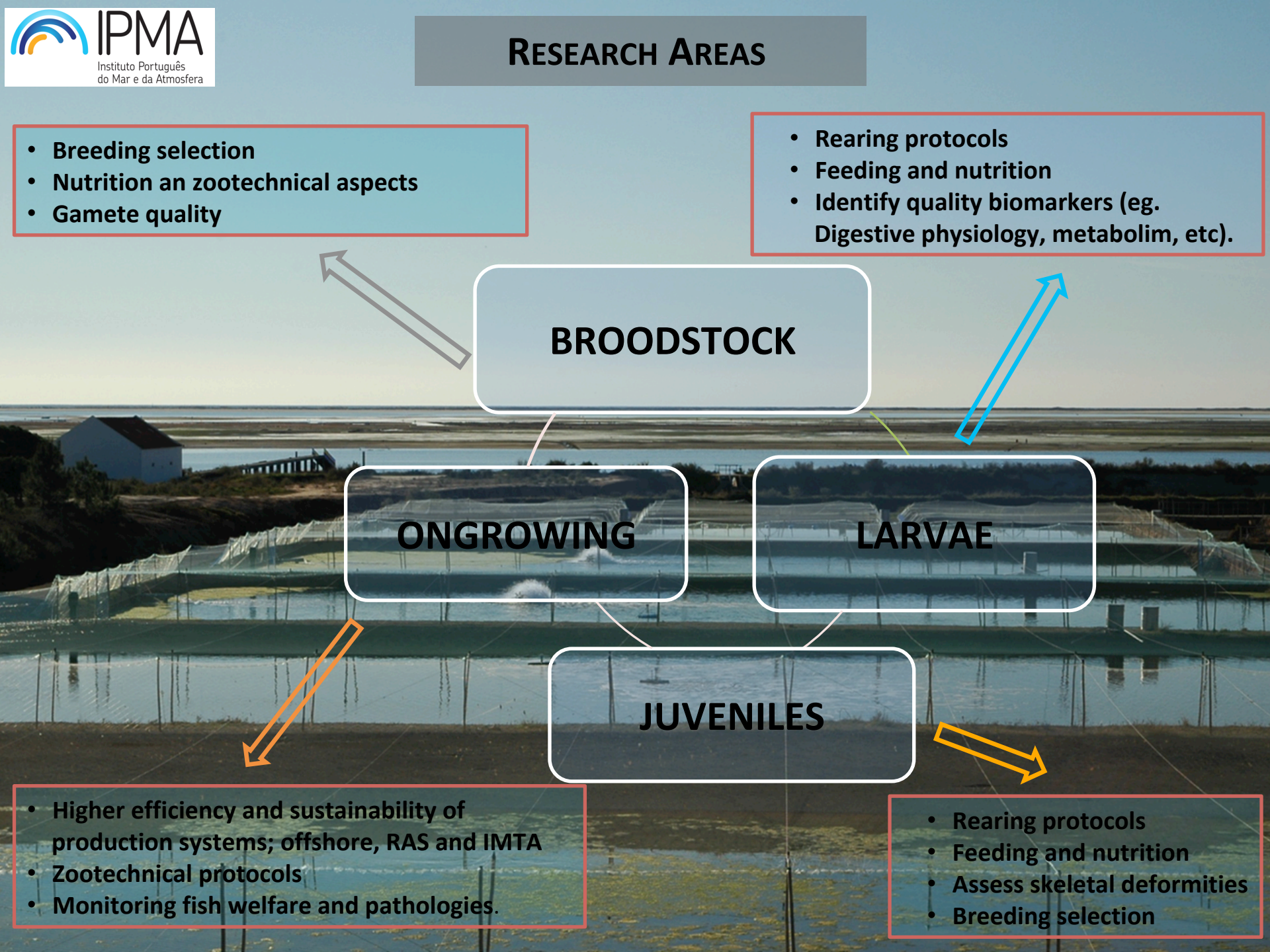
Rearing Location

IPMA – Aquaculture research Station is located in the Ria Formosa coastal lagoon in the Algarve, the Southern province of Portugal

Research Areas

- Breeding selection
- Nutrition an zootechnical aspects
- Gamete quality

- Rearing protocols
- Feeding and nutrition
- Identify quality biomarkers (eg. Digestive physiology, metabolim, etc).



- Higher efficiency and sustainability of production systems; offshore, RAS and IMTA
- Zootechnical protocols
- Monitoring fish welfare and pathologies.

- Rearing protocols
- Feeding and nutrition
- Assess skeletal deformities
- Breeding selection

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Work in progress - species of interest


Solea senegalensis


Argyrosomus regius


Dicentrarchus labrax


Sardina pichardus


Crossostrea gigas


Ulva spp.


Paracentrotus lividus


Sparus aurata


Diplodus cervinus


Epinephelus marginatus


Diplodus sargus

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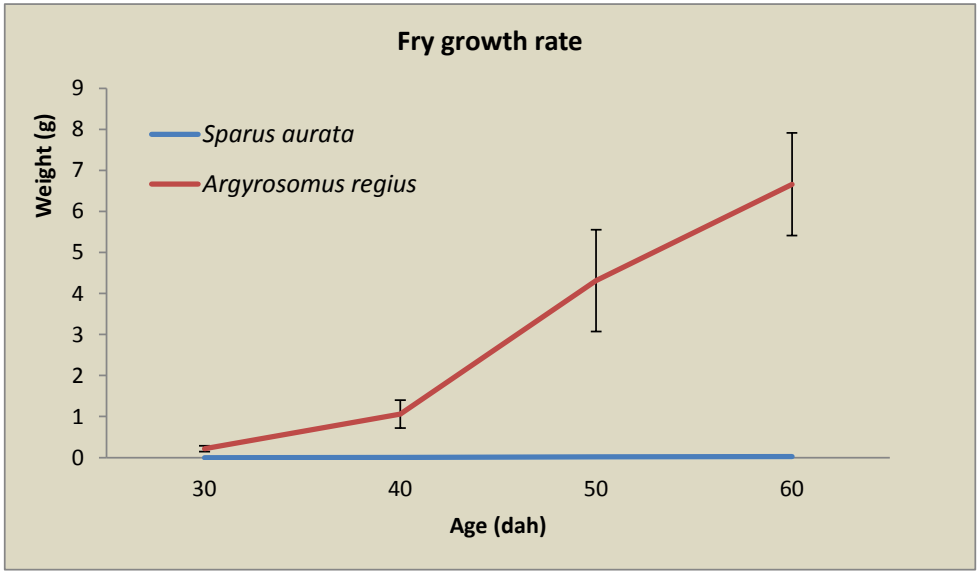

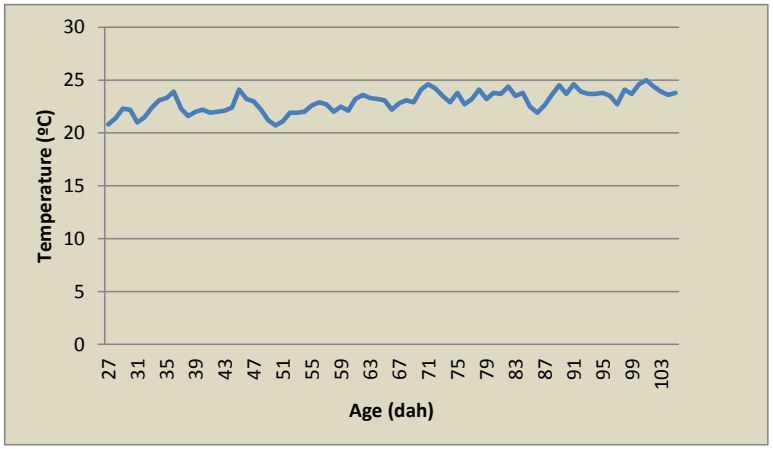

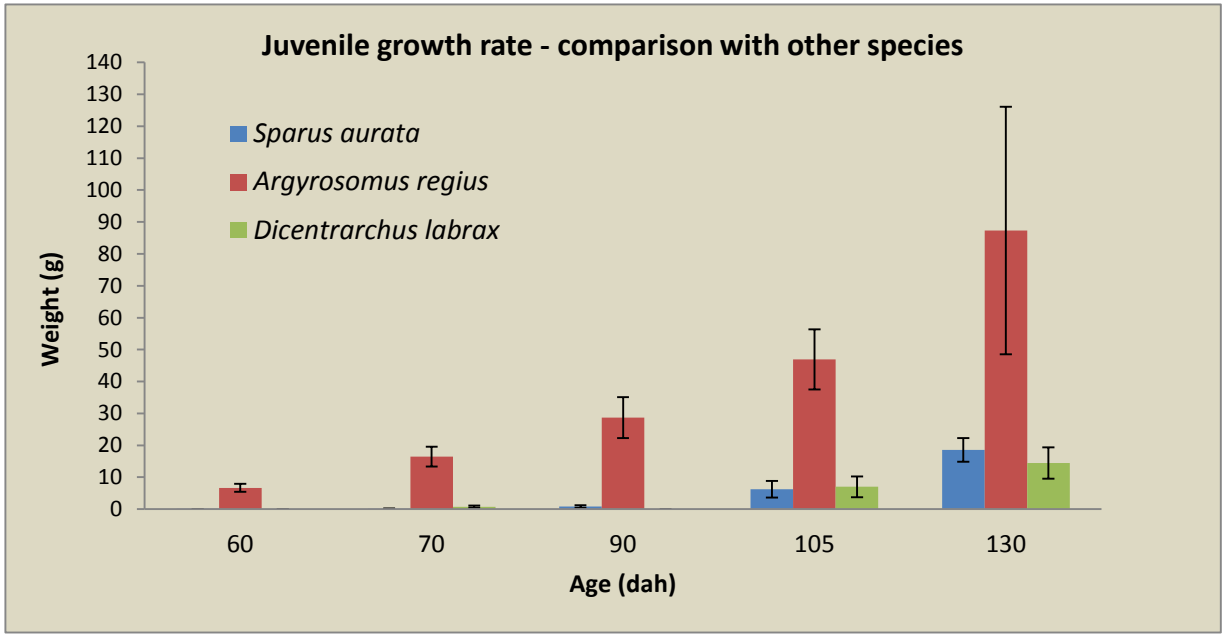

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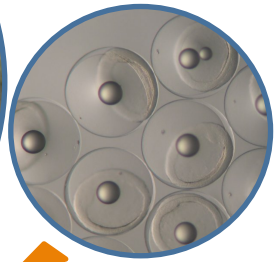
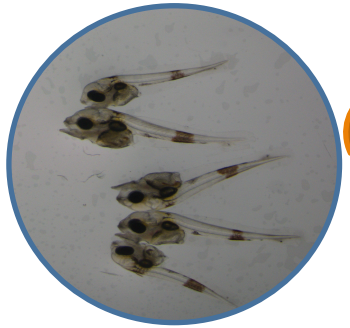
Research to enhance knowledge

FEEDING & NUTRITION

- Optimize feeding protocols
- Optimize nutrition


F1 breeders


eggs


larvae


juveniles


ongrowing

Enhance GROWTH

But

Fish health and welfare

Environmental Sustainability

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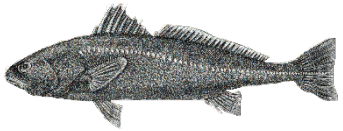
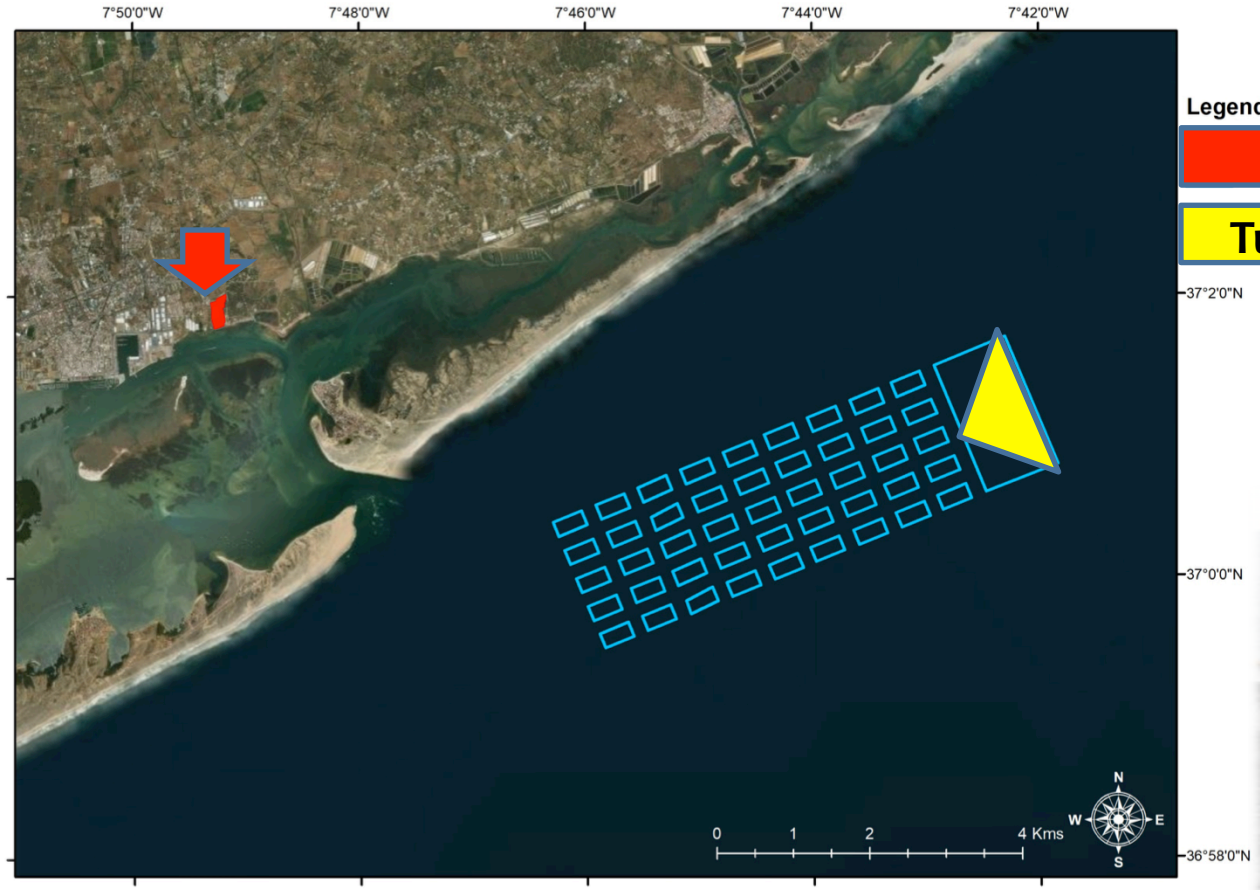
ORIGIN OF THE BROODSTOCK



wild

8 meagre captured between 2007 and 2008, in a tuna trap (Tunipex, Olhão) located 2.5 miles off the SE Portuguese coast.



Legend

EPPO

Tuna trap

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BROODSTOCK ORIGIN

F1, F2

Rearing: Juveniles (300±100g) were moved to earthen ponds to grow until 2.5±0.5kg and selected as breeders

↓

based on physical characteristic

In First generation (F1-2012) and second (F2-2017) generation breeders were rearing at the **Aquaculture Research Center of Portuguese Institute for the Ocean and Atmosphere (IPMA)**

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BROODSTOCK

F1

We maintain meagre F1 stocks in the earthen ponds as backup for future works

Weigth range 5-15Kg

Adapted to inert feed

Provided new stocks for a hatchery in France or others

BROOSTOCK MANAGEMENT
Rearing Conditions

Cylinder tank : 50m^3

- Renewal: 20 L.min^{-1}
- Salinity : 35-38
- Temperature: >14 ºC to < 24 ºC
- Natural photoperiod
- Density : < 5 Kg/m^3
- Protected tanks with shading net

Wild 2007- 2018

Weighed: **15-40kg**

F1 2015- 2018

Weighed: 15-20kg

POUSÃO-FERREIRA, P. *et al*., 2010. Aquaculture Europe'10 Seafarming tomorrow. Porto, Portugal: pp. 1040-1041.

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BROODSTOCK MANAGEMENT

In 2012 - to carry out different trials in triplicate we performed zootechnics adaptations

→ 6 smaller tanks (9 m^3)

→ Smaller individuals (F1)

Trials:

- Different photoperiods
- Nutritional trials
- Semen criopreservation

SANTOS M. *et al.*, 2018. *Aquaculture Research*:1–9.

BROODSTOCK MANAGEMENT

Cubic tank: 9m^3

F1 2012- 2018

6 tanks

Weighed: 2-12kg

- Renewal: 8-10 L.min^{-1}
- Salinity : 35-38
- Temperature:
  Resting season: >12 ºC to 26 ºC
  Spawn season: 18ºC to 21 ºC
- Natural photoperiod
- Density : < 8 Kg/m^3
- Protected tanks with net

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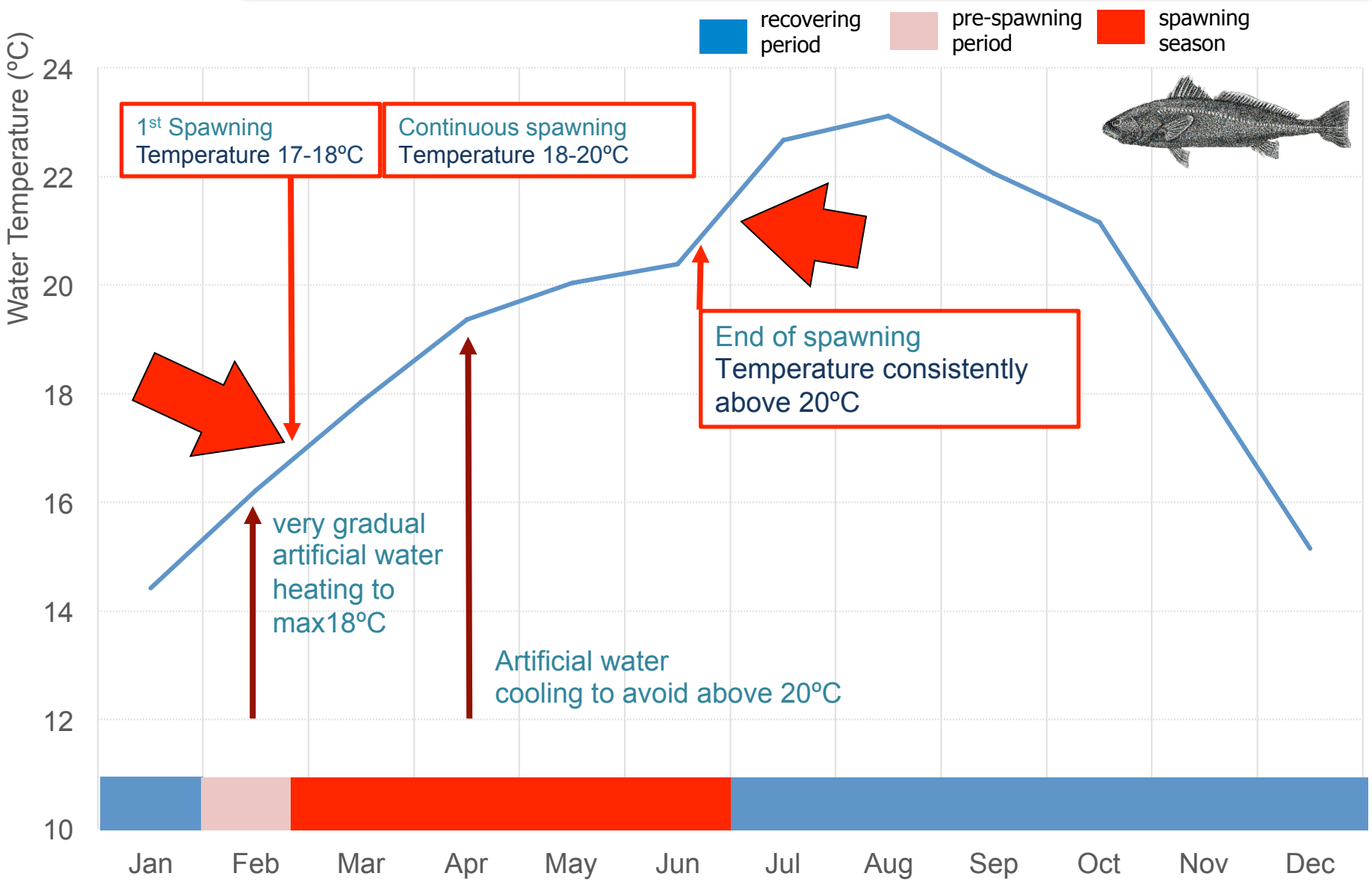
BROODSTOCK MANAGEMENT – Temperature control



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BROODSTOCK – Feeding protocol

Feeding protocol intended to cover the nutritional requirements of the species and promote the fitness of the group in order to obtain good quality brood.

wild

Meagre eat on live mackerel 2 weeks after capture.

Longer period of time to adapt to frozen diet when compared to sparids and seabass.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Illex sp.		***Sardine sp.***		***Illex sp.***		

BROODSTOCK – Feeding protocol

F1 2012 to 2013 → Pellet for seabream (*Sparus aurata*)

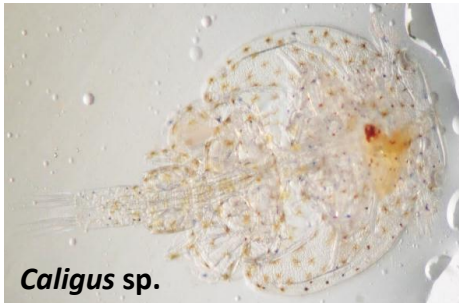
Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Illex sp.	***Pellets***	***Sardine sp.***	***Pellets***	***Illex sp.***	***Pellets***	***Pellets***

F1, F2 2014 to 2018 → Pellet adapted for meagre – Sparos®

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Pellets	***Pellets***	***Pellets***	***Pellets***	***Pellets***	***Pellets***	***Pellets***

BROODSTOCK – Main pathologies

Adults very susceptible to external parasites, in the gills or skin (eg.: *Monogenea* genera).


***Caligus* sp.**

Altered behaviour, such as head above surface and skin darkening were often observed when parasitosis occurred.


***Sciaenocotyle* sp.**

Regular prophylactic baths (Hydrogen peroxide, H_2O_2 – 35%, 1 hour) against parasites in adult fish.


***Diplectanum* sp.**

SOARES, F. *et al.*, 2011. *Bulletin - European Association of Fish Pathologists*, 31(5): 189

SOARES, F. *et al.*, 2012. *Bulletin - European Association of Fish Pathologists*, 32(1): 30-33

SOARES, F. *et al.*, 2018. *Journal of fish Diseases*, 49(4): 1373-1382.

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BROODSTOCK MANAGEMENT - Fish handling

Wild broodstock

Sampling:

1) Fasted for 48 hours;

2) All fish were anesthetized in the tank with 150ppm of 2-phenoxyethanol, sampling was performed inside the rearing tank

3) Tagged with PIT Tags;

4) Measured for total length, weighed

F1, F2 broodstock

Sampling:

1) Fasted for 24 hours;

2) All fish were anesthetized in the tank with 75ppm of 2-phenoxyethanol.

Transfered individually

Vat: 150 a 200ppm of 2-phenoxyethanol.

3) Tagged with PIT Tags;

4) Measured for total length, weighed

BARATA, M. *et al.*, 2015. *Journal of the World Aquaculture Society*. 47 (1)

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BROODSTOCK MANAGEMENT - Fish handling

Sex determination

1. **Apply abdominal pressure** to identified by the presence of milt - Male

2. **Catheterization** by inserting a plastic catheter into the genital pore to collect a **ovarian samples**

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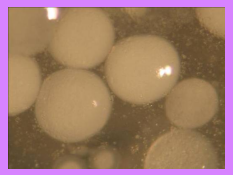
Hormonal spawn induction

Wild Breeders

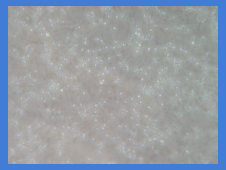
Reproductive season: Spring (March, April, May)

1 ♀ cannulation



> oocytes 600 µm

2 ♂ stripping



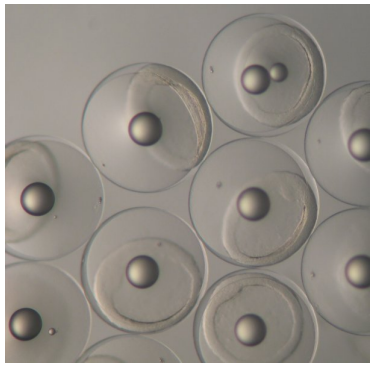
fluent males

GnRH (µg/kg)

20

10

spawning 48h after



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Hormonal spawn induction

Wild Breeders

Wild broodstock were adapted to captivity and spawns were obtained in 2009-2010

	2009	2010
	Spawns	
Parameter	Wild(n=3)	Wild(n=3)
Average (g)	667.7±240.7	502±53.4
Eggs Viability (%)	47.4±11.0	41.7±14.3
Eggs diameter (mm)	0.9±0.20	0.7±0.16
Hatching rate (%)	38	10.0±5.3
Total length at hatching (mm)	2.7±0.2	2.7±0.16

POUSÃO-FERREIRA, P. *et al*., 2010. Aquaculture Europe'10 Seafarming tomorrow. Porto, Portugal: pp. 1040-1041.

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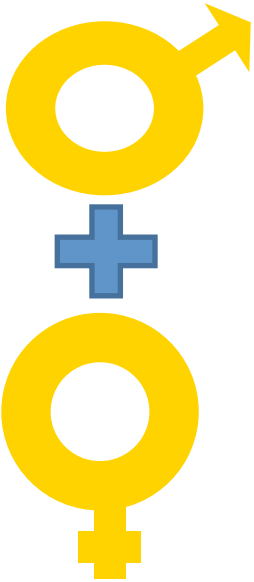
NATURAL SPAWN

F1, F2 Breeders

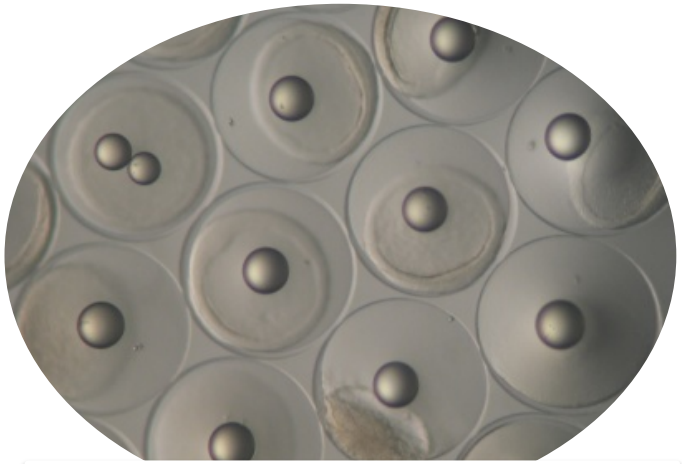
Sex ratio – 1:1

Age > 3 years

Weight > 1,8 Kg



Temperature (18- 20 ± 1,0 ºC)



eggs 1.03±0.03 mm

SOARES, F. *et al*., 2012. Aqua 2012, Prague, Czech Republic: pp.1045

POUSÃO-FERREIRA, P. *et al*., 2015. Atas del XV Congreso Nacional y I Congreso Ibérico de Aquicultura, Acuicultura, cultivando o futuro, Huelva 2015

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NATURAL SPAWN

F1

Introduction of new F1 individuals (3y old fish) from different origins - increase genetic variability

	3y old fish	4y old fish	5y old fish			
Season	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
Fish nº	10	15	78	65	70	70
Nº tanks	1	1	6	7	7	7
Weight (Kg)	2.2±0.2	3.5±0.5	5.1± 1.0	6.9±3.2	5.6±1.9	6.5±1.7
Density (Kg m^3)	5.6	6.4	7.1±0.2	6.9±1.4	5.7±0.4	5.9±1.7
Nº spawns	n=5	n=5	n=44	n=75	n=109	n=102
Spawn biomass (Kg)	0.4	**0.7**	**21.8**	24.6	41.0	45.1

in 2014 reached full maturity:
more fish contributed to reproduction

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NATURAL SPAWN

F1

the hatching rate declines due to zootechnical reasons - large numbers of eggs in the collector

Eggs viability increase with broodstock age

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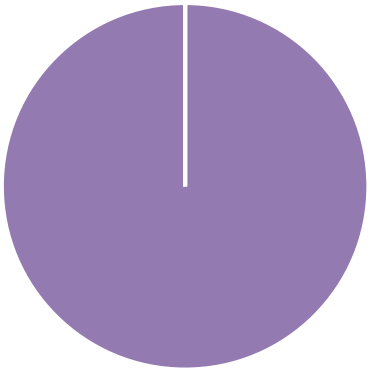
NATURAL SPAWN (kg eggs/month)

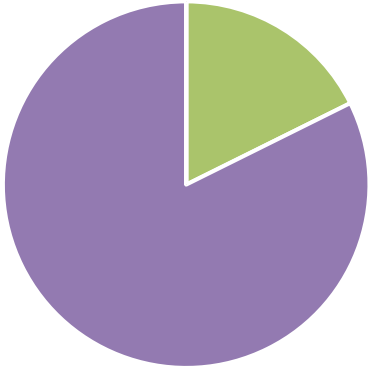
F1

February
March
April
May
June
July
August
October

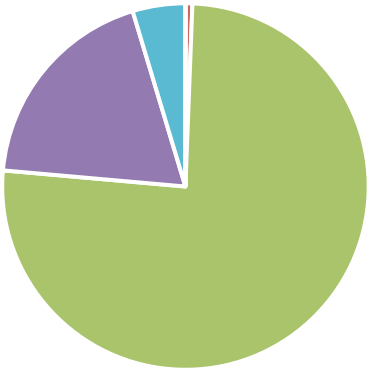
2012



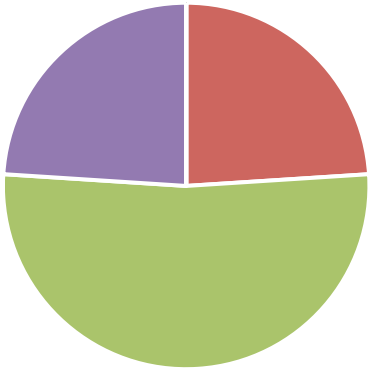
2013



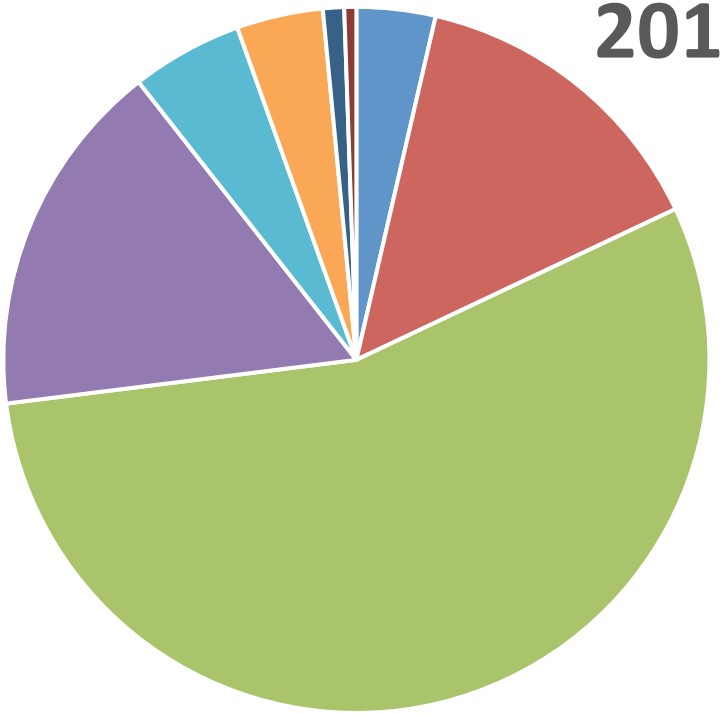
2014



2015



2017



Temperature manipulation from February to August **(18ºC to 21 ºC)**

Wild broodstock *versus* F1 Broodstock

Wild meagre broodstock was adapted to captivity at IPMA.

Successful spawning after hormonal induction.

Low eggs viability and hatching rate.

F1 meagre broodstock was transferred from the earthen pond to indoor tanks

Broodstock kept spawning naturally (without any hormone utilisation) with temperature manipulation.

High hatching rate.

Viability was superior from F1 broostock.
Superior hatching rate from F1 broostock.

The use of F1 broodstock seems a promising tool to obtained spawns naturally and with good quality.

SOARES, F. *et al.*, 2015. *Fish Physiology and Biochemistry*, 41(6)

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NATURAL SPAWN

2018
Atypical year
Climate changes??

F1

the temperature was not controlled

Variability between tanks

Parameters	9m³	9m³	9m³	9m³	50m³
Fish nº	8	10	8	10	12
Weight (Kg)	5.6±1.3	6.9±1.8	7.0±1.5	5.8±1.3	12.0±1.0
Density (Kg m3)	4.2	6.6	6.6	5.5	2.9
nº spawns	1	2	18	-	3
Spawn biomass(g)	37	1064	6050	-	179
Eggs Viability (%)	68	82.4±0.1	78.3±0.1	-	43.9±0.1
Hatching rate (%)	-	-	54.6±19.3	-	32.7

Spawning season in progress

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NATURAL SPAWN

F2

2018 -Variability between tanks

	1 tank - 9m³	1 tank - 9m³
nº fish	18	17
Weight (Kg)	3.9±1.2	4.8±1.3
Density (Kg m3)	8.2	9.7
nº spawns	n=3	n=17
Spawn biomass(g)	854	7 646
Eggs Viability (%)	36.0±0.4	70.6±0.3
Hatching rate (%)	65.4	46.2±16.6
Season duration	2 months	5 months

Spawning season in progress

F1 broodstock *versus* F2 Broodstock

Control tanks

	F1	F2
	F1 – [illegible] years)	F2 – [illegible] years)
nº fish	n= 26	n=35
nº tanks	n= 2	n= 2
Weight (Kg)	5.2±1.0	4.4±0.5
Density (Kg m^3)	7.2±0.2	9.0±0.8
nº spawns	n=44	n=20
Spawn biomass(Kg)	15.6	8.5
Eggs Viability (%)	72.7±17.6	62.6±0.27
Hatching rate (%)	90.9 ± 7.2	50.9±14.8
Season duration	4 months	5 months

Diferent inert diet may affected spawn quality

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BROODSTOCK TRIALS

— Optimize diet formulation

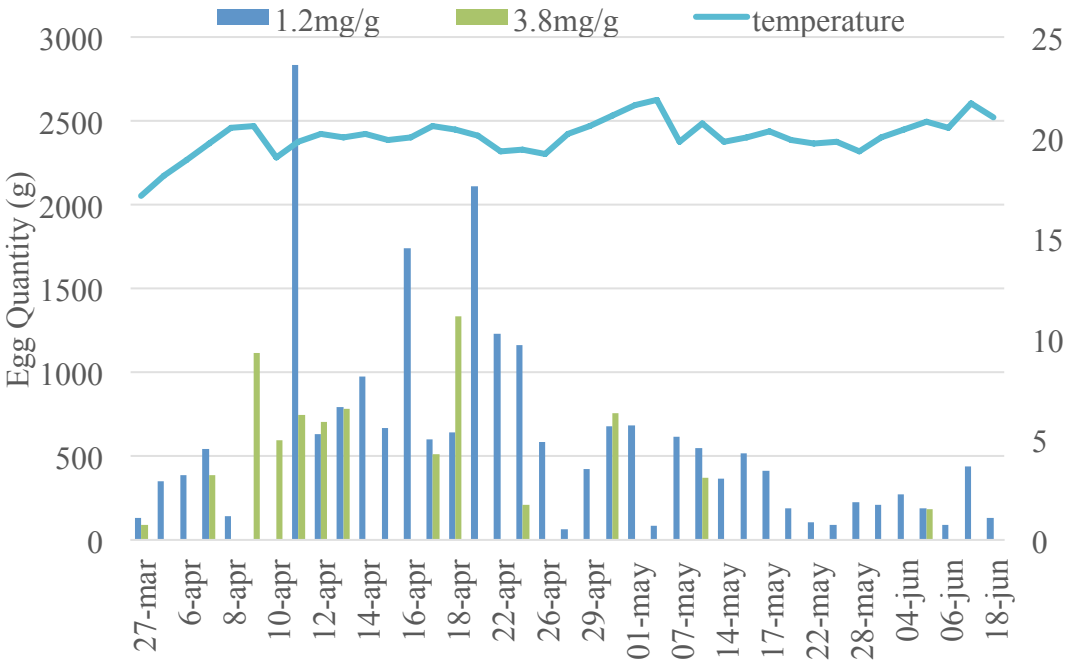
It is known that nutrient reserves of the yolk are dependent of the maternal nutrient reserves and consequently on the daily feeding regime of the broodstock, including nutrient levels and duration.

1) **Arachidonic acid enrichment**;

2) **Phospholipids enrichment**;

BROODSTOCK TRIALS – Arachidonic acid enrichment

Does ARA dietary enrichment improve spawning quality in meagre?



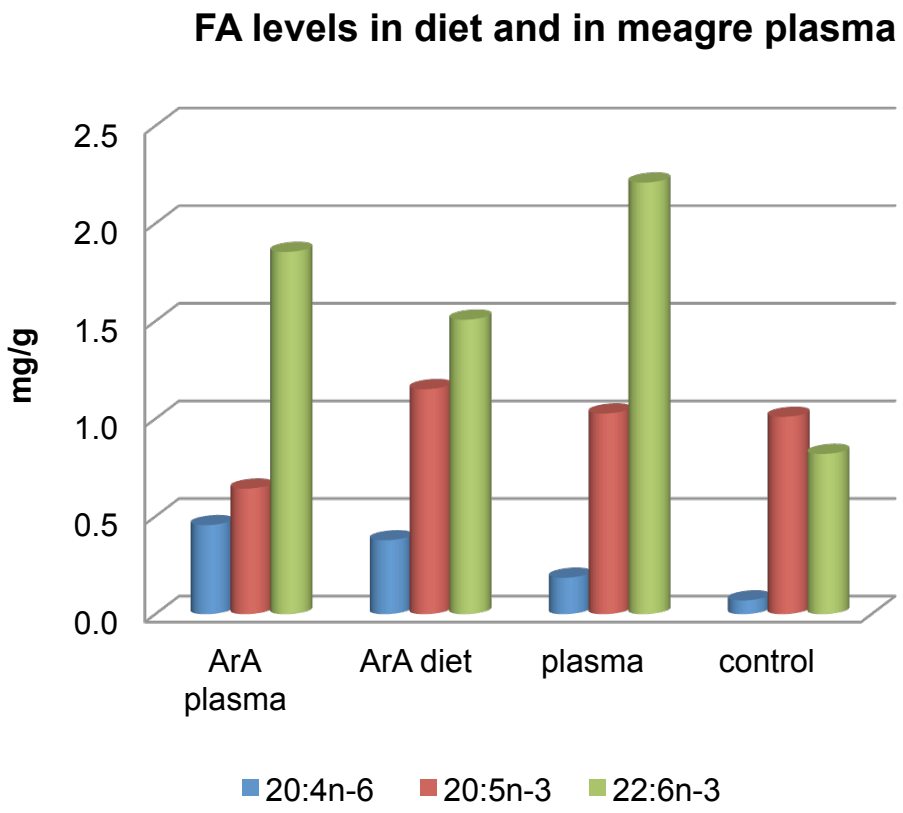
ARA (mg/g)	1.2	3.8
Nº spawns	44	14
Eggs viability (g)	15 683	6 529
Total eggs (g)	21 824	7 780
Eggs Viability (%)	72.7	86.4
Hatching rate (%)	90.9	80.0
DHA/ARA (mg/g)	0.8	1.3
EPA/ARA (mg/g)	13.5	3.0

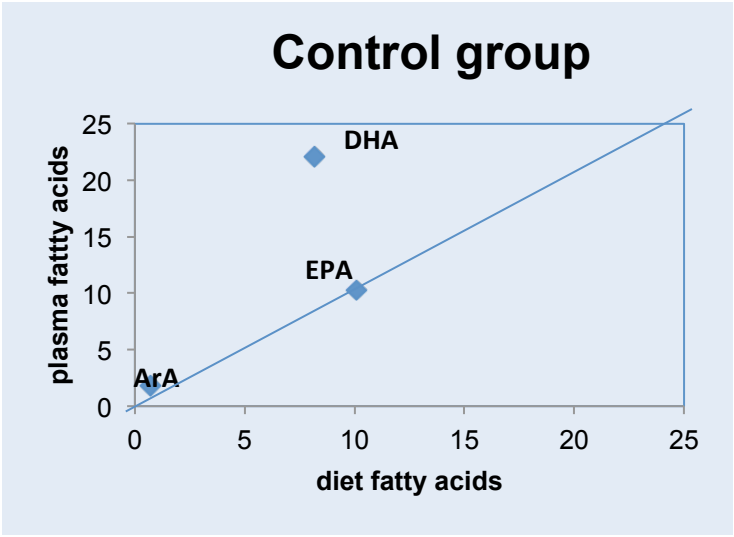
Higher number of eggs and a larger spawning period was obtained when meagre was fed with levels of 1.2 mg/g of ARA in the diet when compared to enriched diet (3.8 mg/g of ARA).

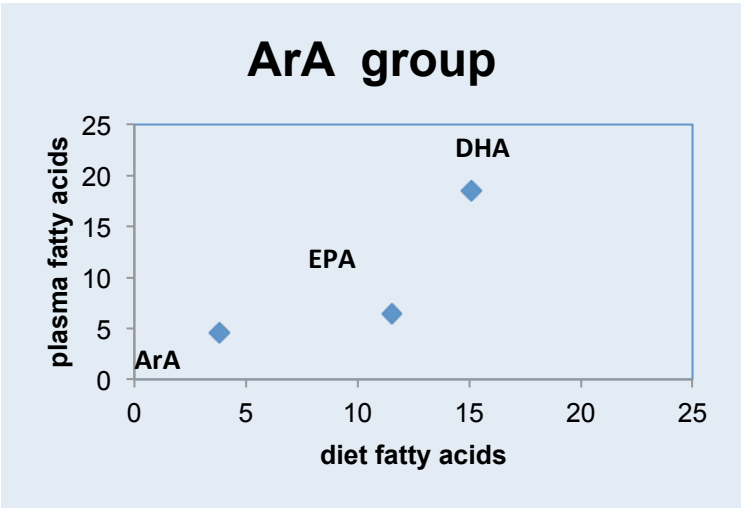
Meagre spawns quality was better without ARA enrichment.

Relation between the FA profile of the diet and the fish plasma

Diet boosted with ARA







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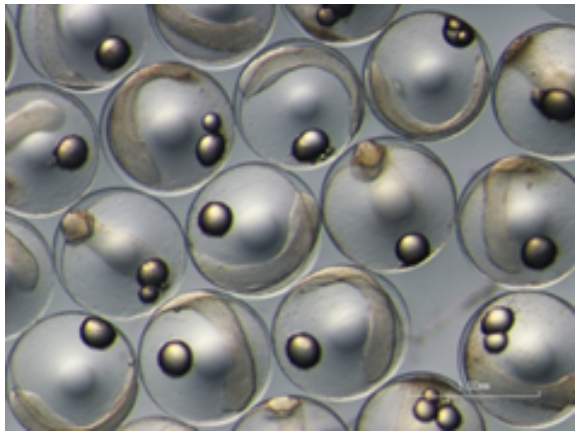
BROODSTOCK TRIALS

Enhance eggs and larval quality trough breeders dietary

Work in progress



Phospholipids effect

Breed diet	Fish oil	Krill+ soy lecithin
Eggs Viability (%)	80± 11	67± 18
Eggs diameter (mm)	1.0±0.04	1.0±0.02
Hatching rate (%)	77± 21	93±9

Hatching and larval development
in batch from breeder with the same diet

Testing photoperiod and temperature

24h dark and 22ºC

Higher larvae FA (ARA, DHA e EPA) profile at mouth opening

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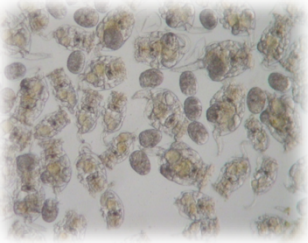
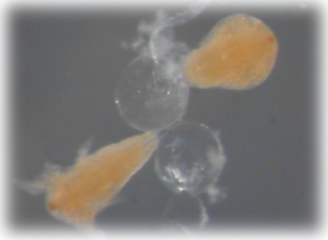
LARVAL TRIALS – rearing larvae

- Functional visual sensorial organs larvae (detection of preys);
- Availability, size, contrast and movement of prey;
- Currents caused by water circulation and aeration;
- **Environmental/zootechnical condition;**
- **Nutritional quality, quantity and digestibility;**

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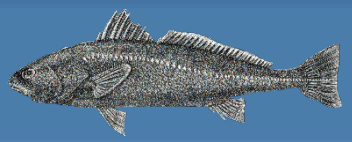
IMPROVEMENT OF MEAGRE LARVAL REARING



Optimizing rearing technics

growth rate

- 1. nutritional
- 2. zootechnic aspects

Optimizing nutritional protocols

feeding

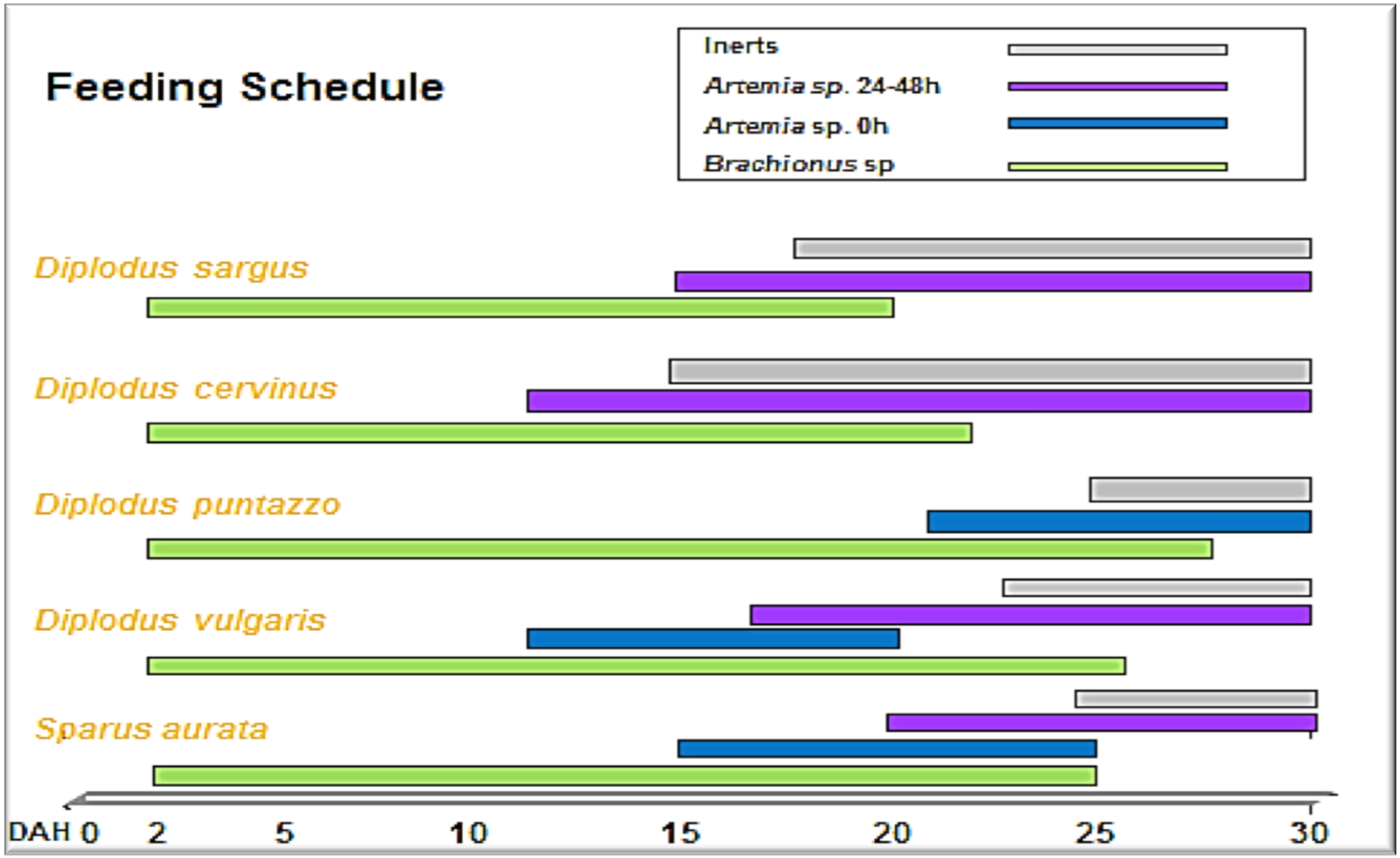
Functional
Reliable

meagre implementation on aquaculture industry

commercial production

LARVAL – Feeding protocols

Feeding protocols should be flexible, in order to promote growth and survival adapted to the each species and to the stage of development



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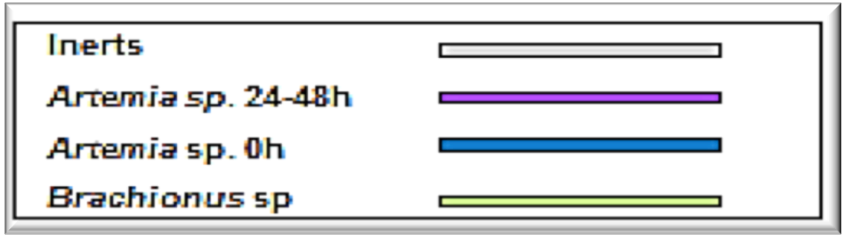
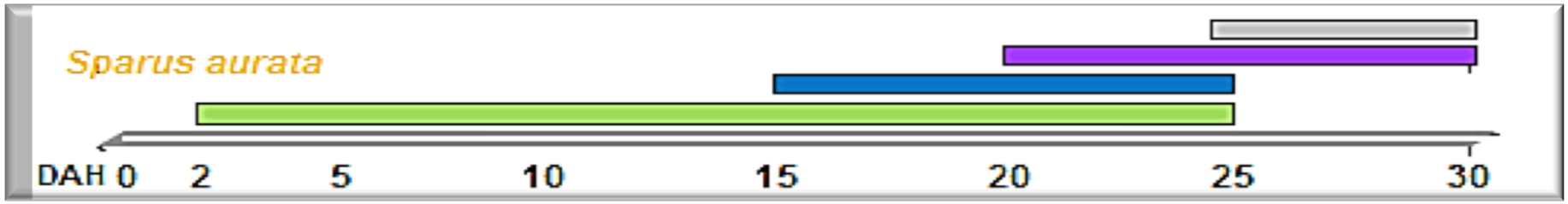
Feeding protocols





Artemia "M" – marine strain (newly hatched)
Artemia "C" – continental strain (enriched)

CTRL
- Rotifers (3-15DAH)
- *Artemia* "M" (6-12)
- *Artemia* "C" (10-25DAH)
- Inerts (20-30DAH)

T1
- Rotifers (3-15DAH)
- *Artemia* "M" (6-12)
- *Artemia* "C" (10-25DAH)
- Inerts (14-30DAH)

T2
- Rotifers (3-15DAH)
- ~~*Artemia* "M"~~
- *Artemia* "C" (10-25DAH)
- Inerts (20-30DAH)

3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

DAH

LARVAL – Feeding protocols

Larval rearing

Density: 44 larvae L^{-1}

Temperature: 22±1ºC

Dissolved oxygen: >80%

Photoperiod: 14L:10D

Light intensity: *ca* 500lux (just above the water surface)

Water renewal (in a semi-recirculation system):

03-10DAH - 20%.h^{-1}

10-25DAH - 40%.h^{-1}

25-30DAH - 60%.h^{-1}

POUSÃO-FERREIRA, P. *et al.*, 2012. Aqua 2012, Prague, Czech Republic pp. 896.

LARVAL TRIALS – Optimize feeding protocols

With the feeding protocols from gilthead seabream

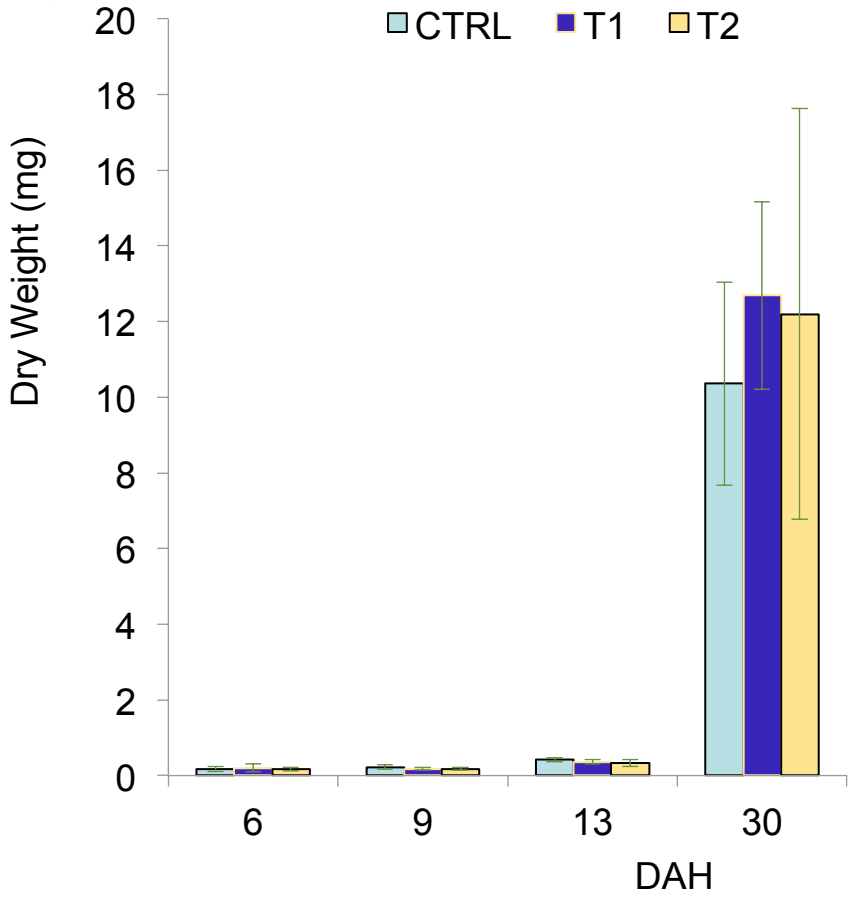
? ? ?

It was not possible to avoid CANNIBALISM

CONSTANT SUPPLY OF FOOD IN QUALITY , QUANTITY AND IN SIZE

T1 (early inerts) presents lower variability

T2 (no Instar I better result than control



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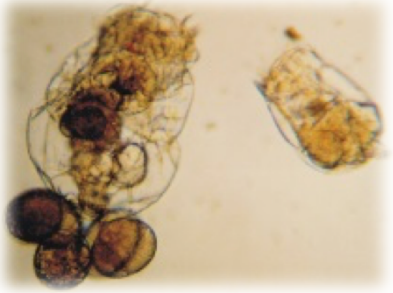
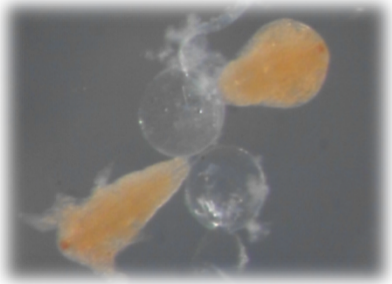
LARVAL TRIALS – Optimize feeding protocols

REDUCING LIVE FOOD DEPENDENCE

LABORIOUS | **EXPENSIVE** | **UNPREDICTABLE**

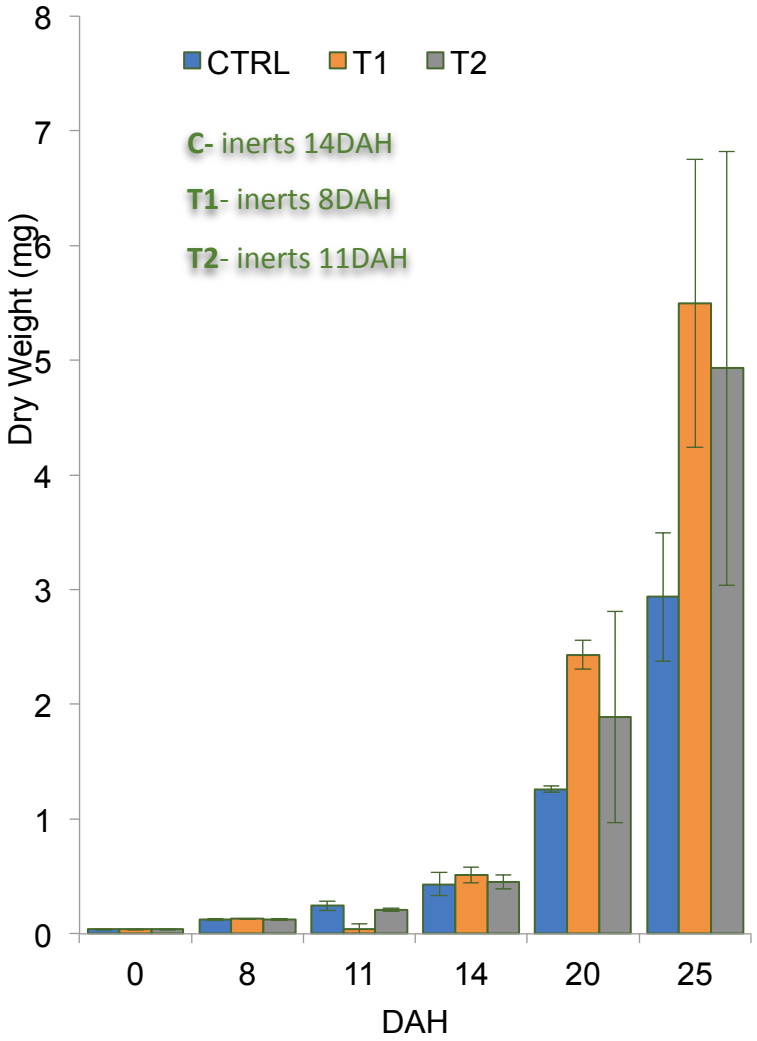


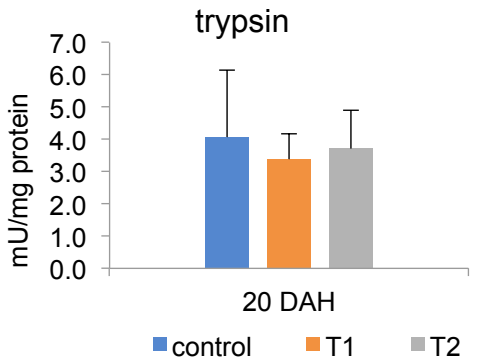


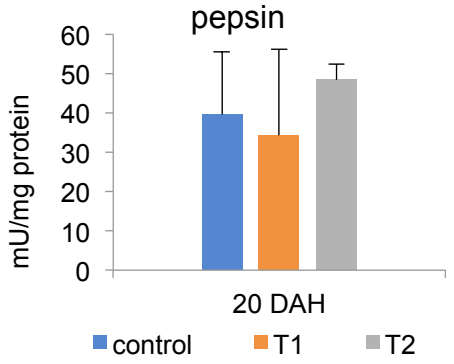
ANTECIPATE INTRODUCTION OF INERT FEEDS

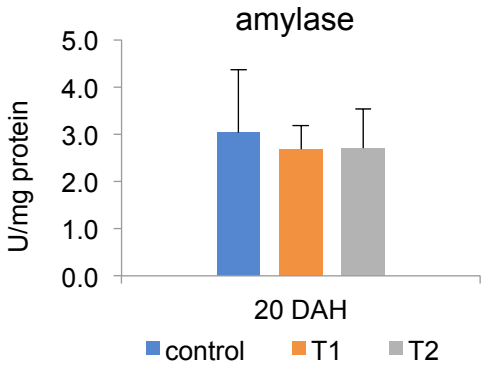
AVAILABLE | **NUTRITIONALLY STABLE**

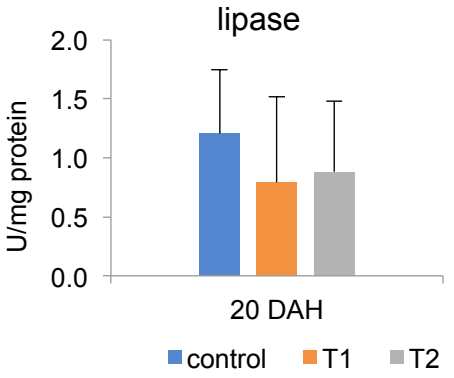
LARVAL TRIALS – feeding trial











20 old day meagre exhibited similar digestive enzymes activities.

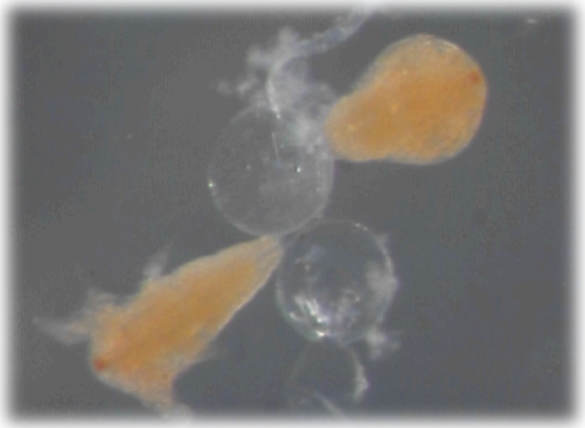
Early introduction of inert diets did not affected meagre digestive capacity

LARVAL TRIALS – Optimize feeding protocols

Possibility to rear meagre without the use of *Artemia Instar* I

Introduction of inert diet as early as 8DAH

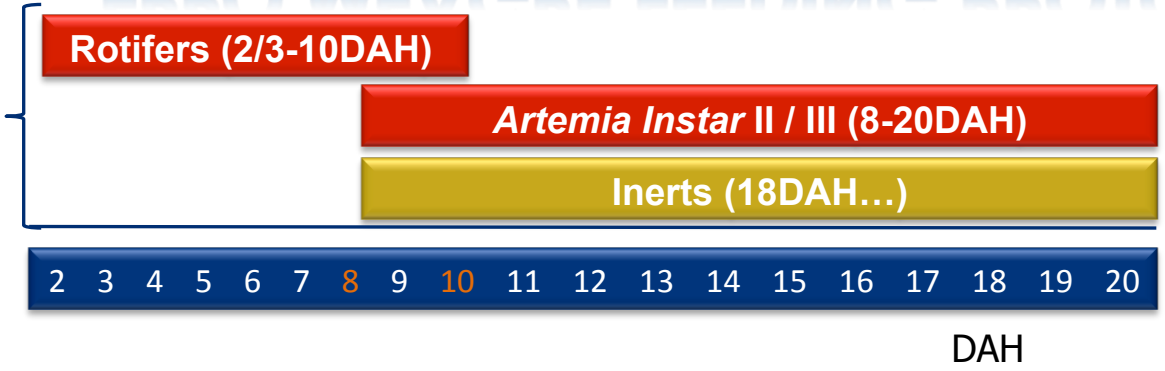
YES



Good results in

- in survival
- fatty acid profile.
- growth;
- in the digestive capacity

EPPO MEAGRE FEEDING PROTOCOL



MENDES, A.C. *et al.*, 2011. Aquaculture Europe 11. Rhodes, Grécia. pp. 163-164

LARVAL TRIALS – Optimization of larval rearing

Rearing strategies ?

To AVOIDING CANNIBALISM

CONSTANT SUPPLY OF FOOD IN QUALITY, QUANTITY AND IN SIZE

Define the best rearing density

50 Larvae/ L

25 Larvae/ L

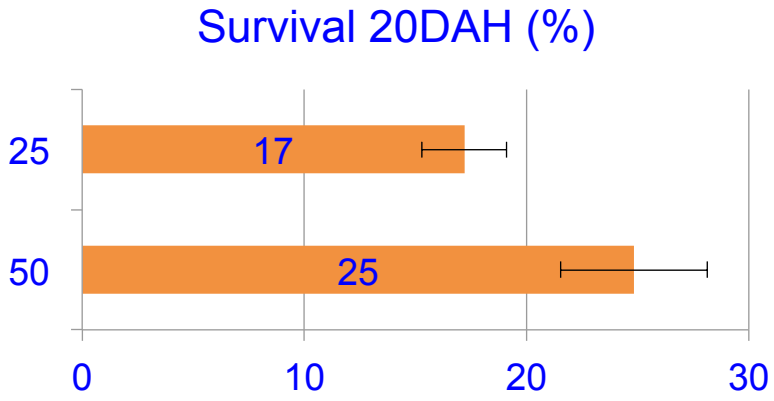
LARVAL TRIALS – Optimization of larval rearing

Testing larval density rearing until 20DAH

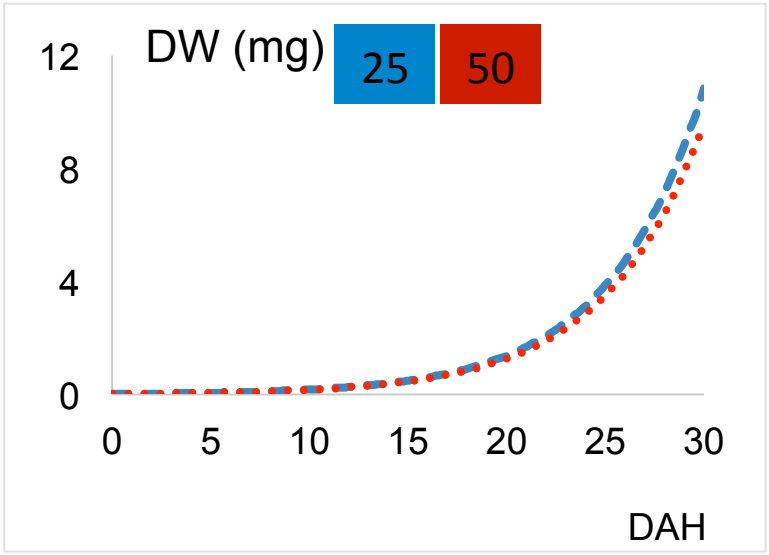
Density 50 higher survival than 25

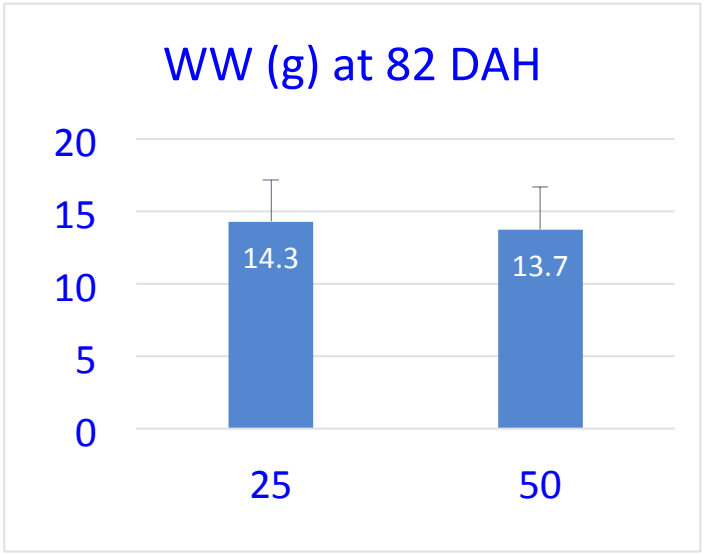
Growth was not affected (p>0,05)

Squeletical malformation not affecteda



long term growth



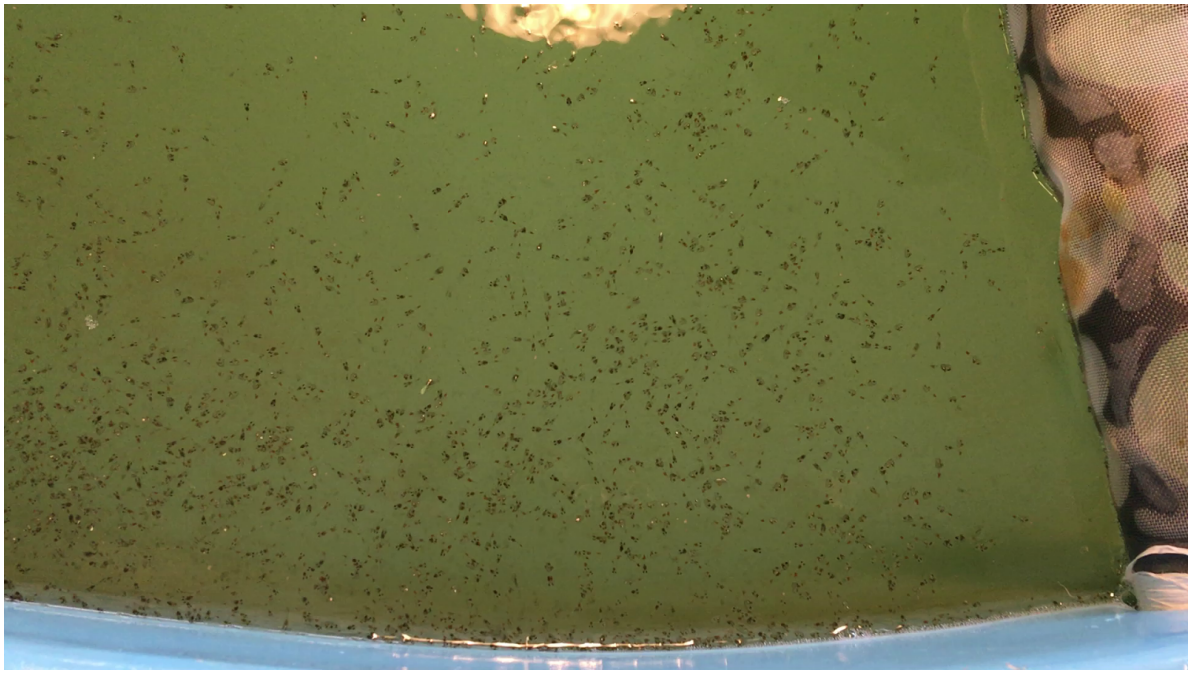


LARVAL TRIALS – Optimization of larval rearing

After 20DAH fish density must necessarily be lowered

Important!!

Very slow and soft transference, avoiding any major disturbance



Better food availability

Cannibalism minimized

Less stress behaviour

Candeias-Mendes, A. *et al.*, 2015. Actas del XV Congresso Espanol e I Congresso Ibérico de Acuicultura - "Acuicultura, Cultivando el Futuro". Huelva

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IMPROVEMENT OF MEAGRE LARVAL REARING

Main conclusion in larval trials

1. Huge importance of feeding protocols and schedules

- ❖ **adjusted to fast growing species**

2. Better results rearing density 35-50 larvas.L^{-1} (first 20DAH)

- ❖ **survival**
- ❖ **similar gowth**

necessarily lower density onwards
VERY GENTLE TRANSFERENCE TO AVOID HYPERINSUFLATION DUE TO STRESS

3. Acute compromise in protein:EFA's in larvae quality and growth

- ❖ **high FA**
- ❖ **high protein**

- ❖ **short term**
- ❖ **long term**

Nutrition | **Zootechnical** | **Health and welfare**

Research efforts have been made to optimize diets for meagre at other stages of biological cycle integrating zootechnical performance results with biochemical, histological, enzymatic and microbiological analysis:

- Protein dietary requirements & Adequate dietary protein:lipid ratio
- Importance of rearing temperature on meagre protein retention efficiency
- Acceptability of ingredients of vegetable origin – impact on fish performance and digestive physiology and intestinal integrity
- Role of certain amino acids on meagre performance and muscle development
- Dietary levels of phospholipids and DHA:EPA ratio meagre diets – effect on fish performance, liver metabolism and fatty acid dynamics
- Protein turnover and synthesis when alternative ingredients to fish meal are used

Received: 3 May 2017 | Accepted: 3 November 2017
DOI: 10.1111/anu.12654

ORIGINAL ARTICLE

Effect of increased dietary protein level in meagre (*Argyrosomus regius*) juvenile growth and muscle cellularity

Aquaculture 447 (2015) 116–128

Review

Effect of vegetable based diets on growth, intestinal morphology, activity of intestinal enzymes and haematological stress indicators in meagre (*Argyrosomus regius*)

Laura Ribeiro [a,*], João Moura [a], Marta Santos [a], Rita Colen [b], Vera Rodrigues [c], Narcisa Bandarra [a], Florbela Soares [a], Patrícia Ramalho [a], Marisa Barata [a], Paula Moura [a], Pedro Pousão-Ferreira [a], Jorge Dias [c]

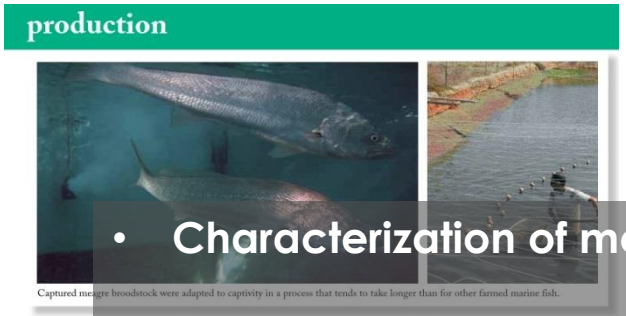
Effect of dietary phospholipids and docosahexaenoic ... growth performance, lipid profile and oxidative stress in Meagre (*Argyrosomus regius*) juveniles

Maria João Magalhães de Almeida Xavier
Mestrado em Recursos Biológicos Aquáticos
Supervisor
Prof. Doutor Pedro Pousão, Investigador Auxiliar, EPPO

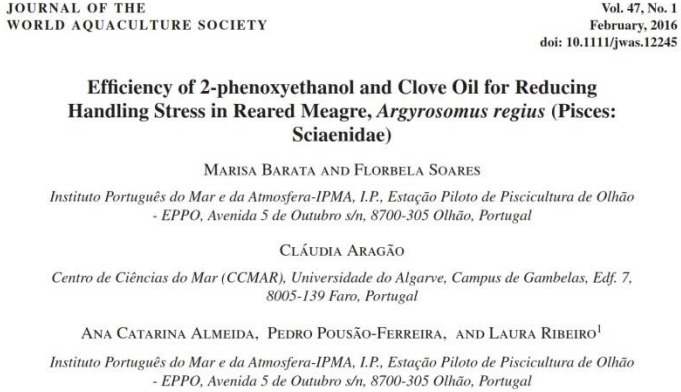
Nutrition

Zootechnical

Health and welfare



- Characterization of meagre in earth ponds in monoculture and polyculture
- Characterization of optimum temperature range for meagre rearing
- Adequate rearing density for juvenile and ongrowing stage
- Development of transport protocols complying with meagre welfare
- Development of anaesthesia protocols



Health and welfare

Nutrition

Zootechnical

Vol. 102: 119–127, 2012
doi: 10.3354/dao02545

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published December 27

OPEN ACCESS

Sarcoma in the thymus of juvenile meagre *Argyrosomus regius* reared in an intensive system

F. Soares, M. Barata[1], S. W. Feist*, P. Pousão-Ferreira[1], L. Ribeiro[1]

[1]IPMA, Av. 5 de Outubro s/n 8700-305 Olhão, Portugal
[2]Cefas Weymouth Laboratory, Weymouth, Dorset DT4 8UB, UK

Bull. Eur. Ass. Fish Pathol., 31(5) 2011, 189

NOTE

Sarcoma in the thymus of meagre juveniles (*Argyrosomus regius*)

F. Soares*, L. Ribeiro, M. Barata and P. Pousão-Ferreira

- Characterization of meagre physiologic answers towards challenging conditions (environment, nutritional, diseases, etc...)
- Elaboration of a data base of the principal diseases (parasitosis, bacteriosis,), skeletal malformations, and others observed in meagre produced at IPMA aquaculture research station
- Dietary challenging ingredients on meagre performance at higher rearing temperature
- Development of biomarkers to characterize fish welfare

28, Bull. Eur. Ass. Fish Pathol., 32(1) 2012

NOTE

First report of *Amyloodinium ocellatum* in farmed meagre (*Argyrosomus regius*)

F. Soares*, H. Quental-Ferreira, M. Moreira, E. Cunha, L. Ribeiro and P. Pousão-Ferreira

DOI: 10.1111/are.13613

REVIEW

WILEY Aquaculture Research

Review of the principal diseases affecting cultured meagre (*Argyrosomus regius*)

Florbela Soares[1] | Ana Roque[2] | Paulo J Gavaia[3,4]

Pedro Pousão-Ferreira, Laura Ribeiro, Florbela Soares, Maria Cunha, Marisa Barata, Margarida Gamboa, Sara Castanho Quental-Ferreira, Anaisa Cordeiro, Narcisa Bandarra, Ana C. M

Thank you for your attention

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