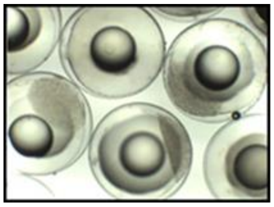
Technical leaflet- Flathead grey mullet (Mugil cephalus)



Species leader: Dr. Bill Koven (National Center for Mariculture (NCM), IOLR, Eilat, Israel)

Workshop Organizer: Dr. Aldo Corriero (University of Bari (UOB), Bari, Italy)

Participating scientists: Dr. Constantinos Mylonas (HCMR, Greece), Dr. Ken Leber (Mote, USA), Dr. Donatella Crosetti **(**ISPRA, Italy**),** Dr. Sherif Sadek (ACO, Egypt), Dr. Dario Vallainc (IMC, Italy), Dr. Alicia Estevez (IRTA, Spain), Dr. Hanna Rosenfeld (NCM, IOLR, Israel), Dr. Iris Meiri-Ashkenazi (NCM, IOLR, Israel), Mr. Dor Israeli (NCM, IOLR, Israel), Dr. E. Gisbert (IRTA, Spain), Dr. Antonella Rosa (UNICA, Italy), Dr. R. Robles (CT-AQUA, Spain), Dr. Thanassis Krystallis (AU, Denmark, HRH, Greece)

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Introduction

Farming of grey mullet has been practiced for centuries, but production of this potentially invaluable source of animal protein in Europe has been small and non-intensive (Nash & Koningsberg, 1981; Pillay, 1993). It is a **euryhaline species, found throughout the world** (Oren, 1981) and is a **rapid-growing, omnivorous teleost** that can be **reared over the wide geographical and temperature range of the Mediterranean basin** (Crosetti, 2015). As it is detritivorous in the wild, it has been stocked in fish ponds to improve sediment quality and avoid oxygen depletion (Milstein et al., 1991). Therefore, it can be an **excellent candidate for the enhancement of aquaculture in earthen ponds, coastal lagoons, and deserted Salinas** that exist throughout the EU Mediterranean countries.

During the autumn and winter months adults migrate to the sea in large aggregations to spawn. When juveniles are 16–20 mm, they migrate to inshore waters and estuaries, where they can be collected for farming operations during late August to early December. Most of the flathead grey mullet fry used in commercial aquaculture are collected from the wild, especially in the Eastern and Southern Mediterranean, Saudi Arabia and Gulf States and South East Asia. Cultured flathead grey mullet are generally grown semi-intensively in polyculture ponds that can include common carp, grass carp, silver carp, Nile tilapia , milkfish and European seabass. Although growth has been reported highest in lower salinity water, they can be successfully reared in fresh water, brackish water and sea water.

Full-scale commercial production of *Mugil cephalus* in monoculture is still in its infancy. Induced spawning and production of fry on a limited scale for aquaculture has been reported in Italy, Israel and Egypt. Hatchery produced juvenile females have been **grown to 1.9 kg in 2 years** on a fishmeal-containing pelleted feed (P4. HCMR). The development of a fishmeal-free feed will reduce the cost of fish production, and will be **more sustainable and environmentally friendly**. This means grey mullet would be more acceptable to an increasingly aware consumer public that demands sustainability and lower environmental impact. Moreover, grey mullet aquaculture has the advantage of providing not only affordable whole fish and fillets, but also **fish roe (bottarga), a high value product (>100 € kg^{-1}),** whose market is expanding around the Mediterranean. Therefore, grey mullet has considerable **economical potential as a species that provides an inexpensive source of sustainable, high quality protein**, **product diversification, and a value added product such as bottarga**.

A market for grey mullet is well established in the Mediterranean where Egypt alone consumed in excess of 129,000 MT in 2015 (Soliman et al. 2015). In addition, the European market for grey mullet is likely to increase in the coming years, due to the demand from established and newly immigrant families originating from North Africa, Middle East and Asia. Currently, **the industry is a capture-based aquaculture, relying almost exclusively on capture of wild fry** (ca 1,000,000,000). It is now recognized that this approach severely reduces natural fisheries and is unsustainable where regulation of this practice is expected in the near future. However, the **future growth of the grey mullet aquaculture is limited by a number of bottlenecks**, which will be addressed in DIVERSIFY. Firstly, **controlling the reproductive cycle and improving egg quality** via broodstock management and nutrition is necessary not only for the production of robust larvae, but also for producing high value bottarga. Secondly, **development of a larval rearing protocol** is necessary to reduce early mortalities, size dispersion as well as increasing metamorphic synchrony, which will lead to a supply of high quality juveniles. Finally, development of a sustainable, economical, **fishmeal-free grow out feed** is needed, which would perform well under different environmental conditions of temperature, pond type, and water quality, thus broadening the geographical range of grey mullet aquaculture in Europe.

Reproduction and Genetics

Hormone based treatments for the synchronization of maturation and induction of spawning

Hanna Rosenfeld, National Center for Mariculture, IOLR, Eilat, Israel

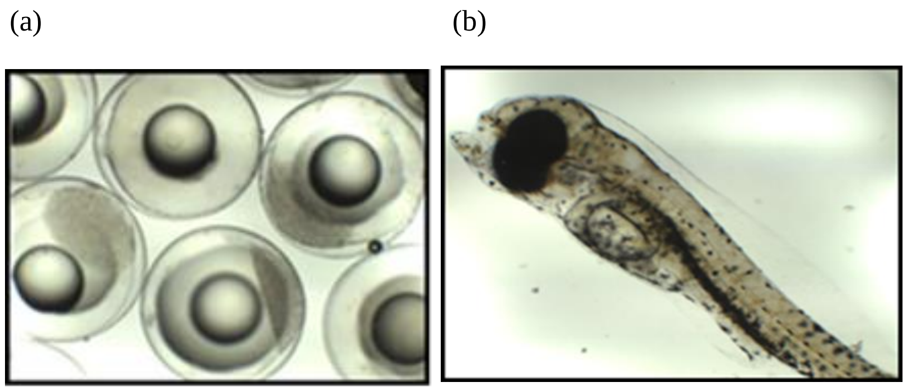

Figure 1 (a) Mullet gastrula stage eggs and (b) first feeding larvae

- Grey mullet broodstock do not spawn spontaneously in captivity, with both females and males exhibiting reproductive dysfunctions.
- Aiming to improve vitellogenesis among captive grey mullet, the effects of combinations of metoclopramide (an antagonist of dopamine D2 receptors; DA) with either rFSH (yeast produced recombinant FSH) or GnRH-EVAc (implants for sustained release of the GnRH analogue) were tested.
- The optimal treatment consisting of DA + rFSH, gave rise to 91% post-vitellogenic females within the treatment-group **(Table 1)**.
- Experimentation with captive grey mullet males evaluated: (a) short term effects of rFSH vs. rLH, and (b) the effect of rFSH as a sole therapeutic agent vs. rFSH use to prime the fish prior to the administration of MT-EVAc (implants for sustained release of methyltestosterone).
- The rFSH was a potent steroidogenic hormone during the early stages of spermatogenesis. It stimulated both gonadal growth and steroidogenic activity, while the comparable rLH treatment had no significant effects. The recognized differences between the two treatments seem to be attributed to rFSH ability to stimulate, at this critical time window, circulating 11-ketotestosterone levels and consequently induce expression of pituitary LH.
- All males that were primed with rFSH and then subjected to MT-EVAc implantation produced sperm. Moreover, the latter group exhibited relatively higher percentages (66%) of fully spermiating males compared to rFSH treated (33%) and control groups (24%).
- To induce spawning, fully mature grey mullet females and males were treated with two consecutive injections consisting of GnRHa +DA given 22.5 h apart.

- The spawning induction trials gave rise to high quality eggs and larvae and later on to large numbers of robust juveniles during natural (September-November) and shifted (January-February) reproductive seasons.

Table 1. Relative abundance of post-vitellogenic females among treatment groups

	Control	DA	DA+GnRH	DA+rFSH
Exp. I	27%	58%	71%	
Exp. II	29%			91%

Effects of captivity on first sexual maturation of wild and hatchery produced fish

Hanna Rosenfeld, National Center for Mariculture, Eilat, Israel; Aldo Corriero, University of Bari, Italy

- Advanced and spontaneous sexual maturity under captive conditions can facilitate grey mullet roe production (bottarga) as a high valued product. Therefore, the current task aims to characterize pubertal development in wild-caught vs. hatchery produced grey mullet subjected to captive conditions.
- The size of 6 year old hatchery-produced fish is equal to that of wild individuals of the same age class.
- All the 2 year old mullets analyzed were still sexually immature. The biometric and histological analyses showed that body growth and gonad development of 2 year old hatchery produced mullets were slightly faster than wild-caught specimens.
- The 3 year old grey mullet exhibit sex related growth and gonadal development patterns (**Fig. 2A**).
- The 3 year old hatchery produced mullet females and males exhibited enhanced gonadal maturation compared with the wild-caught captive-reared fish (**Fig. 2B**), probably as a result of domestication.

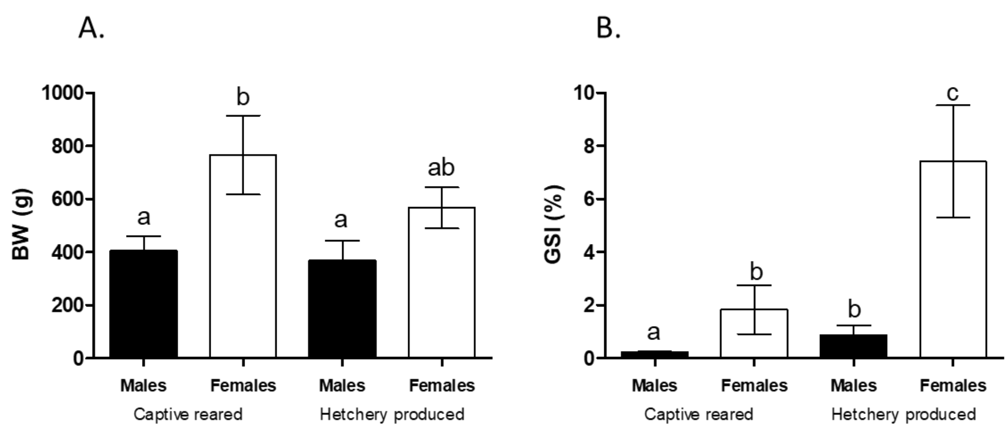

Figure 2 Body weight (A; BW) and Gonadosomatic (B; GSI) of 3 year old hatchery-produced and captive-reared grey mullets. Different letters above bars indicate statistically significant ($P<0.05$) differences between groups.

Nutrition

Improvement of enrichment products, weaning and broodstock diets

Bill Koven, Hanna Rosenfeld, National Center for Mariculture, IOLR, Eilat, Israel

DHA enrichment of rotifers for larval rearing

Figure 3 Experimental system of 18 1.5 m^3 tanks at the IOLR.

- The long chain polyunsaturated fatty acid (LC-PUFA) docosahexaenoic acid (DHA; 22:6n-3) promotes growth more effectively than the other LC-PUFAs (eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ARA; 20:4n-6) in marine fish larvae (Koven et al., 1993, Wu et al., 2002, Izquierdo and Koven, 2011).
- Three rotifer DHA enrichment treatments were tested: low (L; 5.5 %), medium (M; 12%) and high (H; 20%) DHA, as percentage of total fatty acids (TFA).
- After 15 dph (days post-hatching), all larvae were fed *Artemia* nauplii enriched with 5.5% DHA of TFA to 20 dph followed by offering a weaning diet until 40 dph.
- No dietary DHA effect on larval performance in terms of rotifer consumption (mastaxes/larva) and length (mm) was observed**.** This suggests that 5.5% DHA is sufficient.
- Low dietary DHA treatment resulted in highly significant improved larval survival over the other higher DHA treatments on 40 dph (**Fig. 4**), which is 25 days after the rotifer treatments have ceased.
- Emphasizes the importance of feeding DHA at the rotifer stage on survival in later development stages.

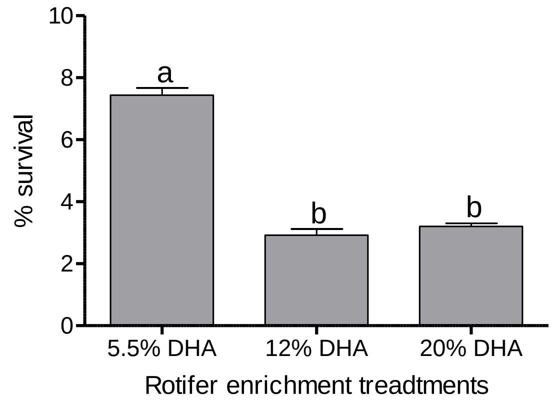

Figure 4 The effect of feeding the low (L), medium (M) and high (H) rotifer DHA treatments on percent (%) survival in 40 dph. Survival values having different letters were significantly ($P<0.05$) different.

Figure 5 Mullet juveniles in 6 m^3 tank at IOLR

Dietary DHA for juvenile rearing

- DHA requirement during the juvenile stage generally decreases compared to larval and brood stock stages (Tocher, 2010), however DHA is still essential and required, particularly if the fish remains strictly carnivorous.
- Mullet become omnivorous in less saline water after metamorphosis and may have some ability to synthesize LC-PUFA (Zouiten et al., 2008), which can effect DHA requirement.
- Three DHA treatments (0.4, 0.8 and 1.2 % DW diet) were tested on 138-222 dph fish (12 weeks), where fish grew at least 200%.
- There was no DHA effect above 0.4 % DW diet on growth and survival (**Fig. 6**).
- Grey mullet juveniles in less saline estuary waters may have some ability to synthesize DHA and other long chain polyunsaturated fatty acids from shorter chain polyunsaturated fatty acids, which may affect its dietary requirement.

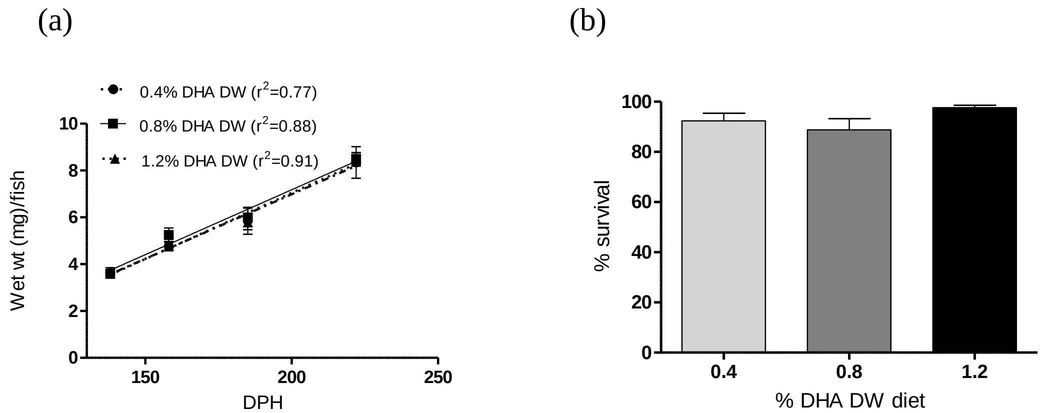

Figure 6. The effect of dietary DHA on (a) growth (mg) and (b) survival (%).

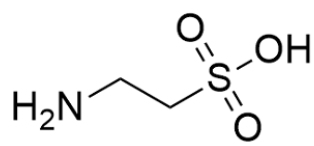

Figure 7 Taurine molecule that is always in a free form and not part of intact protein

Taurine enrichment of rotifers and *Artemia*

- Taurine is a β-amino sulfonic acid involved in bile salt synthesis, anti-oxidative defence, cellular osmoregulation, as well as contributing to visual, neural and muscular function (Fang et al., 2002; Omura and Inagaki, 2000).
- It is unclear if omnivorous fish would have taurine synthesis capability.
- Diversify results showed that only enriching rotifers with taurine (600 mg/l; 6.4 mg/g DW rotifer) has a far reaching and significant effect on larval (12 and 19 dph) and juvenile (44 dph) growth (**Fig. 8**).
- Unenriched *Artemia* have considerable natural levels of taurine. Therefore no added benefit of feeding taurine enriched *Artemia* to mullet larvae was shown.

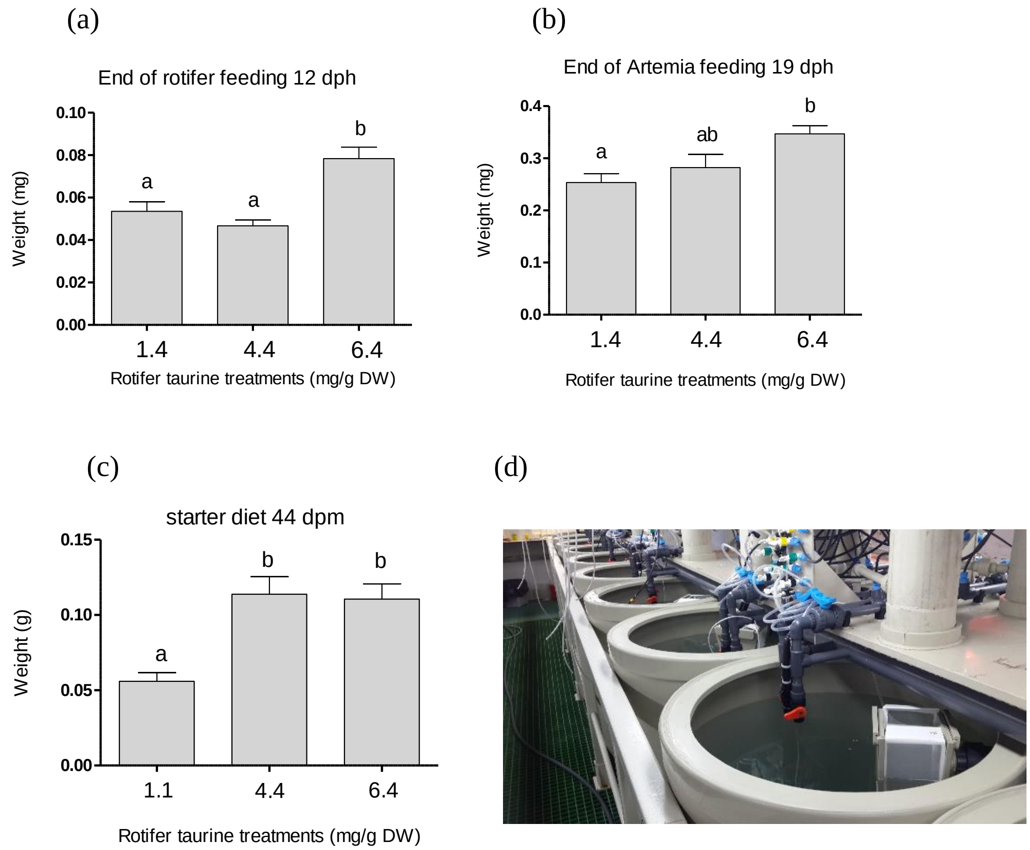

Figure 8 (a) The effect of feeding rotifer taurine treatments to 3-12 dph mullet larvae on larval weight in 12 and 19 dph grey mullet larvae and 44 dph juveniles. Bar values having different letters were significantly ($P<0.05$) different. (d)The experimental 400 l system used for these studies.

Dietary taurine for juvenile rearing

- Tested four 1 mm diameter extruded diets containing different levels of taurine (0, 0.5, 1.0 and 2.0% DW diet) on 126-174 dph mullet juveniles for 48 days where fish grew at least 100%.
- Results showed that grey mullet juveniles have a minimum 0.5% requirement for dietary taurine **(Fig. 9),** which is within the range of taurine requirements measured in a variety of marine species.
- Results suggest that the overall taurine requirement might be higher than 0.5% but part of this requirement may be satisfied by endogenous synthesis of this nutrient.

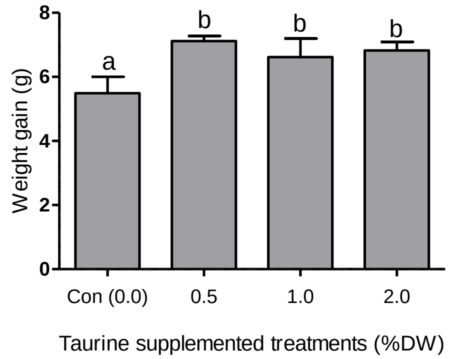

Figure 9. The effect of the taurine diets (control-0, 0.5, 1.0 and 2.0% DW diet) on average weight gain (g). Taurine values having different letters were significantly (P<0.05) different.

Broodstock Diets

- Fish reared in captivity may exhibit reproductive dysfunction that can be overcome by hormonal therapies, modulation of environmental parameters and providing an effective broodstock diet (Mylonas et al., 2010), which should mimic body composition of brooders in the wild (Izquierdo et al., 2001; Rodríguez-Barreto et al., 2014).
- Results showed that gonads from wild and domestic adult grey mullet broodstock differed in saturated, monounsaturated and polyunsaturated fatty acids of the n-3 and n-6 groups.
- Differences were particularly marked in EPA in both sexes and DHA in wild males compared to captive cohorts.
- The effect of replacing soybean oil (VO) with fish oil (FO) in mullet broodstock diets significantly increased percent of hatching of eggs (**Fig. 10a**).
- Larvae from the FO broodstock, regardless of salinity exposure, demonstrated 100% swim bladder inflation by 5 dph, where there was no swim bladder inflation in fish from the VO broodstock, independent of salinity (**Fig. 10b**).
- Adult wild grey mullet showed large ripe ovaries and were in the most advanced oocyte stage while hatchery-produced grey mullets showed signs of reduced reproductive activity.

(a) (b)

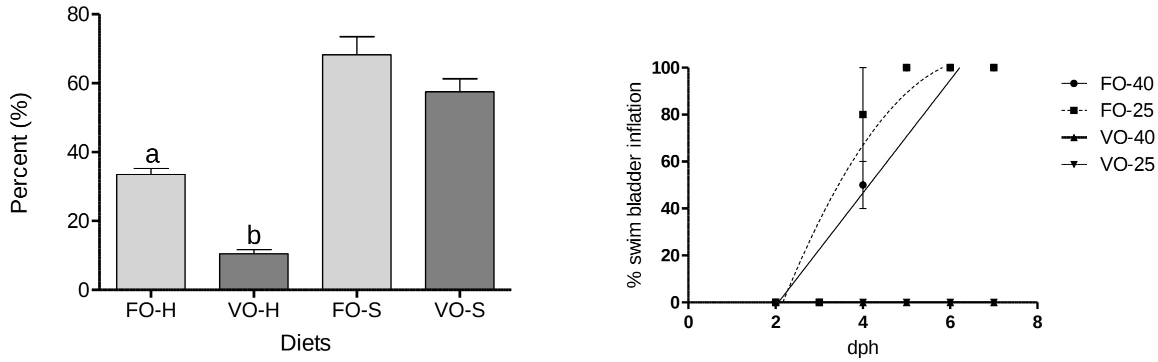

Figure 10 The effect of broodstock diets FO (Fish oil) and VO (vegetable oil) on (a) percent hatching (H) and survival (S) at the end of the day of hatching (T0). Percent values having different letters were significantly different (P<0.05). (b) The rate of % swim bladder inflation increase in 2-6 dph larvae during food deprivation in 25 and 40 ‰ seawater. The regression lines of swim bladder inflation in the FO larvae were highly significantly different (P<0.0001) from the regression lines of the VO larvae but not significantly (P>0.05) different from each other

Larval husbandry

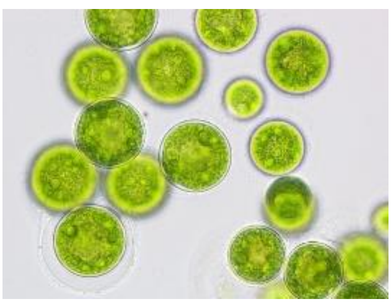

Figure 11 Nannochloropsis cells

Influence of algal type and concentration on larval performance

Bill Koven, National Center for Mariculture, Eilat, Israel

- Tested the influence of algal type and turbidity (**Table 2**) on larval rotifer ingestion, performance and digestive tract enzyme ontogeny.
- Larval ingestion of rotifers improves with algae produced turbidity until 1.2 NTU .
- Maximizing rotifer feeding has long term effects on survival in older larvae and juveniles.
- Ontogeny of digestive enzymes genetically based. But diet composition can influence specific activity of lipase and total alkaline proteases.
- Late age of the carnivorous-herbivorous shift is supported by steadily increasing activity of amylase (25-79 dph).

- 61-79 dph grey mullet juvenile's (*ca* size of juvenile mullet moving to estuaries) capacity to digest protein and starch increases. Allows to exploit starch rich micro- and macroalgae as well as benthic organisms.
- During larval rearing is recommended the use of algae (*Nannochloropsis oculata* or *Isochrysis galbana*) to produce a turbidity of 1.2 NTU in the grey mullet rearing tanks. White clay producing the same turbidity is not as effective as "greening with algae".

Table 2 The microalgae treatments with their concentrations (cells/ml), designations and turbidities (NTU)

Treatments	Designation	Turbidity (NTU)
Control (no microalgae)	Control	0.26 ± 0.01^{a}
Isochrysis galbana A (0.0144×10^{6} cell/ml)	Iso A	0.77 ± 0.01^{b}
Nannochloropsis oculata A (0.2×10^{6} cells/ml)	Nanno A	0.75 ± 0.01^{b}
Isochrysis galbana A (0.0288×10^{6} cell/ml)	Iso B	1.18 ± 0.02^{c}
Nannochloropsis oculata A (0.4×10^{6} cells/ml)	Nanno B	1.20 ± 0.02^{c}

Weaning of grey mullet

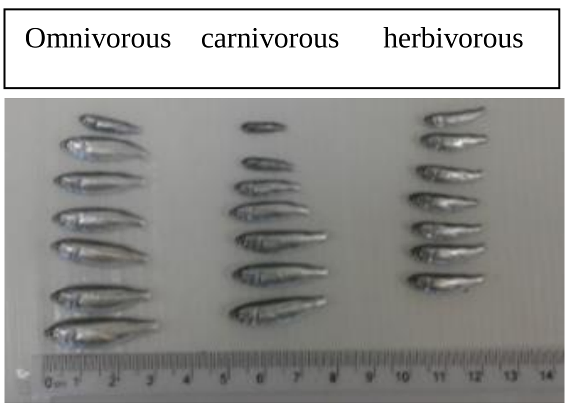

Figure 12 Size differences in mullet juveniles fed an omnivorous, carnivorous or herbivorous diet.

- Grey mullet larvae are strict carnivores feeding on zooplankton. After metamorphosis the juveniles gradually change to an herbivorous/omnivorous diet while searching for less saline estuaries with higher primary productivity of micro and macroalgae.
- Mullet juveniles were weaned off live Artemia to a dry, prepared diet from 24-37 dph.
- Earlier study indicated weaning period overlaps trophic shift from carnivory to omnivory.
- The aim of this study was to evaluate the effect of feeding a carnivorous, herbivorous or omnivorous weaning diet to juvenile grey mullet.
- The omnivorous diet performed best in terms of length, dry weight **(Fig. 13)** and tank biomass.
- The herbivorous diet produced smaller fish (<100 mg), and less larger fish (200-400 mg).
- The omnivorous and carnivorous diet produced morelarger fish (300-400 mg).
- The results showed that the herbivorous diet delays gut maturation.

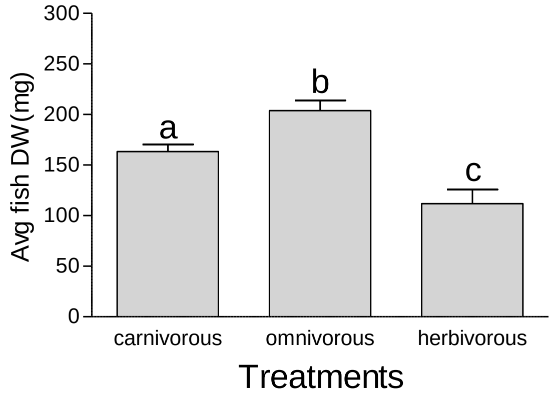

Figure 13 The effect of the carnivorous, omnivorous and herbivorous diets on dry weight (DW)

Grow out husbandry

Figure 14 PVC lined grey mullet grow out ponds at the IOLR with netting to stop bird predation

Effect of different weaning diets on performance and health status

Enric Gisbert, IRTA, Spain

- The aim was to evaluate a weaning protocol from live prey to inert diets with different levels of fish meal substitution (Plant Protein (PP)0, PP50 and PP75%) with a blend of different plant protein sources (corn gluten, wheat gluten and soy protein concentrate).
- Results showed no significant (P>0.05) fish meal substitution effect during the weaning (days 0-30) and the early on-growing period (days 30-60) for flathead grey mullet fry (**Table 3**).
- In this trial, feeds were offered until apparent satiation and palatability was not adversely affected by the level of PP inclusion in diets.
- The activity of pancreatic and intestinal digestive enzymes was not affected by the levels of FM substitution in diets.
- The inclusion of PP ingredients did not affect the integrity, health nor intestinal functionality.
- The results shown indicated that PP50 and PP75 diets were 15.5 and 23.6% cheaper than the FM diet, which was mainly due to the lower inclusion of high quality fish meal (LT 70) in diets.

Table 3. Final body wet weight (BW, mg), standard length (SL, mm), Fulton's condition factor (K), specific growth rate in BW (%/day) and survival (%) of wild flathead grey mullet (*Mugil cephalus*) fry weaned onto diets with different levels of fish meal substitution (FM, no fish meal substitution; PP50, 50% substitution of fish meal with plant protein sources; PP75, 75% substitution of fish meal with plant protein sources). Data is presented for the two distinct phases in which the study was divided: weaning of fry with the above mentioned diets (days 0-30) and the early on-growing phase (days 30-60).

	Day 30 (end of the weaning)				
	BW (mg)	SL (mm)	K	SGR_{BW} (% day^{-1})	S (%)
FM diet	390 ± 15	2.5 ± 0.01	2.64 ± 0.06	1.9 ± 0.1	78.6 ± 5.1
PP50 diet	385 ± 12	2.4 ± 0.02	2.72 ± 0.05	1.8 ± 0.1	73.4 ± 3.1
PP75 diet	375 ± 18	2.4 ± 0.02	2.72 ± 0.05	1.9 ± 0.1	71.1 ± 4.2
	Day 60 (end of the trial)				
	BW (mg)	SL (mm)	K	SGR_{BW} (% day^{-1})	S (%)
FM diet	707 ± 17	3.2 ± 0.02	2.17 ± 0.05	2.1 ± 0.05	74.6 ± 3.1
PP50 diet	661 ± 10	3.1 ± 0.02	2.18 ± 0.04	1.9 ± 0.07	70.4 ± 4.2
PP75 diet	681 ± 20	3.2 ± 0.02	2.17 ± 0.03	1.8 ± 0.09	69.1 ± 3.1

Grow out using a specialized grey mullet diet, under different environmental and geographical conditions in Israel, Greece and Spain

Bill Koven (IOLR, Israel), Yannis Kotzamanis (HCMR, Greece), Rocio Robles (CTAQUA, Spain)

- **IOLR** stocked F2 mullet (8.23 g) mullet in two cement (19.0 m^2) and one polypropylene (3.5 m^2) tanks at densities of 55, 29 and 286 fish/m^2 and daily fed (2% of tank biomass) **IOLR** feed (IRIDA feed formula is based on the IOLR formula).
- Israeli, Greek and Spanish trials demonstrated that a fish density decrease increased average weight.
- **IOLR** found that the effect of feeding the extruded IRIDA diet to 4 and 6 fish/m^2 of F2 fish (ca 108 g) showed no difference in average wet weight gain while **IOLR** stocking 10 and 12 fish/m^2 of wild fish (ca 44.8 g) demonstrated that lower density fish grew better and reflected a more narrow size distribution (**Figs. 8**).
- **CTAQUA** in Spain (earthen ponds) demonstrated a density effect on the final wet weight of the fish. SGRs for the 0.5 and 1.0 fish/m^2 were 0.83% and 0.73%/d, respectively, after a growing period of 533 days. A greater percentage of smaller fish was found in the 1.0 fish/m^2 treatment compared to 0.5 fish/m^2 while a higher percentage of larger fish was found in the 0.5 fish/m^2 treatment compared to 1.0 fish/m^2.
- FCR was improved using the extruded **IRIDA** diet (3.0), which replaced poultry meal with fish meal in the diet, compared to the **IOLR** pelleted diet (3.5).
- **HCMR** in Greece testing the density effect of stocking wild fry at 4 and 6 fish/m^2 (ca 21 g) found no significant differences between treatments in survival, growth performance and size distribution, which largely agreed with the **IOLR** trial using the same densities. However, the Greek fish exhibited only a 30 g/fish gain over the 14 month feeding period.
- Density effect of suppressing growth increases FCR and is a major stumbling block to successful monoculture of this species (**Fig. 16**).

- Extruded feed seems superior to pelleted feed but more research necessary to improve FCR and growth performance of current mullet diet while multiple daily feedings may increase yield and reduce large size distribution.

Figure 15 The participation of Greece, Spain and Israel in the density grow out trials as a function of grow out facility and geographical location; weighing of mullet in Greece, capturing mullet in Spain and the use of cement tanks in Israel

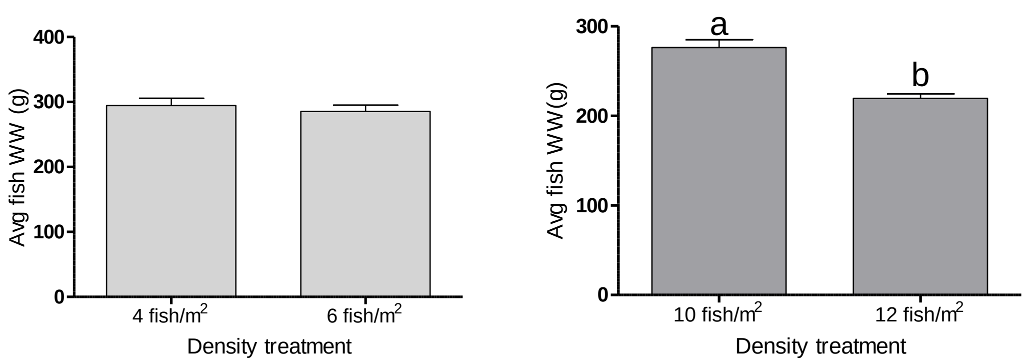

Figure 16 The effect of Israeli stocking treatments (a) 4 and 6 fish/m^2 and (b) 10 and 12 fish/m^2 on the average fish weight. N=86, 58 of the 4 and 6 fish/m^2 treatments, respectively and N=177, 200 of the 10 and 12fish/m^2 treatments, respectively. Average fish wet weight (WW) values having different letters were significantly ($P<0.05$) different.

Market, Consumer perception, new products and business model

Gemma Tacken, Wageningen University and Research, The Netherlands

The socio-economic research in DIVERSIFY includes applied market development approach clarifications on perception of aquaculture products, market demand evaluation, consumer preferences, new product development (**Fig. 17**), value adding and market development. The studies have been performed across five largest European fish markets: France, Germany, Italy, Spain and the United Kingdom,

Market analysis
Machiel Reinders, Wageningen University and Research, The Netherlands

- The market analysis demonstrated that buyers (*i.e.* retailers) in the five countries find it very difficult to position the 6 new species (*e.g.* grey mullet) in relation to the current species in the market.
- Species such as grey mullet are unknown as aquaculture products, as well as wild catch.
- Buyers are open to welcome new species under the following conditions:
    - The product must be cultured in a sustainable way,
    - The product should be available as a fresh product (southern-Europe) and as a frozen product (especially Germany),
    - The product must be easy to prepare and/or ready to eat and
    - The product must be priced competitively.

New Product Development
Marija Banovic, MAPP Centre, Department of Management, Aarhus University, Denmark

- Co-creation with consumers identified the most promising product ideas for new fish products per investigated country.
- Recommendations for new product development of selected fish species, the most important drivers and barriers for the choice of the new product ideas have also been identified.

Sensory characterization of new fish species and consumer acceptance of new product development
Luis Guerrero, IRTA, Spain

- New fish species need to be properly introduced to create a diversification in the current market.
- **Sensory**, compositional, instrumental texture parameters and somatic properties of DIVERSIFY five emerging fish species, namely wreckfish, greater amberjack, grey mullet, meagre, and pikeperch, were examined for characterization purposes.
- Regarding the compositional parameters, fat content was among the most relevant discriminating aspect between species, while hardness was among the most differentiating ones when dealing with texture.
- Greater amberjack was described with sour flavor, pike perch was associated to an earthy flavor and grey mullet was characterized by bitter flavor. Sensory firmness was clearly distinctive for wreckfish, while meagre related to juicy texture.
- The species in this study exhibited a wide range of physicochemical and sensory characteristics that show their potential for being further exploited when designing new products.
- In a **consumer acceptance** test, it was demonstrated the influence of having the product information in advance on the consumer acceptance degree **(Fig.18**).
- In the case of Italy, grey mullet fillet preserved in olive oil and grey mullet smoked fillet showed a different acceptance pattern.

- Fish in olive oil matches the expectations of the consumer since it is a well known preservation method for fish. Smoked fish improved the expectations of the consumer.

Figure 17 Example of the elaboration of some of the products. a) Grey mullet fillets in salting mixture before smoking; b) Grey mullet fillets on smoking trays; c) Glass pots with clean and homogenous pieces of mullet fillets; d) Filling the glass containers with olive oil; e) Bottles with grey mullet fillet and olive oil ready to go under cooking process.

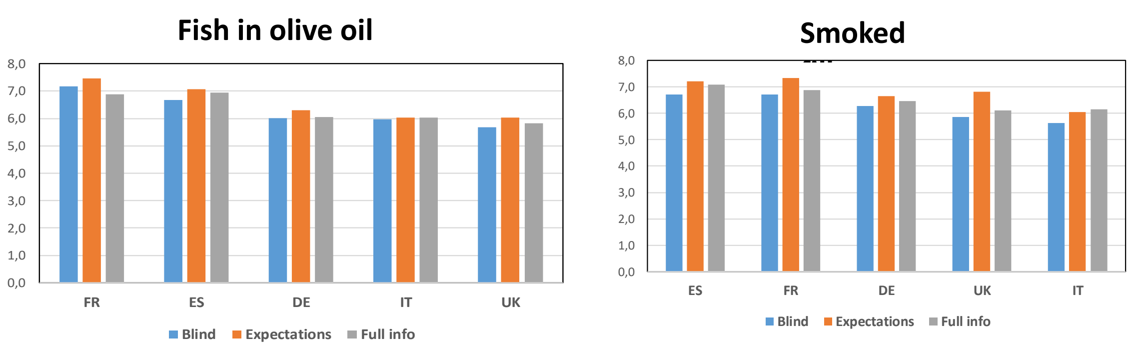

Figure 18. Results of the consumers' acceptance tests for grey mullet new developed products performed in 5 European countries. Consumers were not informed about the product (blue bar), then knowing the product to be tested, they were asked about their expectation once informed about the products (orange bar) and finally they had the full information before tasting the product (grey bar). In Italy, fish in olive oil matched the expectations (graph on the left); smoked filet had a higher acceptance when the consumers had full information of the product.

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