



### Deliverable Report

<b>Deliverable No:</b>	31.34	<b>Delivery Month:</b>	55	
<b>Deliverable Title</b>	“Know-how Transfer” seminar for the aquaculture industry (Italy), presenting the progress achieved in DIVERSIFY in the technology for grey mullet			
<b>WP No:</b>	31	<b>WP Lead beneficiary:</b>	P18. CTAQUA	
<b>WP Title:</b>	Dissemination			
<b>Task No:</b>	31.5	<b>Task Lead beneficiary:</b>	P13. UNIBA	
<b>Task Title:</b>	Full-day seminars on “Know-how Transfer” of the aquaculture for each of the studied species			
<b>Other beneficiaries:</b>	P1. HCMR	P3. IRTA	P4. IOLR	P38. HRH
<b>Status:</b>	Delivered		<b>Expected month:</b>	59
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**Lead Scientist preparing the Deliverable:** Robles, R. (CTAQUA),

**Other Scientists participating:** Corriero, A. (UNIBA), Koven, B. (IOLR), Mylonas, C. (HCMR)

**Objective:** The objective of this Deliverable was to present a full-day seminar directed to the Mediterranean aquaculture industry in Italy covering the knowledge obtained in DIVERSIFY for either meagre, greater amberjack, wreckfish or grey mullet. It was decided that this workshop would be dedicated to grey mullet, since in Italy this species has an important niche market, both for the flesh and for the fish row (bottarga). The seminar included 30 min presentations on selected aspects (*e.g.* reproduction and spawning induction, final product diversification and quality, socioeconomic issues and marketing, etc.), given by DIVERSIFY Partners, but also from any authorities in the species, whose work was not part of the project. Aquaculturists (mainly), but also European aquaculture support companies (feed, pharmaceutical, equipment, engineering, etc.), researchers and educators, government organizations and other important institutions (FAO, Globefish) have been invited to attend this meeting.

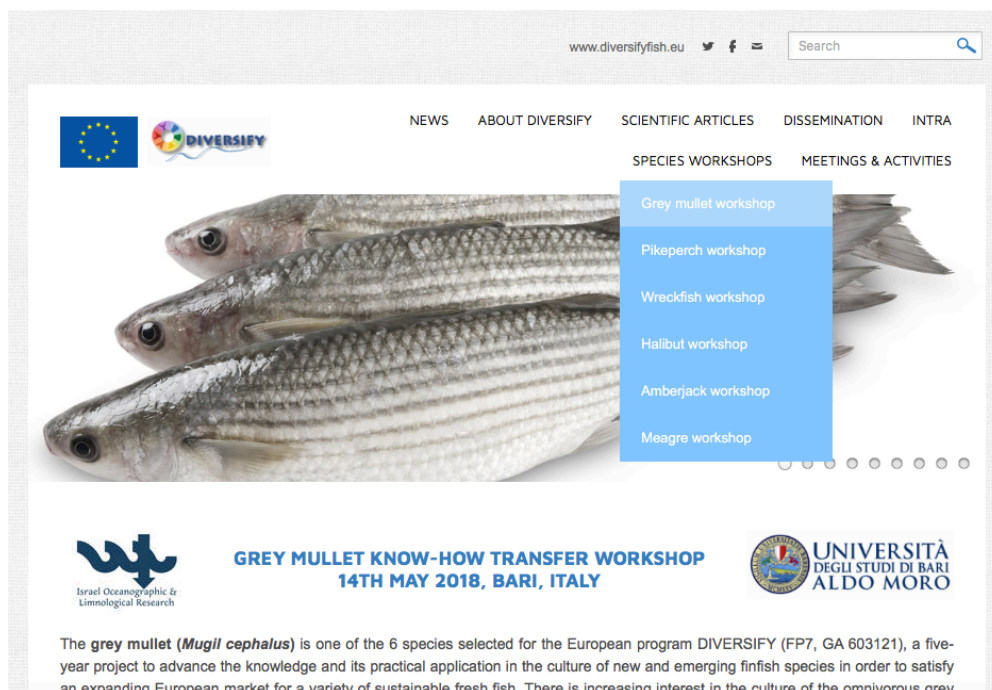
**Description:** The workshop was organized jointly by Dr Aldo Corriero (P13. UNIBA) and Dr Bill Koven (P4. IOLR), the latter being the Species Leader for grey mullet. It was held in Palace Hotel, Bari, Italy on the 14<sup>th</sup> of May 2018, and was advertised via the production of an announcement sent to a number of potential stakeholders, such as National Producers associations, FEAP, EAS, DG-Mare, DG R&I, FAO, etc. (**Fig. 1**), as well as via the DIVERSIFY website, which include a dedicated page (**Fig. 2**).





The flyer features logos for Israel Oceanographic & Limnological Research, the European Union, DIVERSIFY, and Università degli Studi di Bari Aldo Moro. The text reads: "First announcement: Workshop on grey mullet aquaculture: state of the art and perspectives. 14<sup>th</sup> May 2018, Palace Hotel, Bari (Italy)". It includes five images: eggs, a large tank of fish, a single fish, a larva, and a jar of bottarga. The introduction text states: "The grey mullet (*Mugil cephalus*) is one of the 6 species selected for the European program DIVERSIFY (FP7, GA 603121), a five-year project to advance the knowledge and its practical application in the culture of new and emerging finfish species in order to satisfy an expanding European market for a variety of sustainable fresh fish. There is increasing interest in the culture of the omnivorous grey mullet as a high quality source of protein and as a species that requires little or no dietary fish meal. Moreover, the salted and dried roe (*bottarga*) from gravid females is considered a highly prized delicacy in the southern Mediterranean and Asia, and an added value product from the culture of this species."

Figure 1. The announcement of the workshop that was sent to a number of potentially interested stakeholders around Europe (National Producers associations, FEAP, EAS, DG-Mare, DG R&I, FAO, etc.).



The screenshot shows the website www.diversifyfish.eu with a search bar and navigation menu. The menu includes "NEWS", "ABOUT DIVERSIFY", "SCIENTIFIC ARTICLES", "DISSEMINATION", "INTRA", "SPECIES WORKSHOPS", and "MEETINGS & ACTIVITIES". A dropdown menu under "SPECIES WORKSHOPS" lists: "Grey mullet workshop", "Pikeperch workshop", "Wreckfish workshop", "Halibut workshop", "Amberjack workshop", and "Meagre workshop". The main content area features a large image of grey mullet fish and a banner for the "GREY MULLET KNOW-HOW TRANSFER WORKSHOP 14TH MAY 2018, BARI, ITALY". Logos for Israel Oceanographic & Limnological Research and Università degli Studi di Bari Aldo Moro are present. The introductory text is identical to Figure 1.

Figure 2. The dedicated page for the workshop, in the DIVERSIFY website.



The agenda was prepared by the organizers, in collaboration with the WP31. Dissemination leader (Dr Rocio Robles) and the Project Coordinator (Dr CC Mylonas), as follows:

**8:30 – 9:00 Welcome and registration**

**9:00 – 9:15** The DIVERSIFY project (**Constantinos C. Mylonas, Coordinator**, Hellenic Centre for Marine Research, Greece)

**9:15 – 9:45** Traditions and cultural heritage in grey mullet culture (**Donatella Crosetti**, Institute for Environmental Protection and Research, Rome, Italy).

**9:45-10:15** The culture of grey mullet in Egypt: the largest market in the Mediterranean region (**Sherif Sadek**, Aquaculture Consultant Office, Cairo, Egypt).

**10:15-10:45** Stock enhancement and mullet culture in Hawaii (**Ken Leber**, MOTE Marine Laboratory, Florida, USA)

**10:45-11:30 Coffee break**

**11:30-12:00** Overcoming grey mullet reproductive dysfunction in captivity - an expanded tool box for successful breeding (**Hanna Rosenfeld**, Israel Oceanographic and Limnological Research, Eilat, Israel)

**12:00-12:20** Reproduction and rearing of grey mullet for restocking purposes (**Dario Vallainc**, International Marine Center, Cagliari, Italy)

**12:20- 12:40** Sexual maturity in captive and wild grey mullet stocks (**Iris Meiri-Ashkenazi**, Israel Oceanographic and Limnological Research, Eilat, Israel)

**12:40-13:00** Function and practical implementation of algal addition to larval rearing tanks (**Bill Koven**, Israel Oceanographic and Limnological Research, Eilat, Israel)

**13:00-13:20** Salinity as the driving force of DHA requirement in juvenile grey mullet (**Dor Israeli**, Israel Oceanographic and Limnological Research, Eilat, Israel)

**13:20-15:00 Lunch (compliments of DIVERSIFY)**

**15:00-15:20** The transition from carnivorous to omnivorous feeding and its implications in larva and juvenile rearing of grey mullet (**Bill Koven**, Israel Oceanographic and Limnological Research, Eilat, Israel)

**15:20-15:40** Weaning grey mullet fry with diets differing in fish meal levels (**Alicia Estevez**, Institute of Agrifood Research and Technology, Spain)

**15:40-16:00** Nutraceutical properties of mullet *bottarga* (**Antonella Rosa**, University of Cagliari, Italy)

**16:00-16:20** Marketing options for processed farmed grey mullet (**Rocio Robles**, CT AQUA, Spain)

**16:20-17:00** Consumer perceptions and new product development: the case of grey mullet in international markets (**Thanasis Krystallis**, Aarhus University, Denmark and Hellenic Research House, Greece)

**17:00-18:00** Round table discussion on bottlenecks to improved production and defining the farmer's needs (**invited speakers**)

The meeting was attended by 45 people, coming from the Industry and academia with diverse interest in the species. Some fish farmers are getting interested in the culture of grey mullet although there is a number of draw backs such as the marketing difficulties and reproduction control that keep the species away from industrial production.

The morning presentations covered a wide range of grey mullet related topics such as an overview of traditions and cultural heritage in grey mullet culture, reproduction and rearing of grey mullet for restocking purposes and grey mullet culture in Egypt. These talks were given by invited speakers with extensive knowledge and recognized experience with the species. Dr. Donatella Crossetti gave a very illustrative overview of the mullet culture origins. Traditional fishing trap systems used in Italy were well documented in her presentation (**Fig. 3**). Next presentation was given by Dr. Sherif Sadek detailing the production figures of the species in Egypt and the peculiarities of the Egyptian freshwater species production and market (**Fig.**



4). Grey mullet culture could be the first fish species for Egyptian market if there would be a stable supply of fingerlings from industrial hatcheries.



**Figure 3.** Representative slides of Dr. Crosetti's talk showing ancient rock drawings indicating the importance of mullet to aboriginal Australians (left). Fish trap systems used in the North Adriatic to catch grey mullet (right).



**Figure 4.** Representative slides of Dr. Sherif Sadek presentation showing the importance of tilapia culture in the Nile Delta, Egypt (right) and an overview of earthen ponds dedicated to grey mullet culture (right).

Presentations from DIVERSIFY partners focused on the sexual maturation and controlling reproductive dysfunction of grey mullet in captivity and on larviculture optimization. Dr. Hanna Rosenfeld and Dr. Iris Meiri-Ashkenazi (P4, IOLR) disserted about resolving the problems of reproductive dysfunction of grey mullet kept in captivity, addressing in detail the different hormonal treatments tested to obtain broodstock sexual maturation and successful spawning (**Fig. 5**).

Next presentation from Dr. Dario Vallanic from the International Marine Centre in Cagliari (Italy), explained the program on reproduction and rearing of grey mullet for restocking purposes carried out at his institution (**Fig. 6**).



**Major aims**

- 1 To increase the abundance of spermiating males exhibiting high quality milt
- 2 To synchronize gonadal development in- and between sexes
- 3 To induce spawning

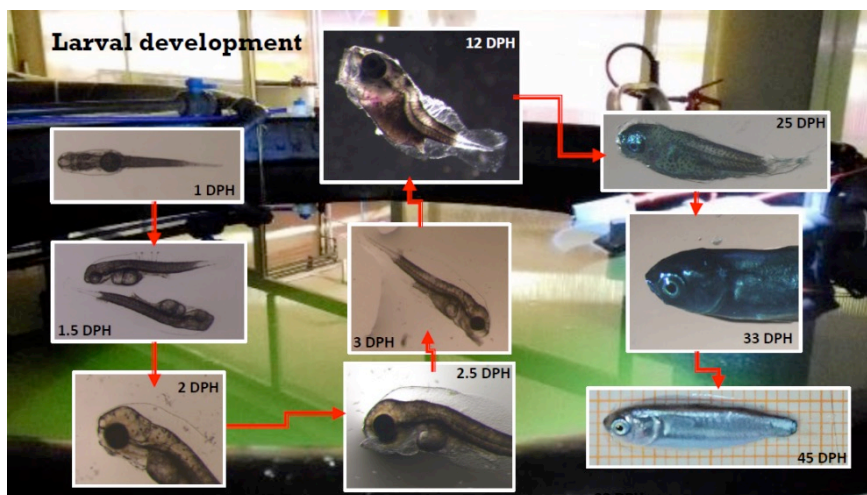
**Specific objectives:**

1. To produce bio-potent recombinant gonadotropins (r-LH and r-FSH)
2. To evaluate their potential to act as therapeutic agents alleviating reproductive dysfunction in captive mullet

**Summary of the spawning data**

- A relatively extended natural spawning season (~3 months).
- Shifted spawning season can be easily achieved via photo-thermal manipulation.
- Improved spawning success (60%) among hormonally induced females.
- Improved synchronization among breeding units increased fertilization rate (>70%).
- Relatively high fecundity (Av. 1.76 ± 0.52 million eggs/kg)
- Hatching rate: 78.84 ± 11.93 %
- Over two hundred thousand fingerlings were produced

**Figure 5.** Representatives slides of Dr. Rosenfeld presentation, showing the objectives of the studies (left) and the final successful results (right).



**Figure 6.** Slide showing the different larval stages of grey mullet (Dr. Vallainc presentation).

Dr. William Koven's presentation (P4. IOLR) illustrated the function and practical implementation of algal addition to grey mullet larval rearing tanks. The main conclusions of their studies are shown in **Fig. 7**.



### Summary

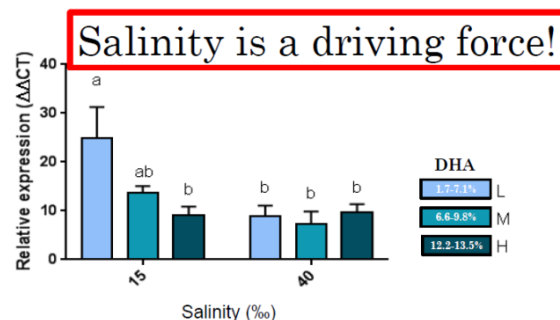
- Most effective turbidity level was 1.2 NTU (prey consumption and survival).
- Rotifer feeding, due to turbidity, has long term effects on older larvae and juveniles.
- Ontogeny of enzymes genetically based. But diet composition can influence specific activity of lipase and total alkaline proteases.
- Late age of the carnivorous-herbivorous shift supported by steadily increasing activity (ca 30x) of amylase (25-79 dph).
- 61-79 dph grey mullet juveniles (ca size of juvenile mullet moving to estuaries) capacity to digest protein and starch -exploit starch rich micro- and macroalgae as well as benthic organisms.
- This study appears to suggest that turbidity plays an important role in prey consumption and larval performance or does it?

**Figure 7.** Summary of the work presented by Dr. Koven regarding the role of microalgae in grey mullet larviculture.

Last presentation in the morning was given by Dr. Dor Israeli and it was entitled “Salinity as the driving force of DHA requirement in juvenile grey mullet”. The data presented show how depending on the salinity of the culture water, grey mullet require a lower amount of DHA in the diet (ca. 7% of total fatty acids), as long as there is a source of linolenic acid. In **Fig. 8** is shown how salinity regulates the expression of SREBP-1 which regulates genes required for glucose metabolism and fatty acid and lipid production and its expression is regulated by insulin.



### Salinity regulates SREBP-1 expression

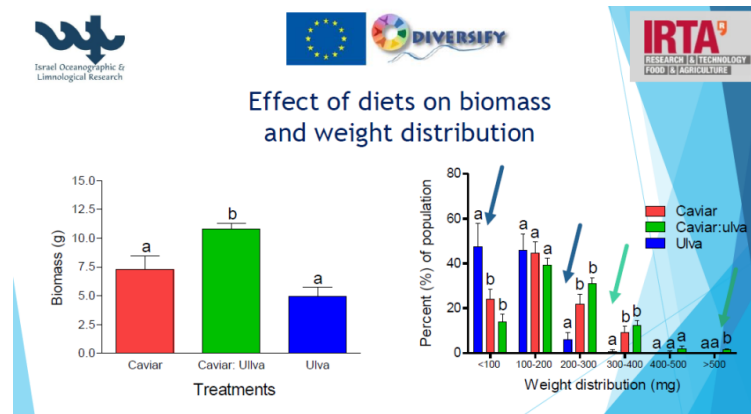


**Figure 8.** Relative expression of SREBP-1 at two different salinities (15 ‰ and 40 ‰) and three levels of DHA inclusion in the diet (1,7-7,1%; 6,6-9,8% and 12,2-13,5%).

Afternoon speakers explained, among other topics, the particularities of the transition of grey mullet fingerlings from carnivorous to omnivorous feeding and how to complete the weaning period with different diets. Dr. Koven described how after metamorphosis grey mullet larvae change from strict carnivores feeding on zooplankton to herbivorous/omnivorous diet searching for less saline estuaries with higher primary productivity of micro and macroalgae. The objective of the study was to evaluate the effect of feeding a carnivorous (commercial feed named Caviar), herbivorous (feed based on *Ulva* sps.) or



omnivorous (Caviar plus *Ulva*) weaning diet to juvenile mullet on growth, survival and population weight distribution. Results are presented in **Fig. 9**.



**Figure 9.** Effect of the 3 different weaning diets: carnivorous (commercial feed named Caviar), herbivorous (feed based on *Ulva* sps.) or omnivorous (Caviar plus *Ulva*) on biomass and weight distribution of grey mullet at 53 days post hatching (dph).

Next presentation was given by Dr. Alicia Estévez (P.3 IRTA). Weaning diets with different fish meal inclusion levels were tested in grey mullet. Fish meal was partially substituted at 50 and 75% with plant proteins (corn gluten, wheat gluten and soy protein concentrate). All the diets were isoproteic (36%), isolipid (16%) and isoenergetic. The only difference in the fatty acid composition of the diets was the higher content of 18:2n-6 (linoleic acid) and total n-6 in PP50 and PP75 diets that contributed to a higher content in total PUFA in these 2 diets and was due to the high content of soybean meal.

The substitution with different plant protein sources at 50 and 75% did not affect growth performance and survival of grey mullet (**Fig. 10**). These findings confirm the capacity of grey mullet to digest plant protein sources at early life stages.

	Day 30 (end of the weaning)				
	BW (mg)	SL (mm)	K	SGR(%/day)	S (%)
Control	390 ± 15	2.5 ± 0.01	2.64 ± 0.06	1.9 ± 0.1	78.6 ± 5.1
PP50	385 ± 12	2.4 ± 0.02	2.72 ± 0.05	1.8 ± 0.1	73.4 ± 3.1
PP75	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(%/day)	S (%)
Control	707 ± 17	3.2 ± 0.02	2.17 ± 0.05	2.1 ± 0.05	74.6 ± 3.1
PP50	661 ± 10	3.1 ± 0.02	2.18 ± 0.04	1.9 ± 0.07	70.4 ± 4.2
PP75	681 ± 20	3.2 ± 0.02	2.17 ± 0.03	1.8 ± 0.09	69.1 ± 3.1

**Figure 10.** Effect of three weaning diets with different fish meal content on grey mullet performance (Control, 100% fish meal; PP50, diet with only 50% fish meal and PP75, diet with only 25% fish meal).

Following talk was given by Dr. Anotnella Rosa from the Department of Environmental Sciences from the University of Cagliari (Italy). Her talk entitled “*Nutraceutical properties of mullet bottarga*” focused on the beneficial effects of bottarga as a source of antioxidants, aminoacids and n-3 fatty acids. A correct preservation methodology is essential to maintain those properties.



The last two presentations of the afternoon were from the socioeconomic area of DIVERSIFY. The talk entitled “Marketing options for processed farmed grey mullet “ from Rocio Robles (P.18 CTAQUA) presented the elaboration of the grey mullet products and the consumer evaluation carried out in five countries with 100 consumer per country: Spain, UK, Germany, France and Italy. Three product ideas were developed and tested for their technical feasibility of production: 1) smoked grey mullet filet, 2) grey mullet preserved in olive oil and 3) fresh fish fillet with different healthy seasoning and marinades. For the consumer evaluation, smoked grey mullet filet and grey mullet preserved in olive oil were used (Fig. 11). Consumer were requested to fill in a questionnaire at three different moments: a) expectation: before tasting the product so they could express their expectations about the product, b) blind: in a blind test, in which they did not have any information of what they were tasting and 3) full info: all the information was provided in advance. The results of these tests are presented in Fig. 12.



Figure 11. Conditions of the smoking process to prepare the smoked grey mullet filet (left) and preparation of the ready-made grey mullet filet in olive oil (right). These two products were evaluated by 500 consumers across Europe (Spain, UK, France, Germany and Italy).

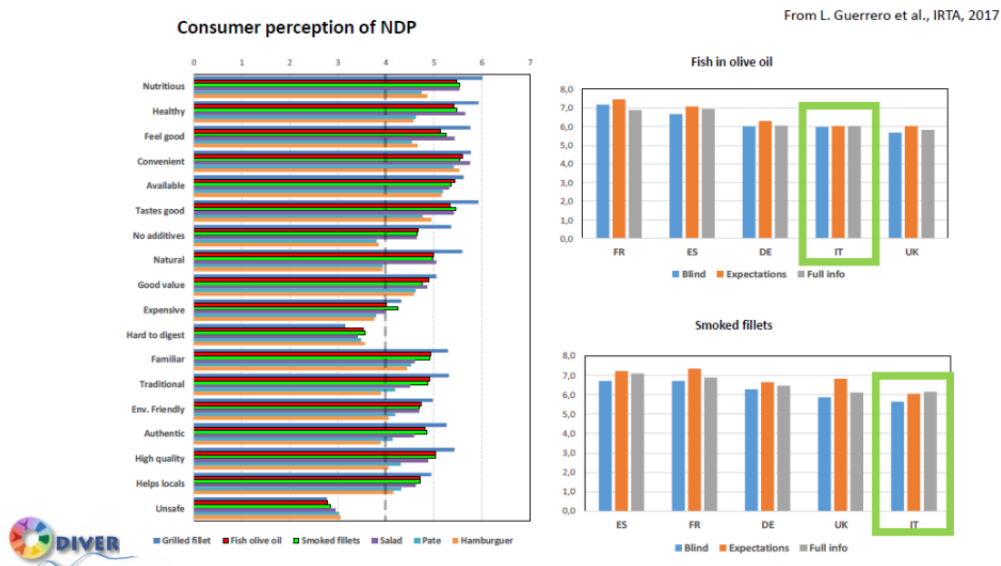


Figure 12. Results from the consumer evaluation of the grey mullet new products developed in Diversify. In the case of Italy, fish file in olive yielded the same results in the three situations (blind, expectations and full info; see text for description). In the case of smoked filet, the more info the consumer had the higher the acceptability of the product.





Last presentation of the day entitled “*Consumer perceptions and new product development: the case of grey mullet in international markets*” was given by Dr. Thanasis Krystallis (P38. HRH). The presentation was based in giving answers to five questions (**Fig. 13**) which are the road map for consumer-oriented high added-value new product development studies in the socioeconomic area of DIVERSIFY.



**Figure 3.** Representative slides of the presentation from Dr. Krystallis including the five questions driving the consumer-oriented product development (left) and some managerial implications to be taken into account when creating new products for the European market.

All the presentations were followed by quite a number of questions from the audience, which promoted interesting discussions among speakers and attendees.

During the following Round Table discussion, several topics were debated between the speakers and the audience. Dr. Koven as species leader started the debate questioning how should we proceed with the investigation towards making grey mullet culture a profitable activity, since the result from DIVERSIFY on the grow out phase were not always promising. Since nowadays the production of the species is based on collection of wild fry, sooner or later there will be not enough fry to support the production. Dr. Ken Leber (invited speaker) pointed out the urgent need to have a correct stock assessment of the species in the Mediterranean area, which is the first step needed to have a clear view of the sustainability of the of the production in the coming years.

Dr.Noah Mozes indicated that for Israel there is a stable production of the species, which relies on the production (and importation) of fingerlings.

Dr. Alicia Estevez (DIVERSIFY, IRTA, Spain) gave a short view on the production in the south of Spain, which is based on polyculture together with aquaculture species such as seabass and seabream. The fry enters in the ponds with the filling up of the “esteros” and they grow together with the cultivated species and with other fish species that enter in the system.

Dr. Constantinos Mylonas and Dr. Sherif Sadek agreed with the need to carry out a stock assessment.

Dr. Donatella Crosseti brought the attention to the fish-eating birds that are a real threat for the mullet culture. Namely cormorants are causing heavy losses in the extensive Italian lagoons. Monoculture of grey mullet does not seem to be an option due to the low profitability of the culture. So polyculture is now the only option.



Sherif Sadek commented that in Egypt the culture of grey mullet is based on polyculture, most of the times with tilapia in cages (there are 22.000 cages along the Nile delta dedicated to tilapia and carp mostly; only 4% would correspond to grey mullet).

Dr. Massimo Caggiano farm manager from the fish producer Panittica Italia srl (Italy), commented that no reference was made during all the presentations to the pathological problems the species may have, confirming that it is a very sensitive species to common aquaculture pathogens such as *Vibrios* spp., *Photobacterium* and *Nodavirus*.

Dr. Donatella Crosetti pointed out that maybe the way to produce grey mullet should be like the Egyptians do. In any case the price of the fingerlings should be lower. Dr. Rocio Robles asked the price of hatchery produced fingerlings, which seems to be around 0,25 dollar per piece, approximately three times the price of a bream or bass fingerling (0, 10 dollar).

Dr. Carlos Mazorra from the Sonrionansa hatchery and farm company (Spain) stated that the main problem of the species is the lack of a defined market in Europe.

Dr. Hanna Rosenfeld (DIVERSIFY, IOLR, Israel) suggested that a multiple production season would facilitate in making the species profitable and supply the market with affordable fingerling prices at the time that wild fingerlings are not available (e.g. spring). Dr. Caggiano pointed out that it does not make sense to have a dedicated mullet hatchery in the Mediterranean as long as wild fingerlings are available at a reasonable price.

The possibility of grey mullet culture in cages was the next point of attention, some of the panellist to locate the grey mullet cages just underneath the seabream and seabass cages. However, it does seem to be feasible.

Ran Epsteen from COLOURS, Israel called the attention of the great interest from countries like China or Indonesia on mullet species. They do have another species of mullet that it seems to have a good market in those countries. So he could not understand how mullet was not in the spotlight of aqua producers.

Dr. Crosetti reported that when she proposes to Italian farmers to grow grey mullet they say that there is still market for more seabass and seabream in Italy, so why to produce a lower value species? Concerning the higher market value of seabass and seabream, Rocio Robles made the point of the lower price of the feed for grey mullet (mullet feed 480 \$ per ton) compared with the feed for those species, which is more than double.

Then some doubts about the suitability of the species for aquaculture production raised a variety of comments on the future of the research for the species. The conclusion was that the species has a great potential due to its omnivorous nature and not requiring fishmeal and fish oil, this being a strong argument for a sustainable aquaculture fish. Dr. Rosenfeld introduced the concept of applying recirculation systems to the nursery phase of grey mullet, which could make the culture more profitable. More suggestions from the audience such as a deeper exploration of culture methods and possibilities were brought into the scene.

Dr. Crosetti stressed the need for identification of farmed grey mullet in the marketing of the species to differentiate it from the non-appreciated harbour associated mullets.

Dr. Mylonas insisted that it is not applicable the business model of sea bass and sea bream to grey mullet.

Dr. Koven finished the round table session summarizing the main issues for this species:

- High potential as sustainable aquaculture species
- Need for a stock assessment in the Mediterranean area.
- Availability of fingerlings at affordable prices
- Need for further and deeper research on the different grow out culture systems.
- Definition and establishment of the business model
- Design of a market strategy for the species.



The Socioeconomic area of the DIVERSIFY project has addressed some of the above points such as the need for further and deeper research on the different grow out culture systems, the definition and establishment of the business model and the design of a market strategy for the species.

**Deviations:** There were no deviations from the DOW.



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