Deliverable Report

Deliverable No:	D31.10		**Delivery Month:**	24
Deliverable Title	Presentations of DIVERSIFY (Y2) at the Aqua Europe meetings (Diversification Sessions) by the Species leaders			
WP No:	31		**WP Lead beneficiary:**	P18. CTAQUA
WP Title:	Dissemination			
Task No:	31.3		**Task Lead beneficiary:**	P1. HCMR
Task Title:	Presentation of DIVERSIFY at the AQUACULTURE EUROPE meetings			
Other beneficiaries:	P3. IRTA	P4. IOLR	P7. IMR	P8. IEO
P9. UL	P18. CTAQUA			
Status:	**Delivered**		**Expected month:**	21
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Other partners participating: Papandroulakis, N. (HCMR), Estevez, A. (P3. IRTA), Koven, W. (P4. IOLR), Norberg, B. (P7. IMR), Peleteiro, J.B. (P8. IEO), Fontaine, P. (P9. UL) and Robles, R. (CTAQUA)

Objective: To make a summary presentation at the European Aquaculture Society's (EAS) annual meeting, presenting the major achievements of DIVERSIFY for each of the included six species.

Description: A Special Session was organized at the AQUACULTURE EUROPE 2015 conference held between 20-23 October 2015 at Rotterdam, The Netherlands (**Fig. 1**), focusing on the work carried out in the last 2 years in the DIVERSIFY project (**Fig. 2, left**). The session was chaired by the Project Coordinator (PC) of DIVERSIFY (Dr. Constantinos C. Mylonas) and the WP31 Dissemination leader (Dr. Rocio Robles). As committed in the DOW, summary presentations (20 min) were given by each of the six Species Leaders (SL) of DIVERSIFY including the meagre (*Argyrosomus regius*) and greater amberjack (*Seriola dumerili*) for warm-water marine cage culture, wreckfish (*Polyprion americanus*) for warm- and cool-water marine cage culture, Atlantic halibut (*Hippoglossus hippoglossus*) for marine cold-water culture, grey mullet (*Mugil cephalus*) a euryhaline herbivore for pond/extensive culture, and pikeperch (*Sander lucioperca*) for freshwater intensive culture using recirculating systems.

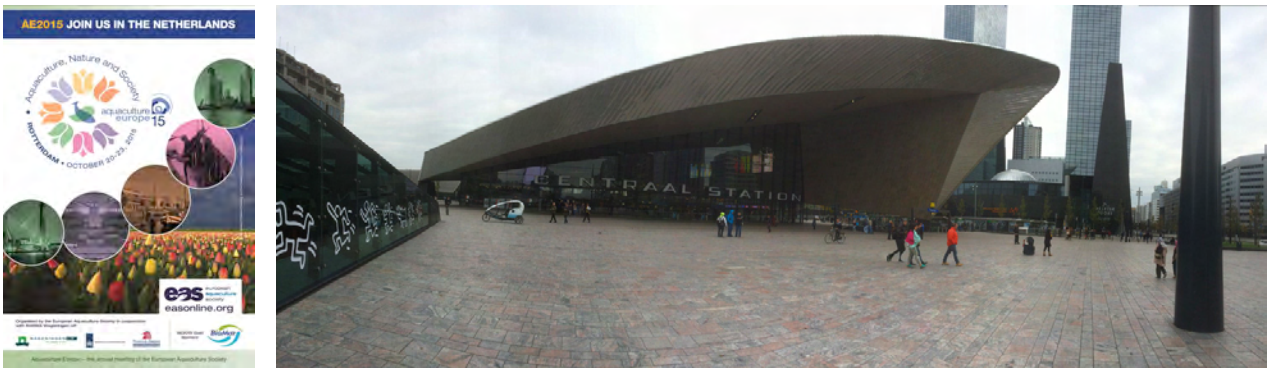

Figure 1. The announcement poster of the AQUACULTURE EUROPE 2015 conference that is organized every year by the European Aquaculture Society, and a panoramic view of Rotterdam, The Netherlands.

The Special Session was titled "New/emerging finfish species (EU Diversify project)" and was organized in the order of the species' work in the DOW. The session opened with a summary presentation for DIVERSIFY, given by the PC of the project -see *Deliverable 31.9 Annual presentation of DIVERSIFY (Y2) at a relevant conference.* Following each of the six Species Leaders summary presentations, presentations were also given by DIVERSIFY researchers on specific Tasks of the DOW. The Special Session lasted for the whole day (10:30 to 17:00) and an estimated of 30-120 persons were present at the different presentations in the designated room.

The first species summary that was presented was for meagre (**Fig. 2**). The presentation contained the results obtained during 2014 and 2015 in the scientific disciplines of Reproduction & Genetics, Nutrition, Larval Husbandry, Grow out Husbandry and Fish Health. There were two main achievements in **Reproduction & Genetics**. Firstly, a study has been completed on the description of the genetic variability of a large number of broodstocks from all the partners, as well as from a number of non-DIVERSIFY commercial operations, demonstrating that the available broodstocks in the Mediterranean region come from three main origins: Spain, France and the Aegean sea. The French lineage is represented in most commercial hatcheries throughout the Mediterranean Sea, as most of them obtained their breeders directly or indirectly from the French hatchery that commercialized meagre in the early 2000s. Secondly, it was shown that meagre can respond to repeated hormonal therapies for the induction of spawning, and a method was developed for the hormonal induction during 17 consecutive weeks of the same female and for the pair mating of specific females with weekly changes of males, in order to produce large numbers of families for the implementation of future breeding programs.

In the area of **Nutrition**, the requirements of n-3 PUFA, vitamin C and E in weaning diets were evaluated, whereas in the area **Larval husbandry** a protocol for early weaning at day 12 under intensive larval rearing conditions (50 larvae l^{-1}) and a survival rate of 5% was achieved. In the **Grow out husbandry**, the results of size variability (slow fish growers and fast fish growers with no compensatory growth after grading), as well as the distribution of fish in sea cages using echo integrator and the results of the use of light or air bubbling as stimuli for the conditioning of fish to anticipate feeding in juveniles were presented, expanding our knowledge of feeding behavior in the species.

Finally, in the area of **Fish health** the results of several trials carried out in 2015 to understand the relation of nutrition and Systemic Granulomatosis and the evolution of Chronic Erosive Dermatopathy were presented. The study of the immune system development in meagre juveniles using histological techniques (for the study of organ development) and molecular biology (to identify selected genes involved in immune response) were presented also in a poster during the same session.

Figure 2. Representative slides of the meagre (*Argyrosomus regius*) presentation at AQUACULTURE EUROPE 2015, presented by the Species leader Dr. Alicia Estevez (P3. IRTA).

The second species summary presented was for the greater amberjack (**Fig. 3**). In **Reproduction & Genetics**, a large number (ca. 140) of wild fish has been acquired, to establish 6 broodstock groups, (2 of them in cages). Progress has been achieved towards a spawning induction protocol providing large numbers of good quality eggs (>75% mean hatching). Maturation and egg collection was possible from stocks maintained in sea cages. Gametogenesis, however, in tanks seems to be problematic in the Mediterranean. In the East Atlantic, both spontaneous and induced spawning was achieved with high quality eggs. Sampling of wild and reared specimens in different gametogenesis phases started to describe the reproductive cycle and detect possible dysfunctions in captivity.

In **Nutrition**, the requirements during first feeding were studied. The enrichment products were improved. Five levels of DHA were tested for *Artemia* enrichment. The higher content (1-2% DHA) resulted in improved performance and minimum bone malformations while excess levels reduced growth. Different sources and levels of LC-PUFA rich lipids were tested for rotifer enrichment. The results indicated that enrichment using marine lecithin provided the best results compared to the lipid composition of wild fish eggs.

In **Larval and Grow out husbandry**, trials were performed to establish the appropriate methodologies for semi-intensive and intensive larval rearing. Experiments were performed to determine optimum larval stocking density and 50 eggs l^{-1} (compared to 25 and 75 eggs l^{-1}) resulted in larger larvae. The effect of light was studied and continuous photophase resulted in better performance than 18L:06D. Furthermore, light intensity and spectrum was studied using 3 background tank colors (white, green and black) without difference between the treatments. To define the feeding pattern of juveniles, an experiment comparing different frequencies (1,2,3,4 or 7 meals d^{-1}) was implemented and results showed that with 1 meal d^{-1} significantly lower specific growth rate (SGR) was observed, while feed intake (% bw) was significantly lower with 7 meals d^{-1}.

In **Fish health**, attempts have been made to isolate pathogens from cultured individuals. Gill parasites were identified (the monogeneans *Zeuxapta seriolae* and *Neobenedenia melleni* and the digenean *Paradeontacylix* sp) in the broodstock populations maintained in the cages and tanks, and in the juvenile population reared in sea cages in Greece. To isolate the aetiological agent of *Epitheliocystis* in larvae, specific rearing was attempted with maintaining larvae in surface seawater without any filtration at the facilities of HCMR (Greece). However, no disease was recorded during the larval rearing period and no related microbial agents were detected. Finally, primers have been designed to study the key immune genes that will be cloned in the course of the study.

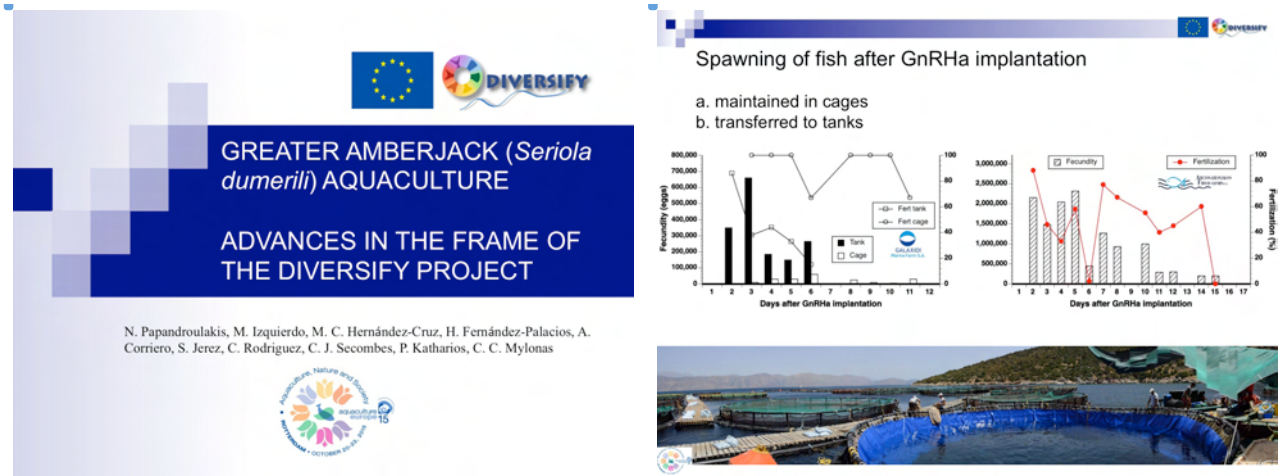

Figure 3. Representative slides of the greater amberjack (*Seriola dumerili*) presentation at AQUACULTURE EUROPE 2015, presented by the Species leader Dr. Nikos Papandroulakis (P1. HCMR).

The third species summary that was presented was for pikeperch (**Fig. 4**), with a synthesis of the main results obtained over the 2014-2015 period. After a short presentation of the main bottlenecks initially identified in collaboration with fish farmers at the stage of the proposal preparation, the species leader presented the four specific work packages (WP) of DIVERSIFY where work on pikeperch was implemented, followed by the major results obtained so far.

In the framework of WP4, **Reproduction and Genetics,** the genetic variability of wild (8) and captive (13) populations from Tunisia to Finland was characterized using a microsatelite multiplex (11 loci). Two genetically differentiated groups were identified (a northern group and a south-central European group), and the key position of the Hungarian population was questioned. It was also shown that the captive populations do not suffer from inbreeding. These new data will be available for use for future breeding programs, with the objective of maintaining a high variability in the domestic broodstocks.

Concerning WP10, **Nutrition**, and WP16, **Larval husbandry,** they focused on larval rearing, and the effects of environmental (light intensity, water current flow, water renewal rate and timing of tank cleaning) and nutritional factors (quantity of live preys, duration of the weaning period, feeding frequency, co-feeding) were specified. Several effects of simple factors or interactions between them were identified, and a first protocol was proposed to improve pikeperch larval rearing, in order to obtain better survival and growth rates, and to reduce cannibalism, which is a major problem in the culture of this species.

Finally, in **Grow out husbandry** a multifactorial experiment has been realized in order to study the effects of husbandry practices and environmental factors on pikeperch growth, physiological and immune status (WP22). The effects of various factors (size grading, fish density, light intensity and spectrum, photoperiod, temperature and diet) and their interactions were determined. As main results, two optimal combinations of factors associated to higher biomass gains in tanks were identified and will be applied on farm conditions in upcoming experiments in a complementary study (P29. ASIALOR, France). At that level, an important objective is to reduce the fish stress and mortality rate. Further processing of the multifactorial experiment results will provide more outcomes in the coming months.

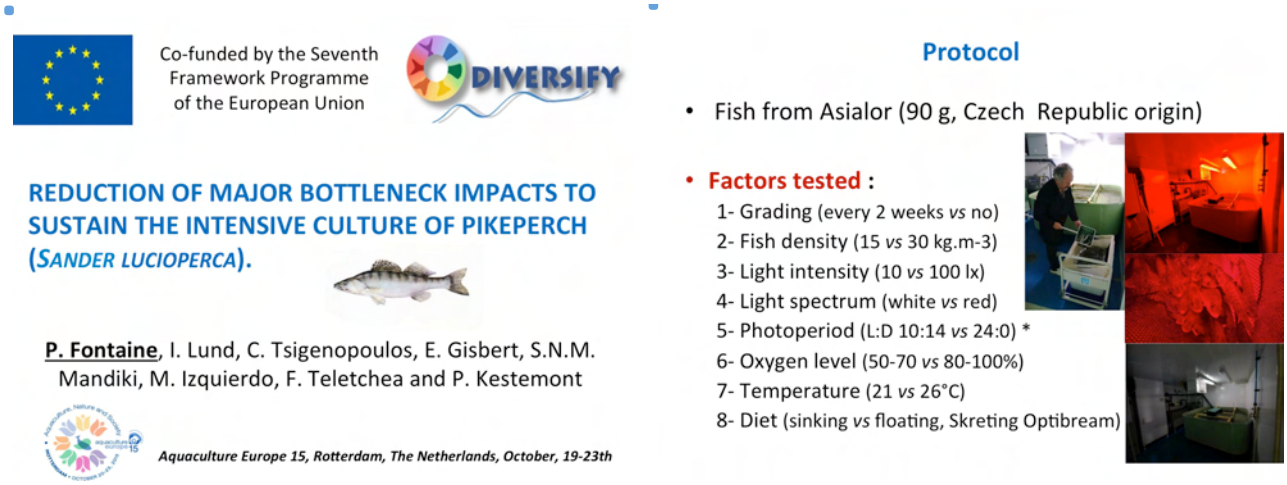

Figure 4. Representative slides of the pikeperch (*Sander lucioperca*) presentation at AQUACULTURE EUROPE 2015, presented by the Species leader Dr. Pascal Fontaine (P9. UL).

The fourth species summary that was presented was for the Atlantic halibut (**Fig. 5**) and included the main results of the work done in 2014-2015. After a general overview of the bottlenecks identified, results from WP 5 (**Reproduction & Genetics**), WP 11 (**Nutrition**), WP 17 (**Larval husbandry**) and WP 26 (**Fish health**) were discussed. In **Reproduction & Genetics**, pilot trials have been carried out on the implantation with gonadotropin releasing hormone agonist (GnRHa) implants to improve and facilitate egg production in F1/F2 broodstock. The results were not conclusive, but suggested that hormone therapy may be a useful tool

to increase egg production in Atlantic halibut broodstock, and synchronizing egg production within a shorted period of time. Documentation of spawning performance in wild-captured, and F1 females showed that F1 females have less regular spawning cycles with higher variability in egg quality as a result.

The work on **Nutrition** and **Larval husbandry** in 2105 has focused on development of a protocol for feeding on-grown *Artemia*, and analysis of the nutrient content of *Artemia* nauplii, on-grown *Artemia* and larvae fed either *Artemia* nauplii or on-grown *Artemia*. Nutrient composition of on-grown *Artemia* reflected the enrichment diet, while nutrient composition in Atlantic halibut larvae remained largely unaffected by the diet during the feeding period chosen. Further work needs to be done to conclude about the usefulness of this method for this species.

In the area of **Fish health**, the virulence of a number of *Vibrio* strains have been tested in challenge experiments in the presence of probiotic candidates. As a first step towards developing a vaccine for these bacteria, expression systems for a capsid protein present on the VNN virus have been developed in bacteria (*E. coli*), a protozoan (*L. tarantolae*) and in tobacco plant (*N. tabacum)*. All systems expressed the capsid, but the protozoan and plant systems need to be optimized. In summary, the work on Atlantic halibut is progressing according to plan and it is expected to deliver all results as planned.

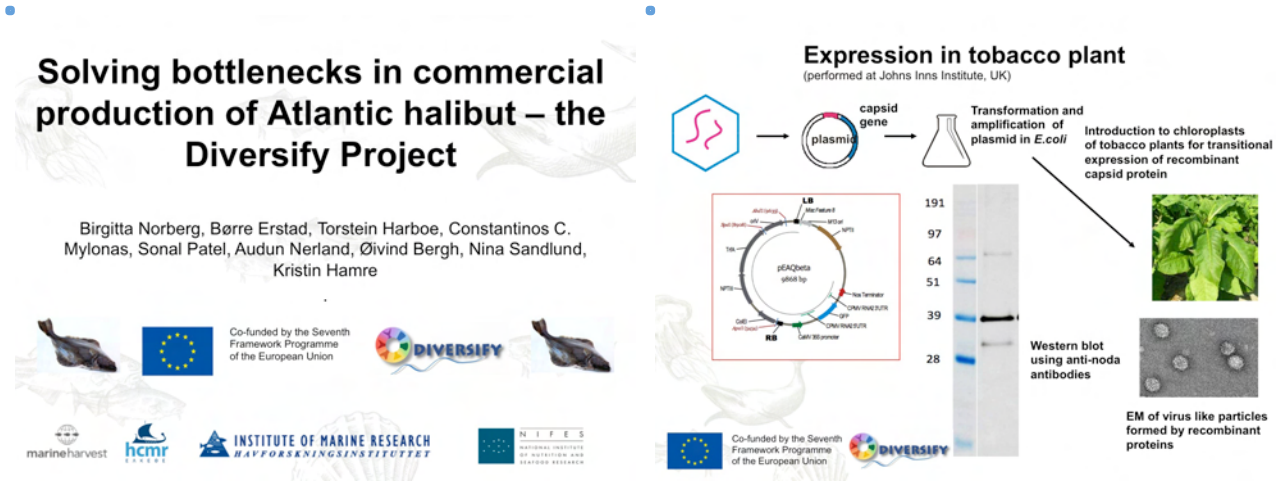

Figure 5. Representative slides of the Atlantic halibut (*Hippoglossus hippoglossus*) presentation at AQUACULTURE EUROPE 2015, presented by the Species leader Dr. Birgitta Norberg (P7. IMR).

The fifth species summary that was presented was for the wreckfish (**Fig. 6**), where results from the research carried out in 2014-2015 were presented. A total of nine partners from the DIVERSIFY consortium have participated in various scientific disciplines, covering WPs on **Reproduction and Genetics** (WP 6), **Nutrition** (WP 12) and **Larval husbandry** (WP 18).

Concerning **Reproduction & Genetics** (WP 6), the most relevant results presented were on the description of the reproductive cycle, where both wild-caught animals from the Azores Islands' fisheries and breeders in captivity were studied. Samples from the flesh (muscle), viscera, fins (from wild caught specimens landed in the Vigo Spanish fish market) and morphometric measurements were taken (from all groups). Also blood, sperm and oocytes were taken from the broodstock in captivity. This delivered important information on biochemical composition, reproductive cycle and several habits regarding this species. Laboratory analysis regarding blood steroids and the relation of levels with gonad histology (ovary and testis) development and maturation are being studied.

Spontaneous spawning was obtained in captivity, producing viable eggs and larvae, which were cultured up to 20 days post-hatching. Hormonal induction (with GnRHa) was also performed, with good results in some cases. Wreckfish sperm characterization was completed, determining biochemical composition, density,

motility characteristics and duration using a Computer Assisted Sperm Analysis system, validated for wreckfish sperm. Sperm cryopreservation was tested, in order to establish an *in vitro* fertilization protocol, as it seems that in most situations wreckfish females undergo maturation and ovulation, but they fail to have reliable spawning in captivity, with poor egg quality.

According to the results, it appears that sperm production/quality is not a bottleneck as males produce plentiful amounts of sperm for a very prolonged period on time. Therefore, it was considered to evaluate the influence of broodstock diet on egg quality. A specific broodstock diet was designed, based on information obtained from the biochemical composition of wild caught specimens. A commercial company produced the diet. The feed is now available for any wreckfish farmer and will be used in the coming years.

Figure 6. Representative slides of the wreckfish (*Polyprion americanus*) presentation at AQUACULTURE EUROPE 2015, presented by the Species leader Dr. Jose Benito (Tito) Peleteiro (P8. IEO).

The sixth species summary that was presented was for the grey mullet (**Fig. 7**). Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of grey mullet sperm was one of the first achievements in the area of **Reproduction & Genetics**. The preliminary results indicate that the major bio-technical settings, such as dilution of sperm, quality of activation and mastering of video recordings have been determined and can now be applied to experimental protocols. The current results will help improve assessment of the effect of different treatments on reproductive performances of grey mullet males.

In the area of **Nutrition**, the effect of dietary taurine on the performance of grey mullet larvae and juveniles was investigated in relation to developmental stage and the shift of grey mullet larvae from carnivory to herbivory. In the first experiment three taurine levels (0, 400, 600mg l^{-1} medium) were used to enrich rotifers and/or *Artemia* nauplii, which were fed to 2-12 and 13-19 dph grey mullet larvae, respectively. All fish were weaned onto a starter diet from 20-44dph. The dry weight of 12 dph larvae fed the highest taurine enriched rotifers (600 mg l^{-1}) grew significantly ($P<0.05$) better than larvae consuming the low and medium taurine enriched live food (0 and 400 mg l^{-1}). The high taurine larvae were still markedly ($P<0.05$) larger than the control at 19 dph, despite the feeding of all fish with non-taurine enriched *Artemia* nauplii for 5 days. In fact, at 44 dph the taurine treatment fish (32 days after the rotifer treatments) continued to be significantly ($P<0.05$) larger than the non-taurine control suggesting the lasting effect on growth of dietary taurine fed during early larval development. Moreover, taurine enrichment of *Artemia* alone or together with the rotifers did not demonstrate a clear growth advantage over the only rotifer taurine enrichments. Nevertheless, better ($P<0.05$) larval survival was achieved when both rotifer and *Artemia* were enriched with the highest level of taurine (600 mg taurine l^{-1}).

In another study, juvenile grey mullet (ca 5.5 g) were fed prepared diets that were identical in lipid, protein and micronutrient composition, but differed in their taurine levels (0, 0.5, 1, 2% DW diet) for a period of 60 days. Dietary taurine supplementation in juvenile mullet continued to give a growth advantage that can be expressed as 2% > 1% > 0.5% >0% suggesting that the fish cannot synthesize taurine at sufficient levels after the mode of feeding shifts, and that juveniles are likely omnivorous in order to ingest adequate taurine levels in nature.

In the area of **Larval husbandry**, the effect of "greening" larval rearing tanks (from 2-30 dph) with one of two different algal species (*Nannochloropsis oculata* or *Isochrysis galbana*) at different turbidities and its consequent effect on prey capture was investigated. As *Nannochloropsis oculata* and *Isochrysis galbana* cells differ in size, each turbidity level (measured twice daily) meant different algal concentrations were used. Although no algal species or turbidity level affected significantly rotifer consumption, linear regressions between turbidity and tank biomass was significant, with *Isochrysis galbana,* but not quite with *Nannochloropsis oculata*, while when turbidity was regressed with survival both *Nannochloropsis* and *Isochrysis* were highly significant. This study concluded that algal turbidity and not algal type was the dominating factor affecting mullet larval performance from 2-30 dph.

In the area of **Grow out husbandry**, a large multi-partner (Israel, Greece and Spain) 1 year study is currently underway evaluating the performance of an improved mullet grow-out diet, in monoculture as a function of stocking density and pond type. The IOLR and the SME DOR (Israel) are feeding the grow-out diet to F1 juveniles stocked at different densities in cement (30 m^2; 4 and 6 juveniles m^{2-1}) and earthen ponds (6000 m^2, 0.5 and 1 juvenile m^{2-1}), respectively. Partner GEITONAS (Greece) will similarly test this diet on wild caught juveniles at the same densities in 6 cement ponds (20 m^2), while Partner CTAQUA (Spain) will evaluate the diet for wild caught juveniles at the earthen pond densities (2 ponds at 1100 m^2 each). The diet will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival.

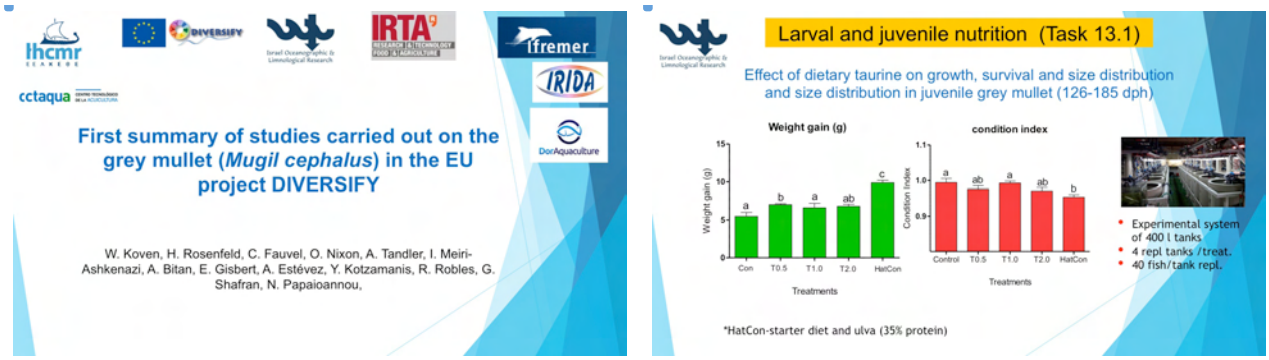

Figure 7. Representative slides of the grey mullet (*Mugil cephalus*) presentation at AQUACULTURE EUROPE 2015, presented by the Species leader Dr. William (Bill) Koven (P4. IOLR).

The last part of the DIVERSIFY special session was devoted to the presentation of some work done within the Socioeconomic WPs (**Fig. 8**). The first presentation was about the importance of innovation for consumers and for the market. The WP 28-New product development, was addressed with the presentation of Dr. Athanasios Kristallis, and was titled "The time is right for fish production innovation: an exploration of European consumer attitudes towards sustainable new fish product ideas". The result of this WP has been the elaboration of a catalogue of 41 ideas for new product development, applicable to the DIVERSIFY species.

The second presentation remarked on the importance of consumer perception towards new products. The work done during 2015 within WP 29 - Consumer value perceptions and behavioral change was presented by Dr. Machiel Reinders. The presentation was titled "Customer value perceptions towards new farmed fish:

European consumer segmentation" and concluded that the consumer segmentation study yielded three groups of consumers: 1) involved traditional, 2) involved innovators and 3) ambiguous indifferent. This study will allow the DIVERSIFY project to target specific market segments and to set the stage for the development of fish products based on new/emerging species for the expansion of the European aquaculture industry.

Figure 8. Dr. Athanasios Kristallis (P11. Aarhus University) and Dr. Machiel Reinders (P6. LEI/DLO) during their presentations at the DIVERSIFY special session.

Overall, this Special session demonstrated that significant progress has been achieved in the study of new/emerging species for the EU aquaculture industry. The six Species Leaders from the DIVERSIFY project (**Fig. 8, right**) have contributed immensely on the success of this Special Session, by presenting all the work relevant to the diversification of the European aquaculture industry.

Figure 8. The Aquaculture Europe 2015 program page showing the Special Session (left) and the six Species Leaders (right). From left to right, Papandroulakis, N. (greater amberjack, P1. HCMR), Estevez, A. (meagre, P3. IRTA), Norberg, B. (Atlantic halibut, P7. IMR), Koven, W. (grey mullet, P4. IOLR), Fontaine, P. (pikeperch, P9. UL) and Peleteiro, J.B. (wreckfish, P8. IEO).

The knowledge acquired so far, and expected to be accumulating in the upcoming years, will allow the incorporation of the new species in the commercial production of the European aquaculture industry, with the objective of increasing their annual production with the inclusion of new species that offer significant biological (faster growth and better FCR) and market advantages (flesh quality, consumer acceptance and world-wide distribution).

Deviations: The deliverable is submitted 3 months later that anticipated in the DOW, but within the month that the Species Leaders' presentations were given at the Aquaculture Europe conference.

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