

Presentation of DIVERSIFY at the Regional Aquaculture Conference, Italy 2014. An international



conference organized by the Italian Presidency of the Council of the EU, the General Fisheries Commission for the Mediterranean, the European Commission and the Italian Ministry of Agriculture took place in Bari (Italy) on December 9-11, 2014. The objective of the conference was to review the progress made by aquaculture in the Mediterranean region and to address the main governance, economic, social and environmental challenges for the sustainable development of the industry. Professor Aldo Corriero (University of Bari) accompanied by Mr. Javier Ojeda (APROMAR, Spain) gave a presentation to the plenary detailing the objectives and activities of the DIVERSIFY project. The presentations attracted great

attention and interest from the audience. More detailed information about the project was available in a DIVERSIFY booth at the conference's exposition area during the 3-day conference.

DIVERSIFY's Annual Coordination Meeting. The 2014

Coordination Meeting took place on November 4-6, at the Palazzo Ateneo of the University of Bari Aldo Moro, Bari, Italy and was hosted by Dr. Aldo Corriero. The meeting was attended by 73 persons, including 7 invited guests from outside the consortium. The Agenda consisted of a common session for all participants during the first day presenting highlights from the six Scientific Disciplines (Reproduction & Genetics, Larval and Grow out husbandry, Nutrition, Fish Health and Socioeconomics, including presentations from the invited guests. Then, six workshop sessions running in parallel were held during the second day addressing the different scientific disciplines. Finally, a third day included a common session dealing with dissemination, Scientific and Financial Reporting and a meeting of the Steering Committee. The presentations from the meeting are available at the project's web site (www.diversifyfish.eu), in the INTRA/Meetings & Activities page.



Spawning induction of greater amberjack (*Seriola dumerili*) and collection of eggs from sea cages. The first spawning induction trials with the Mediterranean stocks were carried out in June 2014, at the Souda Bay sea-cage facilities of the Hellenic Center for Marine Research.

Eleven mature greater amberjack breeders were maintained in a 40-m perimeter, 6-meter deep cage. Part of the stock was maintained for several years, and another part was transferred to the cage location at the beginning of April. All fish were examined for stage of maturation using gonadal biopsy. Upon verification of the maturation stage, breeders were treated with GnRHa controlled-release implants, to induce maturation, ovulation and spawning. Seven males were in spermiation, though no sperm could be collected by abdominal pressure, due to the thick musculature of the abdominal cavity. Sperm samples were collected with a catheter and evaluated under the microscope, exhibiting 40-100% motility upon activation. Three females were in post vitellogenesis, with oocytes exhibiting the early stages of oocyte maturation. A passive egg collector was placed in the perimeter of the cages, and during the following days the cage was monitored for spawning.



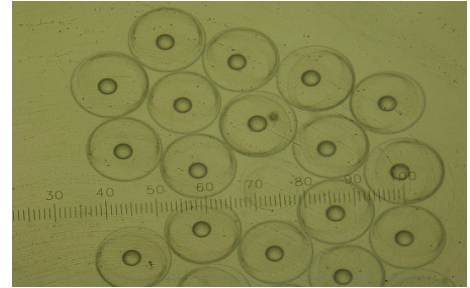
A total of 500,000 eggs (~70% fertilization success) were collected, packed and shipped to the AQUALABS facilities of HCMR. Eggs



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were stocked in tanks in order to begin the implementation of the Work Packages related to Larval Husbandry and Fish Health. During the same time period, eggs were also collected from a collaborating commercial facility in Greece (Galaxidi Marine Farms, SA), from a cage containing a total of 23 mature greater amberjack (11 females and 12 males). The fish were also induced to mature using GnRHa implants. A small quantity of eggs (15,000, ~99% fertilization success) was obtained the first day. Another 400,000 eggs (~95% fertilization success) were also collected the next day. Larger numbers of eggs (~1 million) were obtained from broodstock that were induced to spawn with the same GnRHa implants, but were placed in land-based tanks for spawning.



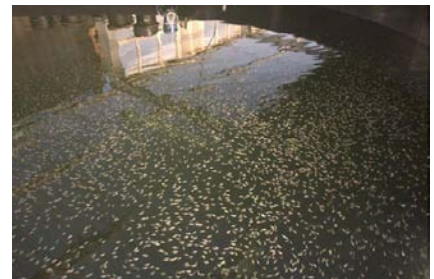
Transfer of greater amberjack fingerlings from the AQUALABS to the Agios Kosmas facilities of HCMR for nutrition studies.

After the successful spawning induction and larval rearing of greater amberjack that was performed at the facilities of HCMR (Greece) in Crete (in collaboration with the Greek national Programme "KRIPIS"), a group of 10-g fingerlings were transferred in September 2014, to the HCMR facilities of Agios Kosmas, Athens, in order to begin the experiments on the nutritional requirements of the species.



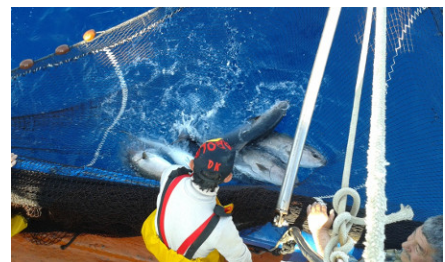
Reproduction control of the Atlantic stock of greater amberjack.

The Grupo de Investigación en Acuicultura of the University of Las Palmas de Gran Canaria (ULPGC), Spain, succeeded in the spawning of greater amberjack, both spontaneously and induced with GnRHa implants or injections, in their facilities of Parque Científico Tecnológico Marino (FCPCT). Twenty-two individuals were kept in three 40-m³ tanks under natural photoperiod, salinity and temperature. In May 2014 the broodstock were biopsied and 12 of them classified as males and 10 as females (average weight 10.7 kg). All females had oocytes bigger than 650 µm. Spontaneous spawning began on June 1st and ended in October 18th, with 19 spawns producing an average of 1.15 million eggs per spawn, with 83% fertilization success. Between June and November, 29 inductions by GnRHa injections were done, obtaining a total of 22 spawns producing an average of 0.5 million eggs per spawn, with 56% fertilization. Fish implanted with GnRHa (12 treatments) spawned 36 times, producing an average of 0,25 million eggs per spawn, with 29% fertilization success. The obtained eggs were used for the implementation of the Larval Husbandry experiments. The results confirm that spontaneous spawning is more effective (higher fecundity and egg quality), but if needed, GnRHa therapies can also be employed successfully for the production of viable eggs.



Sampling campaign of wild greater amberjack to describe their reproductive cycle.

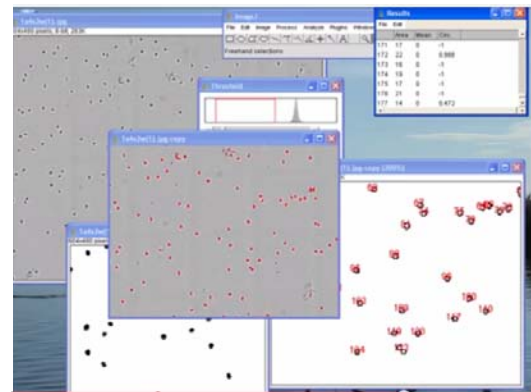
Wild adult greater amberjack caught in Lampedusa (Italy) from a fishing vessel were sampled in May 2014 by the University of Bari (UNIBA, Italy) for blood, brains, pituitaries, gonads, muscle, liver, otoliths and vertebrae (for aging). The sampling continued during June-July and will be completed during 2015. The collected samples will be used for a detailed study of the reproductive cycle. Proliferation and apoptosis of germ cells during spermatogenesis will be examined. Sperm characteristics will be analysed using a Computer Assisted Sperm Analysis (CASA) system. A comparison of liver vitellogenin (Vg) and ovarian Vg receptor gene expression between captive and wild females will be assessed; an analysis of oocyte



yolk accumulation will also be performed. Measurement of sex steroid hormones will be done using ELISAs and the expression as well as the plasma protein levels of the two gonadotropins Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) will be measured. An ELISA for measuring leptin, a key metabolic hormone involved in puberty, will be developed to provide critical information about nutritional status. The nutritional status of wild and captive-reared fish will be compared. Using this information the reproductive activity of this species will be compared with that of reared individuals in order to identify possible dysfunctions occurring in captivity.

Assessment of sperm characteristics of DIVERSIFY species. During spring 2014, several sperm samplings were organized by Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER, France), in order to qualify and perform first trials for successful transport and storage of wreckfish (*Polyprion americanus*) sperm. Samples for sperm quality assessment were taken from 6 wreckfish males from the "A Coruña" Aquarium (MC2, Spain), 2 males from Luso Hispanica Aquicultura and 2 males from the Vigo facilities of Instituto Español de Oceanografía (IEO, Spain). The mean concentration of wreckfish sperm was 250 billion of spermatozoa ml^{-1} . Sperm became immotile after 24 h of raw storage, but exhibited motility if diluted at the time of collection in different storage media, although lower speed and motility duration were recorded compared to fresh sperm. It was concluded that conditions of sperm storage and transportation must be improved, if male and female gamete collection must occur in separate locations or different times.

Concerning meagre (*Argyrosomus regius*), the company Les Poissons Du Soleil (France) offered the opportunity to collect sperm from their broodstock to obtain analytical data and to train personnel from Israel Oceanographic and Limnological Research (IOLR, Israel) on the use of CASA, in order to prepare for the characterization of grey mullet (*Mugil cephalus*) sperm during the next season using the same tools as those developed for wreckfish, meagre and later, greater amberjack. The mean concentration of semen of fish at the peak of the reproductive season was 45 billion spermatozoa ml^{-1} whereas a significant decrease was observed for those at the end of season (males from shifted broodstock) showing only 30 billion spermatozoa ml^{-1} . The initial velocity was $130 \mu\text{m s}^{-1}$, decreasing rapidly to 0 after 50 s. As a preliminary conclusion, meagre sperm behaves roughly as European sea bass (*Dicentrarchus labrax*) sperm. Moreover, diluents formulated for chilled storage of European sea bass sperm were very efficient in sustaining meagre sperm motility for at least 24 h. These preliminary results are very promising for the development of artificial fertilization in meagre, which is planned for the following years.



Influence of wreckfish broodstock feeding regimes for fecundity and spawn quality. A total of 60 fish from the Azores fishing area, were acquired between February and October 2014 in two different markets of Galicia, Spain. Some biometric parameters were measured and samples from the muscle, liver and gonads were collected and analyzed to know the nutritional status of the wild fish, at the Instituto Oceanográfico de Vigo (IEO, Spain). Total weight varied between 3.6 and 18 kg and total length between 56 and 98 cm. No relation was found between weight, sex and peri-visceral fat. Analysis of protein and lipid content in muscle showed values between 74-96% and 3-14% respectively. A high variability was observed in liver and gonads: 19-69% in protein in liver and 10-80% in gonad, while lipid content varied between 15-73% in liver and 9-90% in gonad. In order to assess the influence of the broodstock feed composition on the reproductive development of wreckfish, samples of a semi-moisture diet with a mixture of white fish, oily fish, mussel, squid and fishmeal were analyzed. The effect on the reproductive development of three feeding regimes: semi-moisture diet, commercial dry feed and a new dry feed formulated specifically for wreckfish will be compared in 2015.





Pikeperch (*Sander lucioperca*) grow out husbandry.

In order to identify the main stress factors that may affect the growth performance, physiological and immune status of pikeperch juveniles, two preliminary experiments were started at the University of Lorraine (UL, France) and the University of Namur (FUNDP, Belgium). Scientists have tested the sensitivity of pikeperch juveniles to a single or repeated stress consisting of a complete removal of tank water plus 30 s emersion in air. Such manipulations mimic the grading that is often made along the on-growing phase of pikeperch in farm production. Results concerning the growth response to the emersion stress and some physiological markers are available, but the analyses of immune markers are still ongoing. A second preliminary test is running since November 2014, in order to determine the lethal dose of *Aeromonas hydrophila* or *A. salmonicida*. Results from these experiments will be useful to standardize some analytical protocols for physiological and immune markers for the multifactorial experiment that will start in June 2015.

Development of breeding protocols for DIVERSIFY species. As demonstrated above, the project is on course to provide reliable, predictable spawning protocols and tools for the breeding programs of the studied species, in order to increase their potential for industrial production. Work with greater amberjack offered good quality spawns spontaneously in tanks and from GnRHa-induced spawnings in tanks and cages. The aims are to achieve similar results in all centres and improve egg collection in cages. Atlantic halibut (*Hippoglossus hippoglossus*) work demonstrated that F1 fish could be induced with GnRHa implants to spawn earlier and with improved fecundity. This work will be scaled up and validated with more breeders. Work with wreckfish resulted in spontaneous natural spawning in tanks, GnRHa induced in tanks and stripped gametes for *in vitro* fertilisation. However, few fertilised eggs were obtained. In 2015, work will focus on improving spontaneous spawning and developing reliable protocols for *in vitro* fertilisation. Grey mullet work increased both percentage of maturing fish and synchronised gonadal development using treatments of recombinant FSH and metoclopramide. Spawning was successfully induced with GnRHa and metoclopramide to provide millions of eggs and larvae. However, common problems that need to be addressed are failed ovulation (~42%) and high variation in fertilisation rates (0-90%). Work to describe the reproductive cycle of greater amberjack and wreckfish was initiated with the collection and processing of samples. In order to provide tools for genetic improvement, captive broodstock of both meagre and pikeperch were genetically characterised to prove that the majority of broodstock available to the industry had sufficient genetic variation to be used in breeding programs. Work with meagre also demonstrated that paired spawning (single male and female) was possible to produce known families for a breeding program.



DIVERSIFY project on Twitter. Fresh news about DIVERSIFY project are posted regularly on the website www.diversifyfish.eu and information about specific progress in all Scientific Disciplines is available in the "Research Area" section. Besides the website, from February 2014 the DIVERSIFY project is also on twitter www.twitter.com/diversifyfish, posting daily news about trends and topics in aquaculture and scientific articles with particular attention to the six species and the six Scientific Disciplines of main interest: Reproduction & Genetics, Nutrition, Larval and Grow out husbandry, Fish Health and Socioeconomics. The number of followers increased considerably from September 2014, rising from 18 to 63 in just three months.

