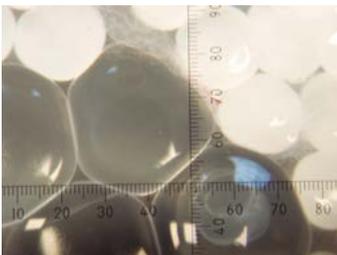


On 29-30 January 2014, the European Commission project DIVERSIFY (FP7-KBBE-2013, GA 603121) had its kickoff meeting at the Hellenic Center for Marine Research (HCMR) in Iraklion, Crete, Greece. The project is coordinated by Dr. Constantinos C Mylonas of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC). DIVERSIFY has a total budget of 11,8 million € for its 5 year duration and it is one of the largest research project in the area of aquaculture funded by the European Commission. DIVERSIFY's consortium includes twenty research and academic institutions, three Large Enterprises, nine Small and Medium Enterprises (SME), five Professional Associations and one consumer NGO.

The project DIVERSIFY (www.diversifyfish.eu) has identified a number of new/emerging finfish species, with a great potential for the expansion of the EU aquaculture industry. These new/emerging species are fast growing and/or large finfishes marketed at a large size and can be processed into a range of products to provide the consumer with both a greater diversity of fish species and new value-added products. The fish species to be studied include **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.

Reproduction and Genetics of Atlantic halibut

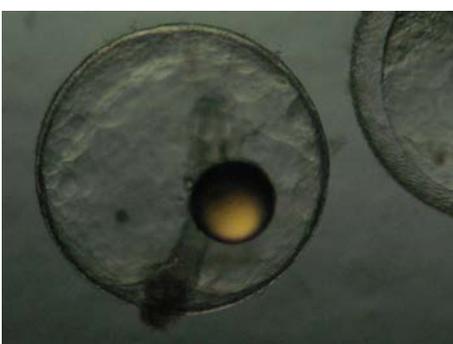
The first experiments on the induction of ovulation in hatchery-produced Atlantic halibut were initiated on 25 & 26 February 2014, at the Austevoll (Norway) facilities of the Institute of Marine Research, with the collaboration of the Hellenic Center for Marine Research (HCMR, Greece). A group of 12 females of an average weight of 15 kg were biopsied and selected to be at the same stage of oogenesis. Fish had completed vitellogenesis and some were in early oocyte maturation, with oocytes with a diameter of >2 mm. All males were in full spermiating condition. The females were allocated randomly to three groups and were treated with one of two doses of GnRH α in a controlled-release delivery system (EVAc) or were sham-implanted and used as controls. Four females were implanted with 50 μ g GnRH kg $^{-1}$, and four females were implanted with 100 μ g GnRH kg $^{-1}$. Control fish were sham-injected. All fish were checked regularly for ovulation, and three more biopsies were taken at one-week intervals.



All females implanted with GnRH α ovulated within two weeks of implantation. One control fish had entered final maturation before implantation, as seen by the occurrence of transparent, hydrating oocytes in the pre-treatment biopsy. This fish spawned spontaneously, while two of the other control fish released their first egg batches 3-4 weeks later than implanted fish. The fourth control fish did not mature during the experiment. Egg quality, measured as fertilization success, was generally low, and only a few batches with >50% fertilization were obtained. The ovarian samples taken by biopsy were split in two and either fixed in Bouin's fixative for histology, or frozen at -80°C for molecular biology. Egg samples were taken from all egg batches, for analysis of cortisol and testosterone content and ratio.

Reproduction and Genetics of wreckfish

Wreckfish broodstock housed in the tank "Nautilus" of the Finisterrae Aquarium (A Coruña, Spain) have been sampled to monitor the maturity state of males and females, and to obtain growth data from their weight and size. There are a total of 24 breeders weighing between 30 and 15 kg. By early May 2014, some of the females were conspicuously mature and the males were in spermiating condition. The specimens are tagged with an electronic chip and with a Floyd tag with a color code, allowing easy identification. By May 16 2014, spontaneous spawns begun to be obtained by this stock, but without any fertilization success. Water temperatures have been rising very slowly this year, contributing to a delay in the maturation and spawning of the



broodstock, in comparison with 2013 results.

Another stock of wreckfish maintained at the HCMR facilities (Crete, Greece), was monitored for maturation beginning on 20 April 2014. Based on the maturation condition of the males (full spermiation) and the female (late vitellogenesis), a spawning induction was attempted about a month later, on 12 May 2014. At this time the female contained oocytes already in oocyte maturation and had release some unfertilized eggs in the tank, in the morning prior to the scheduled induction. Spawning induction was attempted using GnRH α implants, and a pair of breeders was placed in a large (35 m 3) tank and allowed to spawn spontaneously. Spawning (58,000 eggs) was obtained a few days later on 19 May 2014, but fertilization was very poor. Some developing embryos were obtained 3 days after spawning, but no hatching was observed. The pair of breeders was examined the next day and the

female was found to contain some more oocytes in maturation, thus was induced again to spawn and placed in the large tank. No further spawning was obtained and at the final sampling on 17 June 2014, it was found to contain only post-ovulatory eggs (not released) and early vitellogenic oocytes. The results underline that wreckfish do undergo gametogenesis successfully in captivity, with males producing large quantities of good quality sperm, but oocyte maturation is unreliable and tank spawning does not result in a large number of fertilized eggs. It is not known at this stage whether the low fertilization success is due to a problem with the female or the male.

Transport of 30 greater amberjack breeders to HCMR, Crete, Greece.

On 10 April 2014, 31 greater amberjack breeders were transported to the facilities of HCMR in Crete. The fish ranged in body weight between 7.5 and 17.1 Kg and most of them are expected to be mature this coming reproductive season. Twenty-one of the fish were placed in tanks at the AQUALABS facilities, but 8 breeders were moved to the cage facilities of HCMR in Souda Bay, Crete. The breeders will be used for the experiments planned in WP 3 Reproduction & Genetics - greater amberjack, for developing a spawning induction protocol and a method for the collection of eggs from spawning breeders in sea cages. The fish were originally captured from the wild as juveniles (~500 g) by Asteras A.E. in Astakos, Greece and were reared in sea cages for 2 years. They were then transferred to the facilities of Galaxidi Marine Farms in September 2013, where they were "hosted" until space became available at the facilities of HCMR.



Beginning of greater amberjack reproduction experiments - verifying reproductive status of a wild-caught brood stock at ARGO, Greece

On 13 May 2014, the newly acquired stock of wild greater amberjack at the Argosaronikos A.E. facilities in Salamina Island, Greece was examined in order to evaluate its reproductive maturity and stage of reproductive development. The stock consists of 60 individuals and will be used to examine the feasibility of inducing spawning and collecting eggs from sea cages within the work planned for WP3 Reproduction & Genetics - greater amberjack. A total of 30 individuals were examined (biopsied and P.I.T. tagged) ranging in size between 7 and 14 kg. Sixteen fish were identified as females ranging from immature to fully vitellogenic fish and 14 fish were spermiating males. Sperm was collected via a canula, since the muscular nature of the abdominal cavity walls did not allow manual stripping. The sperm was examined under a microscope and was found to be of good motility. The stock will be examined again in the middle of June in order to induce spawning and attempt to collect eggs from the sea cages.



Progress with meagre reproduction experiments



The experiments to induce paired spawning of meagre were initiated in IRTA, Spain on 7 April 2014. Six pairs of breeders were selected based on oocyte diameter and presence of motile sperm. Each pair was induced with a single injection of GnRH α . The pairs of breeders were then placed into separate tanks and allowed to spawn. According to the work planned in WP 6 Reproduction & Genetics – meagre, the selected breeders were induced to spawn every 7-9 days. Before each spawning induction the maturity of the breeders was determined and the males were transferred to a different tank, in order to be cross-bred with a different female. To date, a total of 37 spawns have been collected that represent offspring from 19 different families. Egg quality has been variable, but generally good with fertilisation rates of 85 \pm 15% and many

spawns of over a million eggs. Many of the pairs of breeders have spawned successfully to demonstrate that paired spawning is an approach that must be considered for the production of families for a genetic selection program. The larvae produced are being used for experiments in WP 14 Larval Husbandry & WP 20 Grow Out Husbandry for meagre.

Next DIVERSIFY Annual Coordination Meeting.

The next Annual Coordination Meeting for the DIVERSIFY project will take place on November 4-6 of 2014 in Bari (Italy). Its first day will be open to a number of scientists from outside the DIVERSIFY consortium, based on an invitation or application. The invited/accepted persons must be involved actively in the fish species and areas studied in DIVERSIFY, and will be required to make a short presentation of their experiences/projects. Please, contact the coordinator if you are interested in attending this interesting meeting.

... read about these news and more in <http://www.diversifyfish.eu/news.html>