"DIVERSIFY": EXPLORING THE BIOLOGICAL AND SOCIO-ECONOMIC POTENTIAL OF NEW/EMERGING CANDIDATE SPECIES FOR THE EXPANSION OF THE EUROPEAN AQUACULTURE INDUSTRY

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Introduction

The project DIVERSIFY (FP7-KBBE-2013, GA 603121) is an EU funded 5-year project (2013-2018) with the objective of acquiring the necessary knowledge for the diversification of the European Aquaculture production based on new/emerging finfish species. DIVERSIFY's consortium includes twenty research and academic institutions, two Large Enterprises, nine Small and Medium Enterprises (SME), five Professional Associations and one consumer NGO. Six new/emerging finfish species, with great potential for the expansion of the EU aquaculture industry have been identified by the project (**www.diversifyfish.eu**). These 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. Research has been carried out in six scientific disciplines, including Reproduction & Genetics, Nutrition, Larval and Grow out husbandry, Fish health and Socioeconomics including Final product quality. The combination of biological, technological and socioeconomic research planned in DIVERSIFY is expected to support the diversification of the aquaculture industry and help in expanding production, increasing aquaculture products and development of new markets.

In the area of **Reproduction & Genetics**, the work done for meagre has provided a protocol for the paired spawning with male rotation, that was 76% effective and resulted in the production of a total of 61 families from 10 males and 10 females. In addition, *in vitro* fertilization methods were developed. In greater amberjack, a successful broodstock management protocol was developed. The protocol involves maintaining the fish in the sea cages during the year and then transferring them to land-based tanks for spawning after GnRHa implant treatment. More than 50 million eggs of good qualtiy have been produced from two broodstocks kept in two different locations, allowing the production of ~300,000 juveniles, which were stocked in various research and commercial facilities around Greece. These production numbers are more than has ever been produced before, and now we actually have the capacity to run industrial trials for this species.

In the area of **Nutrition**, meagre larval feeds were optimized in regards to the level of n-3 HUFA, and vitamin E and C content. Weaning diets for meagre must be optimized by increasing HUFA levels up to 3% and vitamins E and C at 1500 and 1800mg kg^{-1}, respectively, in order to spare these essential fatty acids from oxidation. Meagre seemed to be very sensitive to hypervitaminosis D and A, since supplementation with these vitamins lead to a growth reduction. On the contrary, taurine supplementation did not have any effect in meagre larvae performance. In pikeperch, six experimental diets with similar levels of protein and lipid, and increasing levels of phospholipids, EPA and DHA were examined for larvae. The combination of high phospholipid content and high DHA content improved larval growth. Digestive enzyme activity was enhanced by dietary inclusion of PL and LC PUFAs, and trials have shown that pikeperch larvae require both

high dietary inclusion levels of phospholipids in terms of soya lecithin and LC PUFAs to perform optimally.
In the area of **Larval husbandry**, a great success was achieved with greater amberjack in 2016. The results are significant because the very high survival rates achieved are reported for the first time in greater amberjack, indicating a significant technological step in the larval rearing of this species, which will enable its commercial production. In particular, the results from the trials with the modified tank "light environment" are one order of magnitude better than previous reported studies, showing the validity of the tested hypothesis. On the contrary, larval experiments with wreckfish were limited by few and poor quality spawns, which were likely due to an unsuitable diet coupled with unusual environmental conditions. Fertilization varied between 62-97% and hatching rate between 4-56%, while no facility succeeded in growing larvae past 28 dph. Larvae exhibited a syndrome resembling swollen yolk sac, which has been related to poor broodstock nutrition.

In the area of **Grow out husbandry** behaviour profiles of meagre reared in sea cages demonstrated a significant difference between day and night (fish close to the bottom during the day and dispersed throughout the water column at night). Evidence of feeding during the night will be explored to develop feeding methodologies. We also tested the potential of different stimuli (mechanical, optical etc) to be used to entrain the fish to feeding times with automatic feeders. The study demonstrated that (a) meagre is able to learn, to be trained and to remember specific stimuli associated with feeding time, (b) light is an acute stimulus to which the fish respond very quickly (from the second day of its application) and (c) environmental conditions, particularly light intensity, affect meagre feeding behaviour.

In the area of **Fish health**, a study of Systemic Granulomatosis (SG) of meagre showed that vitamin D did not affect the development of SG. Histological assessment of all fish gave new insights into the development of the disease including the possible implication of rod let cells and the unique inflammatory response of the fish. In addition we have seen that both high inclusions of phosphorus and astaxanthin have beneficial effects concerning the severity of SG. In the study of Chronic Ulcerative Dermatopathy (CUD), results confirm the hypothesis that the disease is related to the use of borehole water. In greater amberjack, the work done has included a) morphological studies on the incidence of monogenean parasites in greater amberjack skin, b) determination of environmental conditions that can modulate greater amberjack resistance to parasitic infection, c) formulation of a diet supplemented with mucus stimulation products, and d) standardization of monogenean cultures. In Atlantic halibut, we have managed to express the nodavirus capsid protein and we achieved sufficient and high expression for further use of the protein as antigen for vaccination purposes.

Besides the technical improvement of the selected species, the **Socio-economic** research in DIVERSIFY includes solutions on consumer perception of aquaculture products, market demand, buyer preferences, new product development, value adding and market development. These outcomes will help the EU aquaculture sector and the supply industry in targeted marketing and improvement of its international competitive position. The image of the aquaculture sector has to be improved and new and high added value products have to be developed and SME's have to be more innovative to introduce and develop the market for these new species.

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