

For the third issue of **DIVERSIFY's** newsletter, we have summarized the work carried out during the **first 18 months** of the project. An overview per each DIVERSIFY species is presented here.

ADVANCES IN MEAGRE (*Argyrosomus regius*) RESEARCH



The variety of research activities undertaken with meagre in the first year of DIVERSIFY have been published as a featured article in the [March issue of Aquaculture Europe magazine](#). The article includes a summary of the most relevant results (you can read the full article at www.diversifyfish.eu). Below you can find the highlights of the article.

Reproduction & Genetics: To provide tools for genetic improvement, captive meagre broodstocks coming from 13 research institutions and SMEs from 7 different EU countries were genetically characterized. Results from the evaluation showed an adequate genetic variation to form a base population for a breeding programme with selected families. Besides, protocols for paired hormone-induced spawning have been developed and implemented. Differences in egg production and quality not related to maturity status or repeated spawnings or inductions were observed.

Larval culture: A study to determine the earliest and most cost effective weaning period was carried out during 2014. Larvae were cultured intensively with a standard technique, and live prey was enriched using a commercial diet. Results showed that larvae that had been offered initially a half *Artemia* brine shrimp ration and weaned at 15 days post hatching (dph) onto a dry feed, were not significantly different than controls in terms of growth, whereas survival in all treatments was also not markedly different. However, high cannibalism rates were observed related to early weaning and, therefore, a new trial started at the beginning of 2015.

Nutrition: Results revealed that meagre has a high requirement for Highly Unsaturated Fatty Acids (HUFA) to promote growth, and vitamins E and C to prevent fatty acid oxidation, therefore, indicating a need to enrich commercial weaning diets with these compounds. The specific nutritional requirements of meagre were collected and a basal diet formulation has been developed.

On-growing: Grow out husbandry trials of meagre juveniles were designed with the objective to study and improve growth dynamics. Improved feeding methods and environment-related rearing conditions (depth and light conditions) were tested over several months. The monitoring of vertical distribution of the fish showed that they are usually located in the lower half of the cage during the daylight period while they show a homogeneous distribution in the whole cage volume during the dark period of the day. Besides, it seems there might be a correlation between the depth of the cage net during the on-growing stage and the behaviour of the fish, however no association with growth rates have been established so far.

Health: The study to characterize the ontogeny of the immune response in meagre is being carried out. Several tissues were collected during the larval development and on-growing of meagre, and genes for the characterization of the immune system of meagre are being identified and gene expression assays have been validated. Systemic Granulomatosis has been identified as a major pathological condition for meagre. Since its development is considered to be related to nutritional factors, feeding trials using different levels of vitamin D were evaluated to study the development of the disease in juvenile fish. The initial assessment of the results of a 3-month trial indicated that vitamin D did not affect the development of granulomas at any of the dose.

ADVANCES IN GREATER AMBERJACK (*Seriola dumerili*) RESEARCH



Reproduction & Genetics: A total of six greater amberjack broodstocks were established and spawning induction protocols have been developed. Trials have provided a large number of high quality eggs (>75% mean hatching) from both spontaneous (natural) spawning in tanks and GnRHa-induced spawning in tanks and

cages, and have shown it is possible to induce maturation and collect fertilized high quality eggs from stocks in sea cages. Finally induced spawns from groups formed with F1 females and males were also obtained in the Eastern Atlantic stock, but eggs have not been fertilized.

Larval culture: Trials to establish the best larval rearing methodologies in two rearing systems have started. With this aim, DIVERSIFY tested and compared semi-intensive mesocosm and intensive RAS schemes, using eggs from induced spawning and from different breeders. On day 30, about 950 individuals from semi-intensive mesocosm

larval rearing tests and 2,500 individual from intensive rearing experiments in RAS were transferred for pre-growing. Results were used to establish the methodology for greater amberjack larval rearing. In addition, the effect of stocking density on larval performance was studied and three different larval densities were evaluated in triplicate tanks for a period of 30 days. Feed was based on enriched rotifer and *Artemia* brine shrimp, and an artificial diet that progressively increased in size with larval age. Severe cannibalism and size dispersion were observed from 10-15 dph, resulting in poor larval survival after 15 dph, the medium density trial resulted in significantly larger larvae (5.47 ± 0.64 mm total length at 15 dph) than the ones obtained in other treatments.

Nutrition: Greater amberjack larvae were fed *Artemia* brine shrimp enriched with five different levels of docosahexaenoic acid (DHA) in order to determine larval requirements during early development. Larval performance in terms of survival, growth and welfare was studied and proximate and fatty acid composition of enriched products, live preys and larvae were analysed. Results showed that the lowest DHA content lead to poor survival and growth (total length and body weight), higher contents (1-2% DHA) resulted in improved performance, while excess levels of DHA reduced growth. Furthermore, results demonstrated that adequate levels of DHA in *Artemia* (1-2%) prevented bone malformations. Different sources and levels of LC-PUFA were tested in different rotifer enrichment trials, and results indicated that rotifer enrichment using marine lecithin was the best in terms of LC-PUFA levels. Additionally, experimental broodstock diets have been formulated and will be tested in the next period.

Health: Attempts have been made to isolate pathogens from cultured greater amberjack. Greater amberjack bacteria isolated from skin ulcers have been identified as belonging to *Vibrio harveyi* and *Staphylococcus epidermidis*. Gill parasites were identified as the monogenean *Zeuxapta seriolae*, and a skin monogenean was also identified as *Neobenedenia melleni*. In addition, attempts to isolate the aetiological agent of *Epitheliocystis* in greater amberjack larvae have been made during larval rearing trials. An experimental rearing trial was performed and samples were taken for DNA extraction. PCR analyses using universal *Chlamydia* spp primers, but also specific primers for bacteria associated with previous outbreaks of *Epitheliocystis* disease. However, no *Epitheliocystis* disease was recorded nor any microbial agent related to the disease was detected. Finally, primers have been designed to the key immune genes to be cloned from greater amberjack. Preliminary PCRs have been carried out using the samples collected and promising products have been obtained.

ADVANCES IN PIKEPERCH (*Sander lucioperca*) RESEARCH

Reproduction & Genetics: The genetic variation of over 400 pikeperch breeders from commercial RAS farms in six different European countries was evaluated. Broodstocks appeared to have an adequate genetic variation and were clustered by stock origin. Likewise, samples from eight different wild populations were collected and analysed. Results will be the basis for comparison between captive and wild populations and, therefore, for the definition of how a future genetic breeding program should be established for sustainable optimal performances through domestication of pikeperch.

Nutrition: An experimental study to analyse the effect of different dietary nutrients (mainly phospholipid, HUFA, and PUFA) on pikeperch larval development and performance has been carried out during 2014. On-going research is investigating the influence of salinity on pike perch larvae fatty acid elongation/desaturation capability and metabolism. Results will be available during the second semester in 2015.

On-growing: In addition, DIVERSIFY team worked on the definition of the physiological and immune response of pikeperch when exposed to stressors (light intensity, water renewal rate, water current direction and time for tank cleaning). Results showed a high sensitivity of the species to captive environment, while a long-term application of stress significantly decreased growth performance of the fish.

ADVANCES IN ATLANTIC HALIBUT (*Hippoglossus hippoglossus*) RESEARCH

Reproduction & Genetics: Reproduction trials monitored egg batch sizes, batch intervals, fertilization rates, egg steroid content and total egg production in wild-caught, established spawners and F1 broodstock that were going through their second or third maturation. All individuals had regular and predictable ovulatory rhythms, which resulted in high fertilization rates in eggs from F1 females as well as established wild-caught, spawners. Another group of females were divided in three groups and treated with controlled-release GnRHa implants prior to first ovulation, but in this case ovulatory rhythms were not as regular and fertilization rates did not supersede those achieved by control females.

Larval culture: A larval husbandry study has been implemented with the aim to compare the efficacy of RAS and flow-through system (FTS) for the rearing of Atlantic halibut. Despite RAS potential to offer more stable environmental and chemical water parameters, which might lead to improved larval performance, preliminary results showed higher larvae mortality rates in RAS during the first week after hatching.

Nutrition: In the field of nutrition, a strategy for production of on-grown *Artemia* for Atlantic halibut has been developed, including water renewal, temperature, type of feed, and *Artemia* growth prior to the feeding experiment. Three different commercial dry feeds were tested for early weaning of Atlantic halibut larvae and feed acceptance is being used as an indicator for the trial.

Health: Fish health experiments have progressed well and methods are currently being optimised prior to larger scale production for vaccination of Atlantic halibut against *Vibrio spp.* The challenge study has identified a number of *Vibrio spp.* strains as primary pathogens, capable of inducing infection and significant mortality.

ADVANCES IN WRECKFISH (*Polyprion americanus*) RESEARCH



Reproduction & Genetics: Several individuals identified as potential wreckfish breeders were collected and acclimatized to captivity. Captive broodstocks and wild fish were sampled to describe the reproductive cycle, but captive sampling continued over time to ensure the collection of samples which are relevant to different stages of gametogenesis. Subsequently a total of four stocks were monitored for spawning, two of which matured and produced eggs from spontaneous natural spawning in tanks, GnRH α induced spawning in tanks and stripped gametes for *in vitro* fertilization. However, preliminary results revealed the number of fertilized eggs for larval culture was insufficient and therefore more work is needed to improve fertilization. In May 2015, 6,000 embryonated eggs were obtained and work will continue to improve results, in order to experiment with the larval rearing of the species.

Larval culture: Wreckfish eggs were obtained after spontaneous spawning of the broodstock and were used to study the optimum conditions for larval rearing. Nevertheless, hatching success and larval quality was very poor, possibly due to

extremely variable and changing weather conditions. Measures to improve quality have been developed and will be put into practice during 2015.

Nutrition: A study on the influence of broodstock feeding regimes over wreckfish fecundity and spawn quality has been carried out / started. Wild wreckfish were sampled and analysed for composition, fish size, weight, perimeter and weight of specific organs. In addition, the stage of the reproductive development of each individual was also checked. The effect on the reproductive development of three feeding regimes will be studied and compared in the next years.

ADVANCES IN GREY MULLET (*Mugil cephalus*) RESEARCH



Reproduction & Genetics: Work with grey mullet both increased the percentage of fish maturing and synchronized gonadal development with treatments of recombinant FSH. Spawning was then successfully induced in most females, to provide millions of eggs and larvae. However, the variability in ovulation and in fertilization obtained in the experiments underlined the need to further fine-tune and optimize the breeding protocol for captive grey mullet. Furthermore, preliminary analyses show that mullet sperm motility pattern is not similar to that of sea bass (*Dicentrarchus labrax*) or rainbow trout (*Oncorhynchus mykiss*) in terms of velocity, as well as motility duration. Initial results indicate that the major bio-technical settings have been successfully determined and can now be applied to experimental protocols, which will help improve the assessment of the effects of different treatments on reproductive performances of mullet males.

Larval culture: A study to evaluate the effect of “greening” larval rearing tanks on prey capture was carried out with one of two different algal species (*Nannochloropsis oculata* or *Isochrysis galbana*) at two different turbidity levels. Although no algal species or turbidity levels significantly affected rotifer consumption, linear regressions between turbidity and tank biomass was significant with *Isochrysis*, but not quite with *Nannochloropsis* while when turbidity was regressed with survival both *Nannochloropsis* and *Isochrysis* were highly significant. Moreover, larval survival was markedly improved at a higher turbidity level, however no difference in survival and tank biomass was found between algal species at the high turbidity levels. As a

conclusion, the study showed that it is algal turbidity and not algal type the dominating factor affecting mullet larval performance from 2-30 dph.

Nutrition: In relation to the improvement of larval performance through nutrition, the effects of dietary taurine on grey mullet larval rearing was studied at different stages of development. Results showed a significant, stronger effect of the highest taurine-fortified rotifer diets on larval growth when compared to lower fortified diets or even taurine enriched *Artemia* diets.

On a different study, 40 juvenile grey mullet were stocked in the same experimental system as the larval trial. The fish were fed five extruded pelleted treatments which were identical in lipid, protein and micronutrient composition but differed in their taurine levels. The experiment run for a period of 60 days and results showed that dietary taurine supplementation in juvenile mullet continued to give a growth advantage with higher taurine concentrations.

On-Growing: A large multi-partner 1 year study is currently underway to evaluate the performance of an improved grow-out diet for grey mullet in monoculture, as a function of stocking density and pond type. F1 juveniles stocked at different densities in cement and earthen ponds are being fed the grow-out diet. Similar tests will be carried out on wild caught juveniles at the same densities in 6 cement ponds and 2 earthen ponds. The diet will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival.

ADVANCES IN SOCIOECONOMIC RESEARCH



Identifying market opportunities: The activities have been focused on identifying the institutional and organizational context in which the new species can be introduced and on the first steps of identifying consumer segments for the candidate fish species. The macro-environmental context analysis indicated that the political, economic, social, environmental and legal environmental factors support introduction of new species in the market. This growth can only be realized at the expense of other protein sources, since the protein market in the EU has stabilized the last few years. Concentration of consumer buying has also impact on consumption patterns and consumer choices. The southern EU countries eat more whole fish, while northern EU

countries prefer processed fish. It is expected that products related to health and well-being will be preferred in food choices, next to price/quality relationships.

Competitive analysis indicated that most of the species included in DIVERSIFY are not well known yet as aquaculture products. Meagre is the only species with a substantial production volume. However, in relation to other Diversify species, all species are relatively new and unknown.

Market development: In the market development of the Diversify species a lot has to be done on brand awareness and brand recognition. What is most difficult for new species is production development in accordance with the market development. Industrial buyers state that consumer preferences concerning farmed fish seem to converge, although certain regional differences still exist within the European market. Consumers are increasingly looking for convenience, which is reflected in the assortment of retailers and foodservice providers. Consumers demand fish to be sustainable but are often not willing to pay more for these products, so sustainability is not a market driver. Locality (i.e., region) is also becoming more important – something which will benefit EU farmed fish. Overall, product quality and price are consistently important buying criteria. The overall farmed fish perception of the industry is positive, many buyers see the potential of farming fish to provide reliable supplies, standardized product forms, and relieve pressure from wild stocks. In approaching industrial buyers, farmers should be able to provide full information on their entire production process, feed, and logistics. An encouraging trend is that buyers are increasingly looking to source directly from farmers. Probably the best place to start selling new species would be at fresh fish counters, where customers interact with sales people who are able to inform and persuade them, and display a new type of fish in full sight.

Consumer survey: shows that there are differences between the five countries that were selected for the study (i.e. UK, Germany, Spain, France and Italy) in values and costs attached to a fictitious new fish species. Overall, farmed fish is not perceived as significantly better or worse than wild fish. In general, most consumers in the five countries are open to find out more about a new fish species. Based on the first findings more than one third of the consumers in the five selected countries belong to the segment of “know more about fish and have an open mind to buy new fish products”; and could therefore potentially be open to buy new species. More in-depth analysis in the upcoming year must give insights in the opportunities in the consumer market for the new species and more specific in the five countries.

Find out more at www.diversifyfish.eu