

## 2017 DIVERSIFY Annual Coordination Meeting (ACM) in BARCELONA

For the fifth issue of DIVERSIFY's newsletter, we have summarized the updates of the research carried out in the project and presented during the ACM celebrated in Barcelona during 17-19 January 2017 at the Palau Macaya, hosted by IRTA, Spain. An overview of the advances in each of the Research Areas of the project is presented here.

### REPRODUCTION AND GENETICS

**MEAGRE (*Argyrosomus regius*):** A protocol for strip spawning of meagre females and subsequent *in vitro* fertilization have been developed and evaluated successfully. The optimal ratio of sperm to egg to obtain high percentage of fertilization was >200,000 sperm per egg. Sperm quality was maintained without decline for up to 7 hours in Leibovitz medium and sperm quality did not appear to affect fertilization success. The protocol was successfully used in a large factorial cross of 120 *in vitro* fertilizations using either fresh or cryopreserved sperm. With regard to sperm characterization, the standardized analysis of sperm quality using the open source ImageJ CASA provided a complete dataset for meagre sperm that was used to estimate quality during the reproductive season and to implement protocols for sperm storage either chilled or cryopreserve. These results can be consulted in the following published articles:



Mylonas, C.C., Duncan, N.J., Asturiano, J.F., 2016. **Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality.** *Aquaculture*, 1-26 (online).

Mylonas, C.C., Salone, S., Biglino, T., de Mello, P.H., Fakriadis, I., Sigelaki, I., Duncan, N., 2016. **Enhancement of oogenesis/spermatogenesis in meagre *Argyrosomus regius* using a combination of temperature control and GnRHα treatments.** *Aquaculture* 464, 323-330.

**GREATER AMBERJACK (*Seriola dumerili*):** When compared with wild broodstock, captive greater amberjack breeders display different type of gonadal dysfunctions showing problems of endocrine disruptions (androgens) and gametogenesis sustainability (apoptotic figures). A detailed description of these findings has been already published:

Zupa, R., Rodríguez, C., Mylonas, C.C., Rosenfeld, H., Fakriadis, I., Papadaki, M., Pérez, J.A., Pousis, C., Basilone, G., Corriero, A., 2017. **Comparative Study of Reproductive Development in Wild and Captive-Reared Greater Amberjack *Seriola dumerili* (Risso, 1810).** *PLoS ONE* 12, e0169645.

Nevertheless, good quality spawns from spontaneous natural spawning in tanks in the Canary Islands (Spain) and after induction with exogenous gonadotropin-releasing hormone agonist (GnRHα)-induced spawning in tanks and sea cages have been achieved. During the 2016 summer, approximately 50 kg of eggs have been obtained (ARGO/GMF) and distributed to seven commercial hatcheries in Greece and Cyprus. Moreover 150.000 greater amberjack juveniles produced in HCMR have been supplied to five commercial grow out places in Greece.

**ATLANTIC HALIBUT (*Hippoglossus hippoglossus*):** Wild-caught domesticated females were predictable spawners that consistently gave eggs of very high quality. Farmed females also



produced eggs of high quality when their ovulatory cycles were identified and stripping was carried out close to ovulation. For commercial, as well as breeding purposes, it is not practical to rely on wild-caught females. However, relatively few farmed females consistently produced eggs with fertilization rates >80-85%. As a consequence, it may be necessary to include wild-caught broodstock in future breeding groups in order to ensure a broad enough genetic material.

A protocol for efficient induction of ovulation in Atlantic halibut, based on the use of long-term release implants for gonadotropin releasing hormone agonist (GnRH<sub>a</sub>) has been developed. The use of GnRH<sub>a</sub> implantation offers a logistic advantage to the commercial broodstock management of the species by reducing the spawning season. GnRH<sub>a</sub> implantation may be a useful tool to ensure that all females in a broodstock group reach maturation and ovulation, increasing parentage contribution to the next generation and increasing overall broodstock fecundity, without having deleterious effects on egg viability.

**WRECKFISH (*Polyprion americanus*):** In 2016, work on wreckfish has been focused on improving spontaneous and GnRH<sub>a</sub>-induced spawning and developing reliable protocols for *in vitro* fertilization. Regarding fish composition of wild and cultured wreckfish, results obtained show important differences in perivisceral fat among wild caught fish, which are not related to capture season or sex. Moreover, comparisons of wild and captive wreckfish showed that cultured fish have more lipids in the muscle (27.5% DW) and liver (62%) than those obtained in wild fish with 7% in muscle and 40% in liver. In contrast, protein content is higher in muscle of wild wreckfish than in cultured fish and some differences were also observed in the fatty acid profile with higher values of HUFA and n-3 HUFA in wild than in cultured wreckfish. The DHA values represent 11% in cultured fish and 26% in wild fish. Next issue of the Aquaculture Europe magazine will include a featured article on the species within the research frame of DIVERSIFY.

**GREY MULLET (*Mugil cephalus*):** The obtained results have proven the positive effect of using recombinant follicle stimulating hormone (r-FSH) on pituitary LH synthesis and on 11-KT secretion giving rise to enhanced gonadal growth. Fifty million eggs in total have been obtained with an average fecundity of  $1.76 \pm 0.52$  million eggs/kg. The hatching rate was  $78.8 \pm 11.9\%$  and over 200,000 fingerlings have been obtained.

## LARVAL REARING

**MEAGRE (*Argyrosomus regius*):** It has been shown that larvae can be weaned to artificial diets as early as 10 days post hatching (dph) without compromising nutritional condition and skeletal deformities. Requirements to avoid cannibalism have been determined. Dietary HUFA and vitamin E and C requirements have been documented and already published:

El Kertaoui, N., Hernández-Cruz, C.M., Montero, D., Caballero, M.J., Sale, R., Afonso, J.M., Izquierdo, M.S. **The importance of dietary HUFA for meagre larvae (*Argyrosomus regius*; Asso, 1801) and its relation with antioxidant vitamins E and C.** Aquaculture Research, in press Doi: 10.1016/j.aquaculture.2015.03.020.

**GREATER AMBERJACK (*Seriola dumerili*):** Larviculture has been very successful (survival rate above 20 %). Beside, rearing trials comparing intensive and semi-intensive conditions are being implemented and results show that intensive rearing conditions promote amylase, alkaline protease and peptine activities in 30 days post hatching (dph) larvae, while in earlier stages (12 dph) amylases activity was also higher, in contrast to alkaline protease and lipase activities.

The ontogeny of the greater amberjack visual and digestive systems were also characterised, and the influence of different rearing conditions (semi-intensive versus intensive rearing) on the ontogeny of the two systems were studied. The ontogeny of the retina in greater amberjack was



found to be similar to the general pattern shown in most fish species. Visual acuity – the distance the eye can differentiate between two points - of greater amberjack improved over time, as shown by the histological assessment. The combination of the rearing temperature and the feeding protocol is the key factor in order to achieve better rearing results in the greater amberjack larval rearing.

**ATLANTIC HALIBUT (*Hippoglossus hippoglossus*):** Several research trials have shown that it is possible to wean Atlantic halibut larvae as early as 28 dph; earlier weaning may result in high mortalities. Early weaning should be further tested in rearing systems that are better adjusted to pelagic Atlantic halibut larvae. It seems that the larvae need time to adjust to the prey or feed particle before intake of the particle. Floating characteristics of the particle is also important since *Artemia* cysts are a preferred particle for first feeding Atlantic halibut larvae.

**WRECKFISH (*Polyprion americanus*):** In the case of wreckfish larval rearing, the maximum larviculture period has been 27 days post hatching in all the trials performed so far. Several larval rearing trials were performed. In all cases similar rearing results were obtained. Larvae total length was  $4.70 \pm 0.27$  mm at 1 dph. Yolk sac was consumed by 11 dph at 14-17°C and by 8 dph at 17-20°C seawater temperature. Mouth opening occurred at 7 and 4 dph at 14-17°C and 17-20°C, respectively. Following mouth opening, larvae were fed with enriched rotifers and *Artemia* nauplii, using different enriching protocols. Further knowledge regarding specific nutritional wreckfish larval requirements is being developed during this year.

**GREY MULLET (*Mugil cephalus*):** Extensive research has been performed in relation to the beneficial effect of “green water” in the rearing tanks for larval grey mullet. This beneficial effect was derived predominantly from the resultant turbidity on prey ingestion rate (within the turbidity levels measured in the study) and less so to the algal type or biochemical content (i.e., fatty acid profile). Nevertheless, ingested algae by the larvae may have stimulated and improved gut maturation in early developing larvae resulting in markedly improved survival during the juvenile stage.

**PIKE PERCH (*Sander luciperca*):** It has been demonstrated that weaned juveniles of  $0.5 \pm 0.06$  g mean body weight can be produced in 5 weeks, but survival rates (0.3-2.6%) were very low. It has been possible to improve larval survival up to 10% by implementing several combinations of factors. The regulation of the size or weight heterogeneity is of major importance to limit the impact of cannibal individuals in predatory fish species. Numerous biotic and abiotic factors influence the heterogeneity of a population. The results suggest that a later onset and longer duration of weaning followed by discontinuous feeding will improve larval survival, growth and reduce deformities in pikeperch populations.

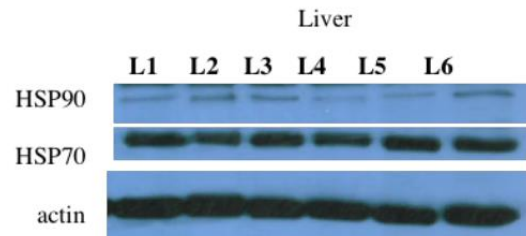
## NUTRITION

**MEAGRE (*Argyrosomus regius*):** Early weaning diets have been improved so far by means of raising the essential highly unsaturated fatty acid (HUFA) levels up to 3 % together with vitamins E and C (over 1500 and 1800 mg/kg) in order to protect these HUFA from oxidation.

**GREATER AMBERJACK (*Seriola dumerili*):**

The optimum levels of lysine in on-growing diets for greater amberjack based mainly on plant ingredients (low fish meal inclusion) have been determined. The dietary lysine requirements which can support maximum weight gain of greater amberjack juveniles fed on a diet based mainly on plant ingredients, containing 45% protein, 18% lipid and 25%

fish meal inclusion was 2.11% of diet. No significant effect of lysine levels on the expression of HSP in liver or intestine was found. Lysine supplementation found to affect the specific activity of CAT in liver and intestine of greater amberjack fed the diet containing 2.11% lysine. The data in this study will be useful in developing balanced commercial diets for greater amberjack, particularly when fishmeal is replaced by plant protein blends.



**ATLANTIC HALIBUT (*Hippoglossus hippoglossus*):** A trial has been set to compare the use of on-grown *Artemia* versus *Artemia* nauplii on Atlantic halibut larval performance. It concluded that the use of on-grown *Artemia* during the critical period of metamorphosis in Atlantic halibut larva did not differ from the use of *Artemia* nauplii with regard to growth, mortality and fry quality. In addition, the production of on-grown *Artemia* is labour-intensive, and high personnel costs may be prohibitive in implementation of this live feed source in commercial larviculture.

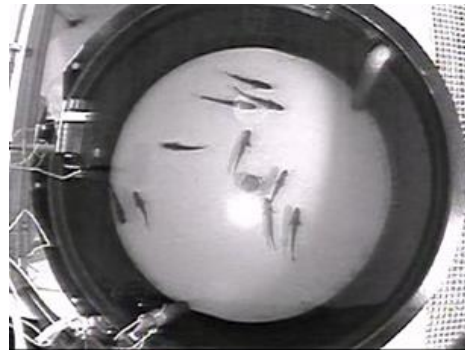
**WRECKFISH (*Polyprion americanus*):** In order to determine the correct broodstock diet for the species, some commercial broodstock feeds have been analyzed, showing that they have a high amount of fat for wreckfish broodstock. A new feed has been formulated on the basis of the data obtained from wild fish. According to the initial results, broodstock feed must contain high amounts of protein, low lipid content and a large amount of n-3 highly unsaturated fatty acids (HUFA). Moreover, the eicosapentaenoic acid (EPA) arachidonic acid (ARA) ratio must be around 1.5. A comparison of feeding of broodstock with semi-moist diet and the new formulated diet was conducted. Results show a clear relationship between fatty acid profile of oocytes from the females and the two diets provided.

**GREY MULLET (*Mugil cephalus*):** An interesting work has been published in which it is documented the evaluation of low fishmeal inclusion diets for grey mullet fry. Fish meal substitution between 50 and 75% by a mixture of different plant protein sources in wild grey mullet fry weaned onto compound diets did not affect their growth performance and survival, as fish fed diets with high levels of fish meal substitution grew similar to those fed the control diet with a high content of fish meal. Similarly, the implemented weaning strategy of wild specimens onto experimental compound diets resulted to be correct in terms of growth performance and survival rates. The complete work can be found in the publication:

Enric Gisbert, Mansour Torfi Mozanzadeh, Yannis Kotzamanis, Alicia Estévez. 2016. **Weaning wild flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish meal substitution.** *Aquaculture*, 461: 92-100.

## GROW OUT

**MEAGRE (*Argyrosomus regius*):** Results of the use of light or air bubbling showed that juvenile meagre is able to learn and remember these specific stimuli related to feeding, indicating that these type of stimuli can be used in an industrial setting, as they can be implemented and managed easily with existing technologies in sea cages.



With the objective to develop a method to avoid size variability of meagre juveniles, a specific experiment has been performed. Results prove that size variability and different growth rate in meagre juveniles exist and seems to have a genetic origin. Moreover, there is no compensatory growth of the small size juveniles when graded and transferred to new tanks and offered enough food. After grading, large fish always show a higher growth rate that it is maintained along the on-growing period. On the contrary, slow growing fish always show a lower growth rate that is maintained along the whole on-growing period causing a delay of approx. 6 months in attaining commercial size.

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**GREY MULLET (*Mugil cephalus*):** a multipartner trial has been performed to compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of F1 juveniles stocked at two different densities in three different locations: cement and polypropylene tanks (Israel), cement tanks (Greece) and earthen ponds (Spain). Results are still under evaluation but it seems that increasing density in larger fish may play a significant role reducing growth and skewing the population size distribution to smaller fish.

**PIKE PERCH (*Sander luciperca*):** Several experiments are ongoing to determine the causes and possible control of the high cannibalism among pikeperch fry. Intraspecific predation, inter-individual variability and ontogeny of the piscivory behaviour are some of the parameters to be evaluated in these studies.

## HEALTH

### **MEAGRE (*Argyrosomus regius*):**

Extensive information has been obtained from a *Nocardia* infection in cage-cultured meagre in Greece and Spain. Microbiological, biochemical and genetic characterization of the *Nocardia* isolates, together with the epidemiological data obtained from the affected fish farms are being used for the development of a *Nocardia* vaccine based on the most appropriate and virulent strain. The study concluded that nocardiosis is present probably in a confined geographical region in Greece, and that it is not the cause of Systemic Granulomatosis.

Although generally, the species does not seem to be very susceptible to common bacterial infections, there are sporadic reports suggesting that several pathogens may become problematic in the future. Vibriosis is expected to affect meagre culture in the future, especially as this intensifies with time. Vaccination has reduced significantly the incidence of vibriosis in other established species, such as European seabass and gilthead seabream, therefore emphasis should be given in developing and testing of vaccines against this disease of major importance.

An interesting article has been published on parasitic infection of meagre:

K. B. Andree, A. Roque, N. Duncan, E. Gisbert, A. Estevez, M. I. Tsertou, P. Katharios. **Diplectanum sciaenae (Van Beneden & Hesse, 1863) (Monogenea) infecting**

**meagre, *Argyrosomus regius* (Asso, 1801) broodstock in Catalonia, Spain. A case report.** *Veterinary Parasitology. Regional Studies and Reports* (DOI: 10.1016/j.vprsr.2016.02.006).

#### **GREATER AMBERJACK (*Seriola dumerili*):**

Severe parasitic infections of the monogenean *Zeuxapta seriolae* have been documented in greater amberjack cage culture, with 100% prevalence in March and a maximum intensity of more than 1000 parasites per fish. It seems that the parasite has a preference for the first gill arch. Fish of one year seem to be the most affected age class. As recommendation for farmers, a good maintenance of the nets and a monthly monitoring of the fish together with the right orientation of the cages with different age class when sick fish are present can be helpful to control the parasite dispersion.

#### **ATLANTIC HALIBUT (*Hippoglossus hippoglossus*):**

An assessment of two eukaryotic systems -microalgae and a protozoan- for the production of nodavirus (Viral Neural Necrosis, VNN) capsid protein was performed for the development of an oral vaccine for Atlantic halibut. The expression of the nodavirus capsid protein was achieved. However, it was only in an *E. coli* system that they achieved sufficient and high expression for further use of the protein as antigen for vaccination purposes. Further optimisation for sufficient expression in plant and protozoan systems and a method for purification of the recombinant protein still remain to be done. The initial testing of possible expression of VNN capsid protein was carried out using non-inducible systems, and the next step is to use an inducible expression kit to increase the production.

### **ADVANCES IN SOCIOECONOMIC RESEARCH**

During the implementation of the project, new products for each species have been selected among twelve ideas that were evaluated with regard to feasibility of production and shelf-life of the final product (grilled fillet, fresh fillet, smoked fillet, frozen fillet, salad, fish olive oil, frozen marinated fillet, hamburger, tartar and pate).

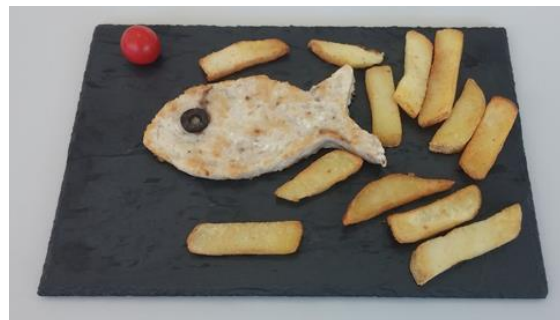
After the evaluation, these are the different prototypes that have been developed:

**Meagre:** fish burger shaped as fish and salad with fish.

**Pikeperch:** fish pate (mainly focused to valorize the pike perch processing by products) and fresh fish fillet seasoned or marinated.

**Grey mullet:** smoked fillets and ready-made fish fillets in olive oil.

**Greater amberjack:** Fresh fish steak for grilling in the pan and fresh fish fillet seasoned or marinated.



Sensory profiling of the different prototypes were carried out in the five countries investigated (Germany, France, United Kingdom, Italy and Spain) and one hundred participants were recruited in each of the five selected countries. All the tests were performed under controlled conditions in a central location. A total of 10 tasting sessions were held in each location in two consecutive days. It was observed that a higher preference for products having the genuine sensory properties of fish existed, probably due to the recruitment procedure that was based on

their regular consumption. French scored all products higher than Italians with the rest of the countries being in between. Grilled fillet was in all cases the best-perceived product in agreement with its higher expected acceptance mentioned above. The fishburger and pate were the two products that were worst perceived regarding the presence of additives.

In conclusion, the study demonstrated that all products were well accepted for consumers with the sole exception of the fish pate. Besides, products with a lower degree of processing were those who generated higher expected scores and higher acceptability in the test. Several publications are already available in the Socioeconomic Research Area:

Banović, M., Krystallis, A., Guerrero, L., Reinders, M.J., 2016. **Consumers as co-creators of new product ideas: An application of projective and creative research techniques.** *Food Research International*, 87: 211-223.

Reinders, M.J., Banovic, M., Guerrero, L., and Krystallis, A. 2016. **Consumer perceptions of farmed fish: A cross-national segmentation in five European countries.** *British Food Journal*, 118(10): 2581-2597.

O. Lazo, A. Claret, L. Guerrero. **A comparison of two methods for generating descriptive attributes with trained assessors: Check-All-That-Apply (CATA) vs. Free Choice Profiling (FCP).** *Journal of Sensory Studies* 31 (2016) 163–176.

Grigorakis, K., 2015. **Fillet Proximate Composition, Lipid Quality, Yields and Organoleptic Quality of Mediterranean Farmed Marine Fish: A Review with Emphasis on New Species.** *Crit Rev Food Sci Nutr*, in press.

All the publications can be found at <http://www.diversifyfish.eu/scientific-articles.html>

### **CROATIA 2016: DIVERSIFY SPECIAL SESSION AT THE AQUACULTURE EUROPE CONFERENCE**



The next Aquaculture Europe Conference will be held in Dubrovnik, Croatia, October 16-20. **DIVERSIFY** will have a Special Session where the most updated findings of the project will be presented. Please be sure to register and prepare your presentation on time. Remember we'll upload the abstracts in our project web. The deadline for abstract presentation is 1st of May 2017. **We hope to see you and your work there!!.**

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