We take a look at the EU project exploring the biological and socio-economic potential of new/emerging candidate finfish species for the expansion of the European aquaculture industry

DIVERSIFY

DIVERSIFY began in December 2013 to acquire the necessary knowledge for the diversification of the European Aquaculture production based on new/emerging finfish species. The project has a total budget of €11.8 million for its 5 year duration (2013-2018), making it one of the largest research projects in the area of aquaculture funded by the European Commission.

DIVERSIFY has identified a number of new/emerging finfish species, with great potential for the expansion of the EU aquaculture industry. These fishes are fast growing and/or large species marketed at a large size and can be processed into a range of value-added products in order to provide the consumer with a greater diversity of fish species and products.

The fish species included are meagre (Argyrosomus regius), greater amberjack (Seriola dumerili); wreckfish (Polyprion americanus) and Atlantic halibut (Hippoglossus hippoglossus). In addition, the omnivorous and euryhaline grey mullet (Mugil cephalus) is also included, as it can be produced in a wide variety of environments and using low cost feed with small amounts or no fish meal/oils, and the pikeperch (Sander lucioperca) as a good freshwater species for recirculating aquaculture systems (RAS).

Each of the species selected for DIVERSIFY has the potential to grow in the market and to produce value-added products. Their biological and economical potential are expected to stimulate the growth of the European aquaculture sector.

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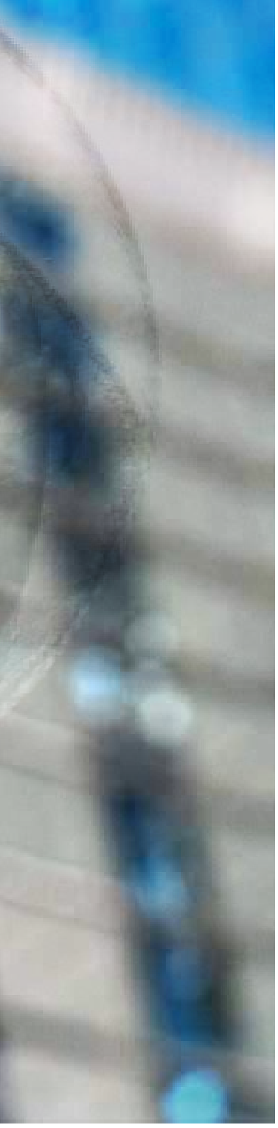
Reproduction & Genetics

Great success has been achieved in the control of reproduction of greater amberjack. Spontaneous natural spawns have been obtained in tanks in the Canary Islands (Spain), while in the Mediterranean Sea stocks the use of gonadotropin-releasing hormone agonist (GnRHa) implants (Fig. 1) has resulted in the production of large numbers of good quality eggs.

In the 2016 reproductive season, >50 kg eggs have been produced from three stocks maintained in sea cages in Greece (Fig. 2), and have resulted in the production of ~150,000 juveniles by the HCMR larval rearing department (Fig. 3). These juveniles have then been supplied to five commercial rearing sites in Greece, for what is the first ever, large-scale commercial grow out trial of this species in the Mediterranean region (Fig. 4).

With regards to work with Atlantic halibut, experiments have demonstrated that F1 fish could be induced with GnRHa implants to spawn earlier and produce higher fecundities compared to controls. This work will be scaled up and validated with more breeders from commercial facilities in the coming years.



Work with wreckfish provided interesting results, with both spontaneous natural and GnRHa-induced spawning in tanks and stripped gametes for in vitro fertilization (Fig. 5). Although a small number of fertilized eggs have been obtained so far, larviculture period had reached 27 days providing important results with regard to critical larviculture parameters.

Although the success is far from what has been obtained in greater amberjack, this is the first time that a substantial amount of eggs of this deep-sea species have been available, for the implementation of larval rearing experiments.

Work with grey mullet resulted in the increase in the percentage of fish maturing and synchronized gonadal development with treatments of recombinant follicle stimulating hormone (Fsh) and metoclopramide. Spawning was then successfully induced in most females, with GnRHa and metoclopramide, resulting in the production of millions of eggs and larvae. However, common problems that still need to be addressed are the observed failed ovulation in many females (~42%) and the high variation in fertilization (0-90%).

For the meagre and pikeperch, in order to provide tools for genetic improvement, captive broodstocks were genetically characterized, demonstrating that they have sufficient variation to be used for breeding programs, and strategies were suggested on how the stocks could be improved. Work with meagre also demonstrated that paired (single male and female) spawning was possible to produce known families for a breeding program.

Figure 2: Greater amberjack breeders maintained in sea cages in Greece and induced to spawn using GnRHa delivery systems *(photo by HCMR)*

Nutrition

The results obtained so far have improved weaning diets for meagre, demonstrating the importance of raising the essential highly unsaturated fatty acid (HUFA) levels up to 3% and vitamins E and C over 1500 and 1800 mg kg^{-1}. Greater amberjack enrichment products were also improved by defining the adequate levels of docosahexaenoic acid (DHA, 1-2%), in order to prevent bone malformations and promote maximum growth and survival.

Moreover, an optimum method for the effective enrichment of rotifers for greater amberjack was developed, and specific diets for broodstock of greater amberjack and wreckfish were formulated based both on bibliographical and analytical studies. Studies to develop optimum weaning-diets also started for pikeperch, focusing on the determination of the requirements for essential fatty acids.

The trials for producing on-grown Artemia for Atlantic halibut have been completed, but have not produced any improvement in juvenile production so far.

Figure 1: Greater amberjack breeders given GnRHa implants to induce spawning during the reproductive season *(photo by HCMR)*

Figure 3: Greater amberjack juveniles produced by the HCMR larval rearing department in 2016 *(photo by HCMR)*

Larval husbandry

Work on meagre showed that larvae can be weaned to artificial diets as early as 10 days post hatching (dph) without compromising nutritional condition and skeletal deformities. However, growth and survival should be considered. Cannibalism could be controlled by increasing the feeding frequency, removing dominant individuals, regular grading and by keeping the larvae in the dark when food is unavailable or in short supply.

In greater amberjack, the larval rearing parameters to be used in the semi-intensive mesocosm method and the intensive method were established, and large numbers of juveniles have been produced (Figs. 3 and 4) and sent for grow out to selected sea cage sites.

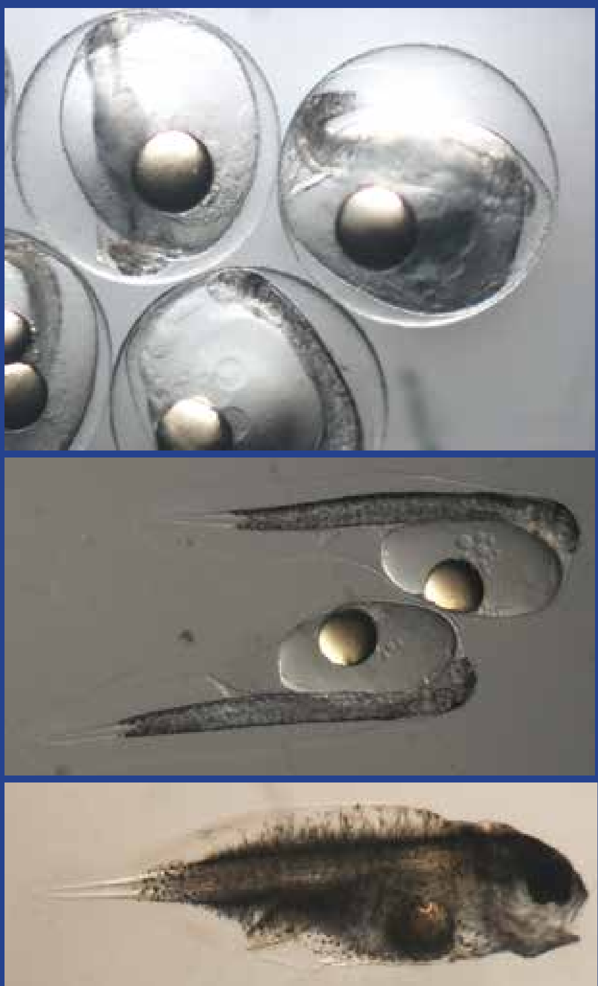
Results until now showed that intensive rearing conditions favour amylase, alkaline protease and pepsin activities in 30 (dph) larvae, while in earlier stages (12 dph) amylase activity was also higher, in contrast to alkaline protease and lipase activities.

In pikeperch, the effects of selected environmental factors (i.e. light intensity, water renewal rate, water flow direction and tank cleaning timing), individually and in combination on larval

Figure 4: Greater amberjack juveniles from HCMR, sent to a number of commercial sea cage sites for on-growing trials *(photo by HCMR)*

Figure 5: Sampling wreckfish (gonadal biopsy) to evaluate reproductive stage of development *(photos by Aquarium A Coruna)*

Figure 6:
(Top) Wreckfish larvae just prior to hatching. (**middle)** 1 dph and **(bottom)** 13 dph during the larval rearing trials at the Aquarium A Coruna, Spain *(photos by Aquarium A Coruna)*



rearing, were examined using a multifactorial design experimental system.

In Atlantic halibut, a study is presently running to compare the efficacy of RAS and flow through (FT) for larval rearing. Larval mortality was shown to be higher in the RAS system during the first week after hatching.

In wreckfish, the objective was to define optimum conditions for the larval rearing. Although, larval survival was poor, samples of larvae were taken out on days 0, 5 and 10 of life to obtain the fatty acid profile of wreckfish larvae and the first results show that the fatty acid profile has little variation in the first 10 days of life.

Moreover, early embryonic and larval development has been documented (Fig. 6). Although the larval rearing still needs further development, these initial larval rearing efforts are very significant in providing information that will enable us to evaluate the potential of this deep-sea species, for commercial larval rearing.

Concerning grey mullet studies, results revealed that rotifer consumption and larval survival were dependent on algal turbidity in the rearing tanks, but independent of algal type added. Higher survival resulted in higher levels of smaller fish, which reduced average fish weight. Also, growth compensation was observed after grading at 29 dph.

Grow out Husbandry

The evaluation of feeding behavior of meagre demonstrated that juvenile fish were able to learn and remember specific stimuli related to feeding (Fig. 7). Small fish of 50-100 g body weight responded very quickly to light stimuli (2 days after the start of the experiment), but responded very slowly to mechanical stimuli (air bubbles).

Larger fish (200 g) responded very quickly to both stimuli. The study demonstrated that both air bubbles and light can be used in an industrial setting, as they can be manufactured, implemented and managed easily with existing technologies in sea cages.

For pikeperch, the husbandry studies focused on the on-growing requirements, emphasizing on the effects of (a) environmental parameters, (b) farm conditions, and (c) domestication level and geographical origin on growth, immune and physiological status.

Finally in grey mullet, the first study that has been completed related to the definition of an optimal weaning diet. It was shown that fishmeal (FM) substitution did not affect any of the performance and condition parameters analyzed and that weaning wild grey mullet fry (which are zooplanktivorous) may be conducted using diets with a high level of FM substitution.

In addition, a grow-out study was initiated in Spain and Greece, using wild-caught fry that are reared to harvest size under different environmental conditions and stocking densities, using a common DIVERSIFY formulated grow-out diet.

Fish Health

In meagre, a first experiment has been made to characterize the ontogeny of the immune response in meagre, with samples collected at various times post-hatch. Samples of different tissues from juveniles have been also provided for analysis of immune gene expression. First attempts to develop a challenge model

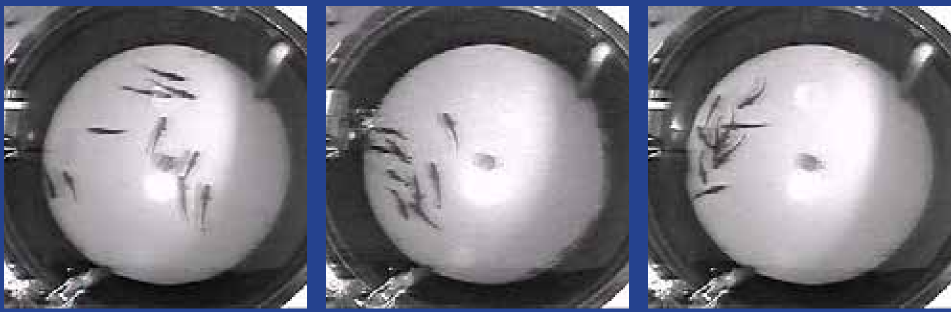


Figure 7: Feeding behavior experiments with meagre juveniles. The fish were usually distributed randomly throughout the tank (left), but when a light stimulus was given (center) they gathered around the light source and consumed immediately the feed that was provided from an electric feeder in the same area (right) (photos by HCMR).

Figure 8: Diplectanum sciaenae from meagre broodstocks **(inset)** and an infection of Paradeontacylix spp. on the gills of a greater amberjack broodstock from sea cages **(large image)** *(photos from HCMR)*

have been performed with Photobacterium damselae subsp. piscicida in meagre and greater amberjack.

Efforts have been made to isolate pathogens from cultured meagre and greater amberjack (Fig 8), and several parasite and bacterial species have been isolated and identified (Epitheliocystis in greater amberjack). The monogenean parasite Zeuxapta seriolae was the most prevalent and important parasitic pathogen.

Apart from Zeuxapta seriolae, what has also been identified is the blood fluke Paradeontacylix sp. to be present in greater amberjack reared in Greece (Fig. 8). There is scarce information on the biology of this parasite and almost nothing is known about its life cycle. A passive collector device has been designed and tested as a method to detect and quantify the level of infestation of monogenean parasites in greater amberjack during rearing in tanks.

With regard to Atlantic halibut, production of Viral Neural Necrosis (VNN) capsid protein has been progressing well, and successful expression in E. coli, tobacco plants and Leishmania has been achieved. However, bacterial cells do not glycosylate the expressed protein, as do higher eukaryotes.

By expressing the capsid protein of nodavirus recombinant in different systems, it should be possible to find out if post-translational modifications influence antigenicity, thereby affecting its ability to induce protection when used as an antigen in a vaccine.

Socioeconomics

The macro-environmental context analysis performed has indicated that most EU countries have a policy to increase fish consumption, whilst seafood consumption is increasing in most of them too.

This growth can only be realized at the expense of other protein sources, since the protein market has been stabilized in the last few years. The southern countries eat more fresh whole fish, while northern countries prefer processed fish.

Consumer preferences concerning farmed fish seem to converge to convenience and fresh standardized products, such as fish fillets, portioned meals and processed foods. Industrial buyers in

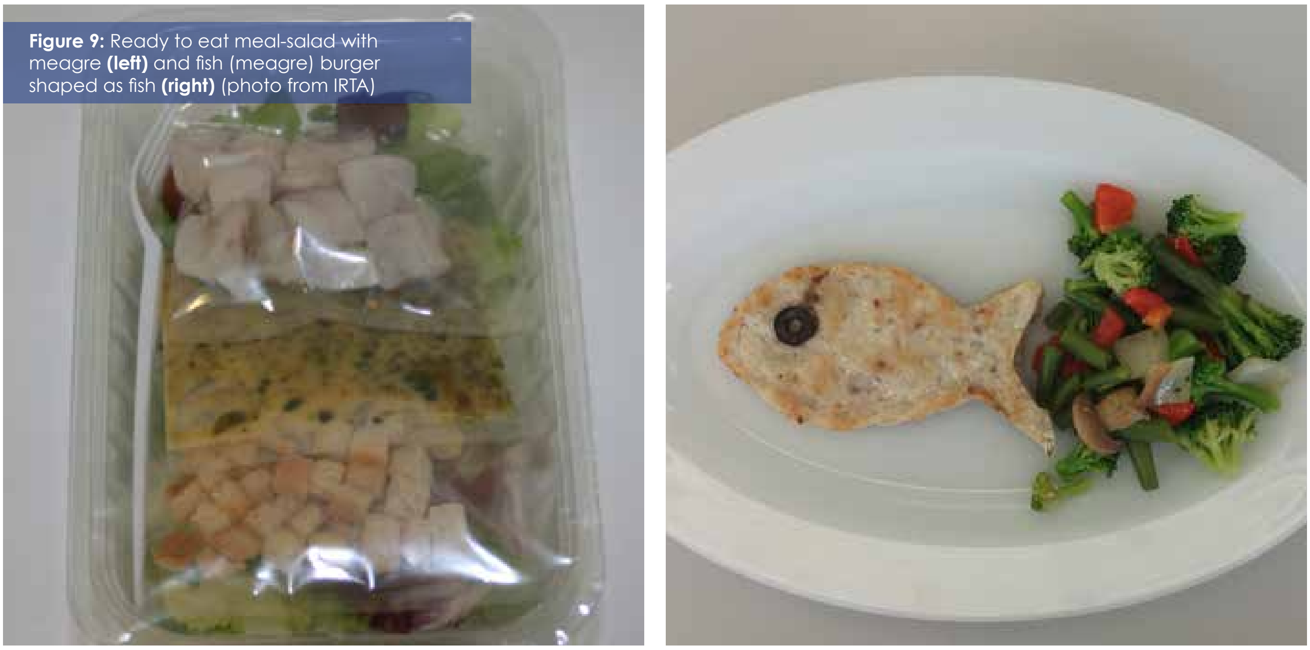


Figure 9: Ready to eat meal-salad with meagre **(left)** and fish (meagre) burger shaped as fish **(right)** (photo from IRTA)

Figure 10: Thin smoked grey mullet fillets **(left)** and ready-made grey mullet fillets in olive oil **(right)** *(photo from CTAQUA)*

northern EU work closely with their trusted suppliers to develop new products, while the southern EU can be determined as seller markets, meaning that suppliers often initiate new product offerings. In approaching industrial buyers, farmers should be able to provide full information on their entire production process.

The consumer survey identified three consumer segments: (1) involved traditional consumers (29%): who know relatively more about fish and buy traditional fish products; (2) involved innovators (36%): who know relatively more about fish and who have a more open mind to buy new fish products and (3) ambiguous indifferent (35%): who know relatively less about fish and who are less open to buy new fish products.

Based on the first findings, more than one third of the consumers in the five selected countries belong to the segment of 'Involved innovators' and could therefore potentially be open to buy new species.

A total of twelve products have been selected from a pool of 41 concepts for new value added product from DIVERSIFY species, based on their different degree of technological complexity and processing and taking into account the appropriateness for each of the species under study: Intrinsic (sensory properties) and extrinsic characteristics (information provided) of the selected products/concepts were assessed by consumers in five countries including France, Germany, Italy, Spain and UK (Fig 9 and 10).

All the results obtained so far have been presented in scientific conferences, as well as in the annual coordination meetings. The next annual meeting will be held in Barcelona in January 2017 (http://www.diversifyfish.eu/2017-annual-coordination-meeting-jan.html)

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www.diversifyfish.eu