EXPERT TOPIC

DIVERSIFY
New species for EU aquaculture

DIVERSIFY have kindly teamed up with International Aquafeed magazine to provide us with the results of the research carried out on the six species of the project: meagre, greater amberjack, halibut, pikeperch, grey mullet and wreckfish.

DIVERSIFY selected these species based upon their economical and biological potential and have carried out innovative research on all these fish species to discover how to create the most profitable, healthy, economical and beneficial final product for consumers, fish farmers and everyone in-between.

For this first article, DIVERSIFY partners are telling us more about the potential of Meagre. Stay tuned next month to find out more about the next fish species that could soon be at the heart of aquaculture.

For more information about the project visit: **www.diversifyfish.eu**

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EXPERT TOPIC MEAGRE

Meagre juveniles during sampling

Exploring the biological and socio-economic potential of new-emerging candidate fish species for the expansion of the European aquaculture industry – the DIVERSIFY project (EU FP7-GA603121)

Constantinos Mylonas and Rocio Robles

The European Union (EU) is the largest importer of fisheries and aquaculture products in the world. Aquaculture provides only 20 percent of the seafood produced in the EU, and capture fisheries provide the rest (Eurostat 2018), while the worldwide contribution of aquaculture towards seafood consumption is already >50 percent.

This situation can be attributed partially to a lack of diversity of aquaculture products in Europe, since European demand increases for a diverse range of fish products, especially for fish fillets and other fish processed products. Nevertheless, aquaculture is undertaken in all EU states, and plays an important role in the supply of high-quality seafood to the European consumer.

The EU aquaculture is a modern industry providing direct employment for 85,000 people, producing 1.3 million tonnes worth €4 billion. Many world-class researchers and facilities exist in research centres and universities throughout Europe, while the private sector employs highly skilled and educated personnel, with modern production facilities.

Therefore, the sector is well positioned to become the world leader in the efficient and sustainable production of safe seafood of the highest quality and nutritional value, considering consumer preferences and lifestyles, and the immense diversity of aquatic products from the wild, to which the consumer is accustomed.

Even though some 35 aquatic species are cultured in Europe, finfish aquaculture production is dominated both in volume and value by a handful of species --such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)- that, in turn, limit the number of aquaculture processed products available in the market.

In fact, the ten most common species account for up to 90 percent of the production and 87 percent of its value (Eurostat 2018). An efficient, sustainable and market-oriented expansion of the EU aquaculture sector based on new species and products will reduce the dependence of the EU consumer on imports from countries of questionable production, health, environmental and social standards, and it will reduce the pressure on over-exploited fisheries in the EU.

The objective of DIVERSIFY -which ran between 2013 and 2018- was to support the EU aquaculture industry in diversifying its production with new/emerging species with important advantages over the ones cultured currently, such as fast growth, large size or low requirement in fishmeal and fish oil.

In addition, the project identified the drivers for market acceptance of the new food prototypes in order to position the EU aquaculture sector in relation to imports from outside the EU. Although the emphasis of DIVERSIFY was on Mediterranean cage-culture, fish species suitable for cold-water, pond/extensive and fresh water aquaculture have been included as well.

The fish species studied were meagre (*Argyrosomus regius*) and greater amberjack

An adult specimen of meagre

(*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 aquaculture systems (RAS).

A strong socioeconomic component was included in DIVERSIFY in order to address important bottlenecks in aquaculture development, beyond biological/production issues. The socioeconomic part of the project had a science based applied market development approach, with a lot of components.

These included the perception of aquaculture products in general and processed products specifically, market potential and demand factors, consumer and professional buyer preferences, new product development, creating added value in relation to raw products and market development. An important limitation in aquaculture consumption is that in many countries and/or segments of the EU market, aquaculture fish have a weaker image than wild fish. Parallel to technological improvement of production methods for the new species, expansion opportunities for the EU aquaculture sector have been identified.

The combination of biological, technological and socioeconomic research activities developed in DIVERSIFY are expected to support the diversification of the EU aquaculture industry and help in expanding production, increasing aquaculture products and development of new markets.

Meagre in the DIVERSIFY Project

Reproduction- The industrial bottleneck to implement genetic breeding programmes for meagre was addressed by genetically sampling over 435 breeders from broodstocks in 13 breeding centres and seven countries using 18 microsatellite markers. The broodstocks originated from a limited number of families from three wild populations or groups.

Although broodstocks appeared to have sufficient variation for

breeding programme(s), the majority required an increase in the number of families. Both genetic tools and protocols to control reproduction for breeding programs were developed. The muscle and liver transcriptome were determined and the first genetic linkage map for meagre was constructed using the ddRAD (double digest restriction-site associated DNA) methodology, which identified 731 markers organised in 27 linkage groups.

The model mapping identified five quantitative trait loci (QTLs) on two linkage groups, which exhibited significant evidence of linkage at the genome level and multiple QTLs were related to differences in body weight and length. Protocols for the induction of tank spawning in paired crossing had a 76 percent efficacy of spawning pairs with male rotation and produced a total of 61 families (full and half-sib) that had >200,000 eggs with >80 percent fertilisation success.

However, a decline in spawning success that was observed with repeated induced spawning with male rotation was a possible drawback that was highlighted. Protocols were also developed for in vitro fertilisation for planned crosses.

Meagre sperm had a mean sperm density of 3.21·1010 ± 1.18 spermatozoa/mL, motility duration was 1:43± 0:18 min, mean percentage of initial motility of spermatozoa was 48.17 ± 2.80 percent and the mean initial spermatozoa velocity (VAP) was 90.69 ± 5.76 μm/s.

Different sperm storage methods and cryopreservation techniques were modified to provide protocols for meagre sperm. The optimal period for stripping eggs was 38-39 hours after the application of gonadotropin releasing hormone agonist (GnRHa) and a ratio of 150,000 motile spermatozoa to egg was recommended.

The results of DIVERSIFY provided the technology required to implement industrial breeding programs and scientific advances in the reproductive control of marine fish in general, as well as sperm characterisation and genetic resources for meagre and related species.

Nutrition

Despite the interest of meagre for aquaculture diversification in the last decade, there is a lack of information on nutrition during larval development. Dietary HUFA levels of three percent improved larval growth and lipid absorption and deposition. Besides, among fish fed three percent HUFA, increases in vitamin E and vitamin C improved significantly body weight, as well as lipid and HUFA contents in the larvae.

Thus, weaning diets for meagre must be optimised increasing HUFA levels up to three percent and vitamins E and C >1500 and 1800 mg kg-1, respectively, in order to spare these essential fatty acids from oxidation.

A 0.4 percent dietary HUFA is not enough to cover the essential fatty acid (EFA) requirements of meagre larvae. It is also important to supplement meagre weaning diets with 2.4 mg/kg vitamin K, since the absence of this vitamin reduced markedly larval survival. Meagre seemed to be very sensitive to hypervitaminosis D and only mildly sensitive to hypervitaminosis A, since supplementation with these vitamins lead to a growth reduction. Taurine supplementation did not have any effect in meagre larvae performance.

The nutritional requirements and optimum levels of HUFA for meagre fingerlings were examined, evaluating its effects on survival, growth performance, feed utilisation and fish composition. Meagre showed the ability to selectively conserve key fatty acids (FA), particularly Docosahexaenoic acid (DHA; 22:6n-3) and Arachidonic acid (ARA; 20:4n-6) over other FA, in response to essential FA-deficiency.

Newly hatched meagre larvae

Furthermore, meagre seems to have active Δ6 desaturases and Elovl5 elongase, but their activities were insufficient to produce DHA and Eicosapentaenoic acid (EPA; 20:5n-3) from PUFA precursors to sustain fast growth. The EFA deficient meagre in the present study also showed a higher incidence of granulomas than fish fed two percent n-3 HUFA. Based on our results, DIVERSIFY showed that the requirement for HUFA for meagre fingerlings is at least 2two percent Dry Matter (DM) in diets containing 16.5 percent DM lipids, a ratio of 0.9 EPA/DHA and 0.4 percent ARA of total FA content.

Larval husbandry

The main task for meagre larval research in DIVERSIFY was to provide the industry with an early weaning protocol for this species, that included co-feeding live prey with artificial micro diets. The standard method for meagre larval production is to start weaning the larvae around 20 dph (days post hatching).

In the trials carried out in DIVERSIFY we showed that weaning time can be advanced successfully to 15 and 12 dph using a commercial micro diet with a gradual transfer from live prey to the artificial diet over a minimum period of five days. However, larval survival was low due to cannibalism, a major problem in the culture of many marine fish larvae.

Size variation is the primary cause of cannibalism in larval fish, together with factors such as food availability, larval density, feeding frequency, light intensity, water turbidity and shelter. This project demonstrated that meagre larvae have the same capacity to digest live prey and microdiets and they can be weaned earlier reducing the production costs if some measures to reduce cannibalism are in place.

These measures include increasing feeding frequency, removing dominant individuals and keeping the larvae in the dark when the food was unavailable or in short supply.

Grow out husbandry

The development of size variability in meagre culture was

described and was observed at all stages including early juvenile stages when cannibalism was a problem. There was no compensatory growth of slow-growing fish and it was recommended that slow growing fish were not selected for grow out as an economic analysis indicated that these fish would need six months more to attain 500 g.

Genetic differences were observed between fish that grow at different growth rates and genetic markers were identified that were associated to growth. A wide range of husbandry parameters did not alter the variable growth rates and consequently did not alter the wide size distribution obtained.

These included light conditions (shaded or unshaded cages), depth (eight- or six-metre cages), feeding methods (self-feeding, hand feeding or automatic feeding), time of feeding (night or day) and depth of feeding (surface or bottom of the cage).

In addition, the studies indicated many aspects that can improve feeding methodologies: mortality and feed conversion ratio (FCR) were lower in deeper cages (eight metres). High light intensity from natural sunlight had negative effects on feeding behaviour. The structure of the visual system indicated that meagre are a nocturnal species that prefers low light intensity environments.

A total of 50 percent of the stomach content had been transferred to the rest of the digestive channel eight hours after feeding. Self-feeding fish feed during the entire 24-hour period throughout the year. Feeding behaviour was stimulated by both visual (light) and mechanical (aeration) cues.

In conclusion, variable growth rates appeared to be related only to genetic differences, which suggested that genetic breeding programs and domestication might be the solution to this problem. The information obtained, indicated that an optimal feeding methodology should adjust to the biological characteristics of meagre by feeding when light intensity is low (dusk, dawn and night), using stimuli to ensure a good feeding response from fish that often cannot be observed and fish should be left to digest during periods of high light intensity (daytime – particularly mid-day).

Fish Health

During the course of the DIVERSIFY project, all major diseases and health-related issues of meagre were recorded and studied. Through the various tasks, studies of key disease states, development of appropriate treatments, and a first characterisation of the meagre immune system/immune responses were carried out.

One of the most important bottlenecks of meagre production is Systemic Granulomatosis (SG), a pathological condition affecting the majority of farmed populations. Through various tasks we tried to identify the etiology of the disease; we have run various feeding trials to identify potential nutritional causes of SG and we monitored meagre populations farmed in various locations in order to isolate and identify *Nocardia* spp., or other granuloma-associated pathogens.

The general conclusions from these tasks were that nocardiosis is not the cause of SG; the addition of vitamin D3, selenium and manganese did not stop the development of the disease, while high dietary content of phosphorus, vitamin E and C in a fishmeal-based diet seemed to improve the condition.

From the pathological assessment performed on various fish samples, a diagnostic protocol for SG was created based on the results of visual inspection, histopathology, electron microscopy and on the assessment of selected blood biochemical parameters.

Meagre is one of the fish species that are sensitive to Chronic Ulcerative Dermatopathy (CUD). The results of the studies in DIVERSIFY indicated that CUD is induced by the use of borehole water; however, neither pH nor CO2 are the underlying causative agents. The causative agent is still unknown, however a full description of the syndrome in meagre was made using histology and SEM (Scanning Electron Microscope), as well as osteoclast activity using molecular markers.

Most of the currently important diseases in meagre are of parasitic etiology, such as the monogenean *Sciaenacotyle panceri*. One of the tasks was focused on the use of essential oils with vermicide properties in order to test their efficiency as parasiticide. Overall, cinnamon showed immunostimulant properties and a clear potential to treat a parasitosis with *Sciaenacotyle panceri*i when administered orally to juvenile meagre. Furthermore, the first report of *Diplectanum scianae* infecting cultured meagre and the first report associating this parasite with fish mortality was recorded within the project framework.

Regarding the other pathogens of meagre, a wide range of molecular protocols for diagnosis by PCR have been developed that can be used for the detection of *Vibrio anguillarum*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*, *Photobacterium damselae* subsp. *piscicida* and *Nocardia* spp.

Moreover, based on the results obtained of the occurrence of different pathogens and experimental challenge test, recommended protocols have been developed with the recommendations on specific antibiotic dosages and treatment regimes. Furthermore, since meagre is a relatively new species to aquaculture it was necessary to document the immune response of specific genes under conditions of vaccination and against pathogens of significance for commercial aquaculture, such as *Vibrio anguillarum* as it is a pathogen with broad host-range and likely to be of concern for intensive rearing facilities of meagre.

In total, 28 assays have been developed for measuring the expression of genes related to the immune function in this species. Moreover, two different vaccine preparations were evaluated in the trials performed that appeared to stimulate positively immune responses of a diverse repertoire. Further, immersion vaccination against *V. anguillarum* showed that it

conferred protection in vaccinates when challenged with *V. anguillarum*.

These data, and related published work from this project, show the potential to modulate immune responses in meagre in culture, such as by delivery of immuno-stimulants, to enhance particular immune pathways at a time of disease risk such as prior to transport.

Socioeconomics

There are some important bottlenecks in aquaculture consumption in Europe, such as the fact that a large proportion of EU consumers is product-loyal in buying food and, therefore, also in fish products and that in many countries and/or segments of the EU market, aquaculture fish have a weaker image than wild fish.

Thus, the introduction of new species requires in depth market research, as it has been done in DIVERSIFY. So, parallel to the technological improvement of production methods for the new species, expansion opportunities for the EU aquaculture sector have been identified.

Market research identified market potential for aquaculture fish products in cross-cultural consumer segments, with increased-to-strong interest in new products in the main EU fish markets (France (F), Germany (D), Italy (I), Spain (ES) and United Kingdom (UK)). In this project processed new fish products were developed and sensory and conceptually tested by consumers in the five selected countries.

This resulted in a sensory positioning in regard to other species in the market and framing suggestions for marketing. Buyers and consumers would welcome new species, if they are a) sustainably farmed, ideally in domestic or EU waters; b) fresh (especially southern-EU) or mildly processed (northern-EU); c) easy to prepare and/or ready to eat; and d) competitively priced.

Concerning product development from the DIVERSIFY species, the first steps were to consult and discuss with different focus group, discussions with consumers and interviews with experts in the selected countries of the project (UK, D, ES, F, I).

This research provided a list of ideas for new product development that were further tested in the experimental and quantitative research that touched upon the two main areas of the work, namely related to development and selection of new product concepts from selected fish species, and evaluation and optimisation of newly developed fish products. Interviews with experts were conducted by using a structured questionnaire in each of the five countries to explore the possibility of creating new fish products from the ideas gathered from the focus groups.

Experts from different countries agreed that the created products were attractive and feasible ideas that have potential in the market. They considered that overall these ideas could increase fish industry profits due to the higher diversity of choice.

New product concepts, generated combining information of the market perceptions and the technical limitations and the economical prospect efficiencies, were submitted to a quantitative screening. From this screening, 12 concepts or ideas out of 43 that acquired the highest scores were suggested for product development. These 12 ideas covered different options: mass market products, products targeted to specific market segments and added-value products.

Meagre has been studied for its fillet composition, technical yield, fillet sensory properties and mechanical texture. The physical prototypes from the developed meagre products were designed based on the market potential, the product concept, consumer value perception and segmentation, physicochemical characteristics of the raw material, the technical properties of the products and the process and the availability of similar products in the market.

Breeder specimen at HCMR facilities

Sperm collection for in-vitro fertilisation

Meagre was used for the development of the “frozen fish fillets with different recipes”, “fish burgers shaped as fish” and “ready to eat meal: salad with fish”. The necessary information to obtain these new products, as well as a number of guidelines, processing conditions, technical specifications and troubleshooting were also described.

In addition, basic information regarding the packaging of the food products, conservation conditions, preliminary product shelf life and consumer handling/cooking specifications were provided as well. The technical feasibility suggested that it was possible to produce these products at an industrial scale, which was corroborated by the presence of other similar products in the market.

The technical quality of the developed products was also assessed. The total proximate composition of the products (protein, lipid, moisture, inorganic and carbohydrates content), the energy contents of the selected products, the quantitative nutritional value in aspects of fatty acids and the sensory profile of each of them was determined.

As expected, processing had an effect on both the proximate composition and fatty quality of the products when compared to the raw fillet tissue. However, the effect depended on the processing method used as well as the inclusion of additional materials (such as olive oil) during the product formulation. Processing generally had a negative effect on nutritional quality reducing the proportion of essential fatty acids, i.e. EPA and DHA, of the majority of products when compared to the corresponding fish fillets.

Regarding sensory properties, all processed products exhibited unique sensory profiles. The processed products showed a more complex sensory profile, with more attributes than the unprocessed cooked fillet of the species. The developed characteristics of the processed products in their majority were connected to the added materials and/or the processing method.

Finally, the correlation between the fish dietary history (e.g. dietary fat and protein levels, fat sources, etc.) or other rearing parameters (e.g. rearing system, temperature, or density) and the end-product quality was evaluated. Results indicated that filleting yields and protein contents did not seem to be influenced significantly by rearing and dietary histories at grow-out stage. Meagre filleting yield and protein content were quite attractive.

Its total fat contents did not seem to be highly influenced by the dietary or growing history, displaying low contents of fat, which is an attractive feature for low fat dietary regimes.

Business model and marketing strategy

Each of the DIVERSIFY species has advantages in relation to the current aquaculture fish assortment in EU stores. The selection of species has been broad and diverse. However, production challenges make production process outcomes still uncertain for some of the species (e.g. wreckfish and grey mullet) and a constant and high-quality supply is therefore still difficult.

Consequently, selling to large retail chains could be difficult or impossible at this stage. This channel demands a continuous stream of production. Based on this, suppliers of the experimental species are advised to begin by selling to smaller retailers/parties and local restaurants.

This strategy will help generate cash flow that can be invested to further professionalise production. For these producers, collaborating with innovative channel partners (for co-creation and co-investment) is the best bet. The more promising business opportunities and thus business models concern meagre, greater amberjack, pikeperch, and Atlantic halibut.

For these species, most bottlenecks in production have been addressed satisfactorily. The challenge now is to grow customer demand and market acceptance. The newly developed products can help give an impulse to this effort. The results showed a coherent business story for these four species, which is the first litmus test for any viable business model.

The value propositions for meagre is based on the fact that meagre is a white-flesh fish with an attractive shape that offers lean fillets with excellent texture and mild flavour. Its firm texture makes it very versatile; it is suitable for a large variety of recipes. Its rapid growth rate allows producers to farm them to larger size than many other farmed competitors (e.g. European sea bass or gilthead sea bream).

It is appropriate for filleting and further processing. Promotion of this marine fish could stress high omega 3, leanness, and excellent taste. The fact that the species is known under different names in different countries requires attention.

While very good possibilities exist for the above production-ready four species, results did show that firms should increase their attention for marketing and relationship building with channel partners. Although farmers will benefit from enhancing their production processes to further increase quality/growth and decrease cost, investments in marketing and sales/channel management generally lag behind.

This could jeopardise the chance of penetrating the market and reaping profits from their efforts. However, only with a buy-in from distribution partners and adequate marketing efforts can consumers be reached and convinced to adopt and continue purchasing these new species and products.

Marketing can benefit from using country/region of origin branding and health claims (e.g. high omega 3), among others. Certification issues also need to be remedied, particularly since, for example, super markets consider this a key purchasing requirement. Without proper certification, market development may proof difficult at best.

A technical production manual and a fish health manual have been produced for meagre, and can be downloaded from the project's website at www.diversifyfish.eu