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aquaculture europe

VOL. 44 (1) MARCH 2019

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THE MEMBERS' MAGAZINE OF THE EUROPEAN AQUACULTURE SOCIETY

1st Semester 2019
Afgiftekantoor: 8400 Oostende Mail

VOL. 44(1) March 2019

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Subscription to the Aquaculture Europe Magazine: €85

For air mail add €16 to prices above

Design: James Lewis, Capamara Communications Inc.

Printed in Belgium ISSN 0773-6940

Results from the DIVERSIFY project

REARING OF GREATER AMBERJACK

(*Seriola dumerili*)

INTRODUCTION

At the end of November 2018, the project DIVERSIFY (www.diversifyfish.eu) concluded, after 5 years of research with the aim of contributing to the expansion of the European aquaculture industry by diversifying its production with new/emerging species (Mylonas et al., 2017). The species selected in DIVERSIFY have important advantages over the ones cultured currently, such as fast growth, large size or low requirement in fishmeal and oil. In addition, DIVERSIFY determined the drivers for market acceptance of the new food prototypes in order to position the EU aquaculture sector as a leader in aquatic food production.

The European Union (EU) is the largest importer of fisheries and aquaculture products in the world. However, aquaculture provides only 20% of the seafood produced in the EU, while the worldwide contribution of aquaculture towards seafood production is already >50% (FAO, 2018). An efficient, sustainable and market-oriented expansion of the EU aquaculture sector based on new fish species and products will reduce the dependence of the EU on imports, reduce the pressure on over-exploited fisheries and explore new segments and tailor-made products for the EU market. DIVERSIFY has identified six new/emerging finfish species, with a great potential for the expansion of EU aquaculture (Mylonas et al., 2017). One of the selected fishes was the **greater amberjack** (*Seriola dumerili*), which is suited for warm-water marine cage culture (Figure 1). Research was carried out in different scientific disciplines, including Reproduction and Genetics, Nutrition, Larval and Grow out husbandry, Fish health, Socioeconomics and Final product quality.

Figure 1. The greater amberjack (*Seriola dumerili*)

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Figure 2. Wild amberjack broodstock captured around the island of Lampedusa, Italy.

The greater amberjack is a highly valuable commercial species and a popular game fish. The total worldwide catch of this species has increased tenfold since 1990, still only reaching 3,287 tonnes in 2009 (FAO, 2018). The greater amberjack flesh is much appreciated by consumers and its market quotations are high, ranging between 8 and 16 euro per kg in Europe and reaching $20–30 per kg in Japan. In the late 1980s, a greater amberjack farming activity initiated in the Mediterranean basin, based on the grow-out of juveniles caught from the wild and cultured in tanks and cages (Lovatelli and Holthus, 2008; Ottolenghi et al., 2004). Greater amberjack aquaculture production has an enormous potential for expansion: the total value of the global aquaculture production was just $808,051 in 2007 (source: Cultured Aquatic Species Information Programme, *Seriola dumerili*, FAO, 2016), despite its high-quality flesh and high consumers' appreciation. Its rapid growth rate, worldwide market demand and high quotations make the greater amberjack a very promising aquaculture species. However, a proper commercial aquaculture production of the species has not developed so far, mainly due to its inconsistent and unpredictable reproduction in captivity, which prevented the development of hatchery production of juveniles and the conversion of this capture-based farming activity into a true aquaculture industry.

In this article we present a summary of the results obtained in DIVERSIFY. A technical "Production Manual" for greater amberjack, has been also produced by the project and is freely available in the project's website (https://www.diversifyfish.eu/amberjack-workshop.html), and can be used by the industry to begin investigating the potential of greater amberjack as an alternative marine species for European warm-water aquaculture.

REPRODUCTION

In order to facilitate the broodstock management of greater amberjack in aquaculture, important life history traits of wild fish were first determined. Fish are 35-40 cm in length (fork length, FL) and 1 kg in weight (body weight, BW) at age 1; 60-70 cm FL and 3-5 kg BW at age 2; 80-90 cm FL and 7-10 kg BW at age 3. Male greater amberjack are reproductively active at the age of 3 years and females reach the first sexual maturity at 3-4 years of age. The spawning season of the wild greater amberjack population from the western Mediterranean is extended from late May to early July (Figure 2). When greater amberjack reared in sea cages in the Mediterranean were handled as other captive species, they exhibited poor gonadal development, low pituitary gonadotropin gene expression, low gonadotropin and sex steroid plasma concentrations, atresia of vitellogenic follicles, reduced proliferation and increased apoptosis of male germ cell (Pousis et al., 2018; Zupa et al., 2017a; Zupa et al., 2017b). As a consequence of the spermatogenesis impairment, greater amberjack confined in captivity showed low sperm quality, in terms of sperm density, motility and velocity, as well as ATP content and membrane integrity (Zupa et al., 2017a). The observed reproductive impairments are likely related to the handling stress, the lack of optimal conditions required for reproductive maturation and/or to nutritional imbalances caused by the lack of specific broodstock diet for the species. In fact, gonads of captive-reared greater amberjack had different lipid and fatty acid contents compared to wild individuals. An overall improvement of rearing technology, particularly as it relates to husbandry operations (*e.g.* fish handling and transferring) together with a better formulation of dietary ingredients (Sarih et al., 2019) is suggested to overcome the observed dysfunctions and improve greater amberjack reproductive performance.

Greater amberjack reared in sea cages in the Mediterranean without any handling during the reproductive period (Figure 3), were then successfully treated with the reproductive hormone gonadotropin releasing hormone agonist (GnRHa) via sustained-release polymer implants and injections (Fakriadis et al., 2018). Treatments with GnRHa implants at the dose of ≈50 μg kg^{-1} body weight were more effective that injections in promoting the proper endocrine pathways leading to multiple cycles of oocyte maturation, ovulation and spawning and allowed production of more eggs without altering their quality in terms of fertilization, embryo survival, hatching and larval survival. This method helped minimize the handling stress (*i.e.* one handling every two weeks as opposed to one handling every week) and was very effective in producing eggs of adequate numbers and quality for the implementation of larval rearing at commercial scale.

Greater amberjack caught from the wild in the eastern Atlantic (southwester coast of Gran Canaria, Spain) when they were 3-5 kg in body weight and reared for two years in indoor tanks under appropriate environmental and nutritional conditions (Sarih et al., 2019), were able to undergo normal gametogenesis, and spawned spontaneously large quantities of high-quality eggs (Sarih et al., 2018). In the same stock, hatchery-produced F1 greater amberjack (15-30 kg body weight) reared in outdoor tanks in Tenerife (Spain) underwent normal gametogenesis and were induced successfully to undergo maturation, ovulation and spawning through the administration of GnRHa implants at doses of 50 and 75 μg kg^{-1} body weight (Jerez et al., 2018). The repeated administration of GnRHa implants resulted in multiple spawns of high quality fertilized and viable eggs for an extended period lasting from May to September. Consistent egg production is now available for this species, and has enabled the further development of larval rearing methods within the project.

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Figure 3. Sea cages in Greece where greater amberacjk breeders were maintained

Therefore, thanks to the experimental work carried out within DIVERSIFY, a set of tools to reproduce greater amberjack reared under different conditions in the Mediterranean Sea and in the eastern Atlantic is now available, and this represents a fundamental step towards the large-scale aquaculture production of this species. We expect that these spawning induction methods will enable the incorporation of this species in the aquaculture industry in Europe and its large-scale aquaculture production.

NUTRITION

To improve larval enrichment products for greater amberjack (Figure 4), the optimum levels and ratios of essential fatty acids and combined PUFA and carotenoids in greater amberjack enrichment products were determined (Roo et al., 2019). The highest greater amberjack growth was obtained when larvae (17-35 days after hatching, dah) were fed *Artemia containing* Docosahexaenoic acid (DHA; 22:6n-3) in a range of 5-8% Total Fatty Acid (TFA), with a maximum around 7% (1.5 g 100 g^{-1} DHA DM). The essential fatty acid (EFA) requirements of larvae are similar during rotifer and *Artemia* feeding, as reported for larvae of other marine fish species. Requirements of amberjack larvae for DHA (1.5 g.100 g^{-1} DHA DM) were higher than those found in other marine fish species and similar to those for other fast-growing species. Increases in DHA tend to improve larval resistance to handling. Even the highest DHA levels in the enrichment emulsion (70% DHA in TFA) resulted in reduced incorporation of DHA into *Artemia lipids* (11% DHA in TFA). Despite that Eicosapentaenoic acid (EPA; 20:5n-3) levels in *Artemia* increased from 0.87 to 6.81 % TFA, EPA levels in greater amberjack larvae were only increased up to 5.2% TFA, denoting a saturation process that could be associated with the fulfillment of the EPA requirements. On the contrary, DHA levels in greater amberjack larvae showed a linear increase. Dietary DHA was linearly related to skull anomalies, dietary DHA levels over 2 g per 100 g^{-1} inducing a higher incidence of skeletal malformations, particularly those related with skull development.

It is well known that raising the ratio of Phospholipids (PL) to Total Lipids (TL) in larval feeds may enhance growth. Rotifers enriched with marine lecithin (E1) displayed a fast incorporation of polar lipids particularly rich in DHA. Although the role of carotenoids in embryonic development is not very well established, there is evidence that the presence of carotenoids mitigates deleterious oxidative damage to the developing embryo. Larvae fed diets with astaxanthin below 5.3 ppm were found to have marginal growth, whereas those fed levels above 5.3 ppm had a better performance and significantly higher lipid levels. Rotifers enriched with polar rich emulsion containing a marine natural lecithin LC60 combined with 10 ppm of Naturose also resulted in a significant advantage in larval growth, survival and welfare compared to rotifers enriched with other emulsions. Thus, DIVERSIFY established the following recommendations for enrichment products for greater amberjack larvae culture: DHA in enrichment products for *Artemia* 10-17% TFA, EPA 14-20% TFA, and DHA/EPA ratio 1-5. For rotifers (*Brachionus sp.*), DHA in enrichment products 14% TFA, EPA 6% TFA, and DHA/EPA ratio 2.3. Carotenoid levels in enrichment products must be around 10 ppm.

Regarding greater amberjack juveniles, the dietary lysine requirements, based on the Broken-line model, 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.

In broodstock diets, the requirements of essential fatty acids were determined to obtain improved spawning quality (Sarih et al., 2019). Broodstock fed a diet containing 1.57% EPA+DHA showed high fertilization and egg viability, higher number of eggs per spawn and kg of female, with the highest percent of fertilization, egg viability, hatching rate and larval survival. Egg fatty acid composition was shown to be influenced by broodstock diets. A diet containing 14-15% EPA+DHA of total fatty acids (corresponding to 2.5-3% in a dry diet) resulted in the best spawning performance in greater amberjack broodstock. Increasing dietary EPA+DHA contents did not improve spawning performance. Histidine contents in broodstock diets from 1 to 1.5% and Taurine in broodstock diets increased the reproductive performance of greater amberjack.

So, the nutrient requirements for greater amberjack larvae were determined, improving larval growth and survival and reducing skeleton deformities. Recommendations for enrichment protocols for both *Artemia* and rotifers have been

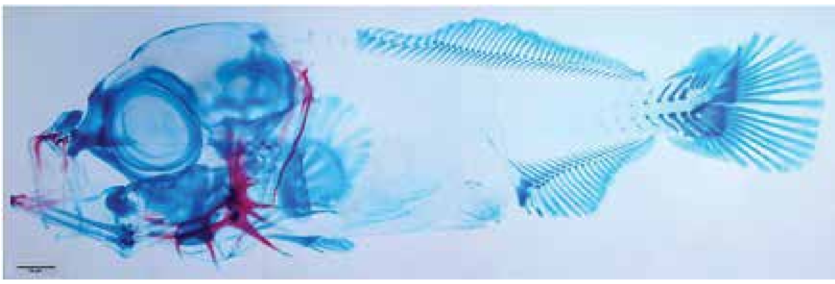
Figure 4. Stained larvae of greater amberjack (5.64± 0.11 mm).


Figure 5. Hatchery produced greater amberjack juveniles

produced, improving the quality of the fingerlings produced. The lysine requirement has been determined for juveniles, improving the feed conversion ratio (FCR) and growth of juveniles in sea cages. For broodstock diets, important improvements have been done, determining the levels of essential fatty acid, histidine and Taurine to improve the spawning quality of this species. The different results obtained will be indispensable for the feed production companies that will be called to produce specialized feed for the emerging greater amberjack aquaculture industry.

LARVAL HUSBANDRY

The objectives of DIVERSIFY for larval husbandry were to (a) study the effects of different feeding strategies on larval performance in intensive systems, and (b) develop feeding protocols and rearing methodologies in semi-intensive systems for the industrial production of the species. Starting with the ontogeny of the digestion and the vision system to acquire the basic biological information, our studies focused on the prey enriching diet and feeding regime and finally to critical parameters (tank type-shape, duration of the photo phase, tank background color and light conditions, stocking density) of the rearing process. The results were evaluated in terms of growth, survival, skeletal deformities, biochemical composition, stress and larval condition.

The results indicated that larval rearing in large tanks and low initial stocking of eggs-larvae improved the growth performance and survival of greater amberjack. Egg stocking densities >25 eggs l^{-1} affected negatively the results. For the different environmental parameters the ranges considered as optimum can be summarized as follows. The recommended photo phase is 24L:00D from 1 to 20 days after hatching (dah) and 18L:06D between 21 and 30 dah, with light intensities of 800, 1200, 1000 and 500 lux at 3, 6, 12, and 20 dah, respectively. A renewal of filtered seawater (5 µm) at an increasing rate ranging from 15-40% day^{-1} at 1 days after hatching, 30-40% at 10 dah, 100-120% at 20 dah, and 200-240% at 30 dah ensures a good quality of the rearing environment. Dissolved oxygen ranged between 4.9 and 8.2 mg l^{-1}, but must be preferably > 6.0 mg l^{-1}, salinity between 35 and 40 psu, pH between 7.8 and 8.5, and temperature between 23.5 and 25.0ºC. Furthermore, the feeding protocols used have to be coordinated with the rearing conditions and the larval development. The larva has to be able to see, ingest and digest the food, and therefore needs the coordinated development of vision and digestive system. In general, the addition of live microalgae at 150-300 x 103 cell ml^{-1} from 1 dah, enriched rotifers two or more times a day, from 3 to 25 dah, at densities between 3 and 10 rot ml^{-1}, *Artemia* nauplii at 12 dah and enriched 1-day-old *Artemia* EG at 14-18 dah, followed by commercial weaning diets (200-800 µm) from 18 dah can be a good sequence. Moreover, the enriched emulsions of prey supplemented with PL, carotenoids, Arachidonic acid (ARA; 20:4n-6) and immune modulators such as *Echium* oil and black cumin oil improved the larval rearing of greater amberjack, so enrichment that results in these characteristics would give better results.

During larval rearing, and especially following 20 dah, high size variability occurred in all rearing systems tested to date. This high variability has been managed until now with early sorting of the reared groups to appropriate size classes. Applying standard methods and equipment available in all hatcheries, the sorting procedure resulted in significantly higher survival compared to unsorted groups. Unsorted groups between 20 and 30 dah had >90% mortality, while

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Figure 6. Grow out of greater amberjack in sea cages in the HCMR facilities in Crete, Greece

for the sorted groups it was limited to approximately 10%. During sorting, transport of the individuals is also required. Individuals of less than 15 mm do not tolerate netting and transfer should be performed with care and avoid air exposure of the larvae. After reaching 20 mm in total length individuals can be netted normally. Husbandry practice with larger individuals (>0.5-1 gr) is easier, although in some cases light anesthesia may help.

Therefore, as a result of DIVERSIFY a commercial larval rearing protocol has been developed, with a considerable increase in larval survival over previous attempts (Figure 5). This protocol enabled the industry – both partners in DIVERSIFY, but also non-partner companies – to obtain juveniles from the research partners or to produce juveniles themselves, and then start pilot sea cage rearing.

GROW OUT HUSBANDRY

For the grow-out tasks of greater amberjack, development of methodologies emphasized cage technology (Figure 6). The feeding pattern of different age classes has been studied, while trials to define optimal stocking densities were implemented. Furthermore, there were trials aiming to study temperature effects on growth performance of greater amberjack. Cage rearing is important for the commercial production of greater amberjack, but appears to be challenging. Several trials have been performed at an industrial scale and during all trials fish accepted commercial feed of appropriate composition, i.e. high protein (of fish origin) without problem. There was also no problem during the standard husbandry practices of net cleaning/changing and although the stocking density was not high, a value of ~ 5 kg m^{-3} is considered acceptable for a pelagic fish. Regarding the growth performance, during the first 4 months the growth was high (5 g d^{-1}) while it decreased later by 50%. Significant variations in growth were observed among individuals resulting in size variability of almost 100%, a problem that requires further investigation. Another significant difficulty during grow out is the occurrence of parasitic infestations by monogenean worms *Zeuxapta seriolae* and *Neobenedenia girellae* (figures 8 and 9). Even though treatment with peroxide was effective, the application is not easy and appropriate methodologies suitable for large cages should be developed. The species is also facing bacterial infections and in the case of Greece incidences with *Vibrio harvey* were reported, causing significant mortalities.

Environmental temperature was shown to affect significantly the performance of greater amberjack. Juveniles of 5 g held at 26°C showed significantly higher body weight compared with fish held at 22°C or 17°C (Fernández-Montero et al., 2017). Morphological analysis showed that the increase of temperature led to an elongated fish body, especially of the head. Also, the specimens reared at 26°C showed significant swimming differences compared to the individuals reared at 17°C and 22°C, while there was no difference between the later groups. For individuals of 350 g body weight, fish held at 21°C showed significantly higher growth compared to fish held at 26°C, while fish held at 16°C showed the lowest final body weight. The survival rate was higher at 16°C, but there was no significant difference in the FCR for the whole experimental period of 3 months. Nutrient digestibility coefficients were high, indicating the good quality of the diets. Although temperature is one of many parameters affecting gut transit time it did not affect energy fat, protein and dry matter digestibility of greater amberjack. Finally, fish of 500 g showed no significant differences for the temperature studied (20ºC and 23ºC) on feed intake and growth.

Regarding stocking densities, results showed that it affects growth rates and feed intake. Fish maintained at a high density (7 kg m^{-3}) had lower specific growth rate, while the condition index presented no difference between the groups for individuals of 5 g. Further to this, feed intake was significantly lower at low density (2.5 kg m^{-3}) for individuals of 150 g at the beginning of a 4-month trial, a tendency that changed in the fourth month during which the feed intake decreased with the increase of density. No negative effects on growth were observed in fish of 150 g initially stocked at 3.2 kg m^{-3} and reaching a final stocking density of 6.8 kg m^{-3}. Results from immunological parameters suggest that greater amberjack reared at the higher stocking density are not under a stressful condition. For the feeding pattern, studies showed that greater amberjack juveniles grew less when fed at 2.5% body weight d^{-1} compared to fish fed 3.5% d^{-1} or at apparent satiation. Furthermore, animals fed one meal daily showed lower growth compared to those fed 3 or 4 meals per day. Similarly, FCR was higher for fish fed 2.5% body weight d^{-1} or once daily compared to the other conditions tested. For larger individuals (200 g), between the feeding frequencies tested (1, 2, 3 and 7 meals d^{-1}) the best results in growth and FCR were obtained with 7 meals daily. The absence of changes in the hematological and biochemical parameters among the different feeding frequencies suggests that greater amberjack juveniles were able to adapt to the different feeding frequencies under the particular culture conditions. However, results from immunological parameters revealed differences in the immune status among fish subjected to different feeding frequencies that could influence the health status of fish.

The results obtain in DIVERSIFY are of great importance, as several barriers for the industrial production of the species have been removed. The information accumulated during the project enriched the specific knowledge base with a range of parameters appropriate for the biological requirements of the greater amberjack (temperature range, feeding rates, stocking densities, innate immune status). Of particular importance was the involvement of private companies in the implementation of the task and the associated direct transfer of research results in the everyday husbandry practice in the farms. Worth noting is the initiation of the species commercialization in Greece by project partners and follow up by more companies. Despite the difficulties identified related to husbandry and health management (see below), the grow out of the species is feasible.

FISH HEALTH

Fish health is a key aspect to be optimized in cultured fish. The effect of the developmental stage, rearing conditions and nutrition on the capacity to modulate specific immune

Figure 7a. Secondary lesions after *Neobenedenia girellae* infection

Figure 8. Heavy infestation of *Zeuzapta seriolae* on greater amberjack

Figure 9. A *Paradeontacylix spp* infection on greater amberjack

responses will help predict vaccine responsiveness and fish health. New tools have been developed in DIVERSIFY, to study the immune system of greater amberjack, with the design of 20 primer relevant genes (IL-1b, IL-8, IL-10, IL-17AF, IL-17D, IL-22, Defensin, Hepcidin, Piscidin, RAG2, IgM, IgT, Mx prot, iNOS, IFN1, IFNg, TNFa, EF-1a, b-actin, MUC-2). Similarly, the characterization of greater amberjack piscidin, as an important antimicrobial peptide (AMP) gives new insights about the function of this species' immune system. These tools are an important advance in our ability to evaluate the health status of this species, which is highly susceptible to parasite infections.

Neobenedenia girellae is a monogenean parasite of the skin, and causes the main health problem for Atlantic populations of greater amberjack in aquaculture (Figure 7a and b). This monogenean has been described in relation with water temperature increases in sea cages around the Canary Islands, Spain. New insights about the relation of this parasite with its host shows the mechanical damage that the fixation causes, resulting in thickening of the epidermis, vacuolization of epidermal cells, disruption of cellular layers, recruitment of goblet cells, and mononuclear cell lymphocytic type mobilization to the adhesion regions. Because of this, secondary infections appear and could result in 100% mortality. New prevention strategies have been developed, such as the inclusion in the diet of mannan oligosaccharides (MOS and cMOS), which enhanced mucus production and increased the immune response, reducing the parasite load and growth (Fernández-Montero et al., 2019). A functional diet has been formulated to increase resistance of greater amberjack to the monogenean parasite *Neobenedenia girellae* and could be applicable for other monogenean parasites as well. This diet was based on a high protein inclusion (required for fast growing species) and the utilization of the mentioned additives with immunostimulant properties. This important milestone will provide a tool to reduce the incidence of this parasite in sea cages, reducing mortality of greater amberjack juveniles in farms.

Zeuxapa seriolae is another monogenean parasite of greater amberjack, considered the main health problem for greater amberjack culture in the Mediterranean region. This parasite gets attached to the gills (Figure 8), being hematophagous, producing important gill anaemia and inefficient oxygen exchange. Due to its rapid lifecycle and its increase with water temperature, it could cause the demise of the whole production. Treatments with hydrogen peroxide at 75 ppm during 30 min have been reported to be efficient for killing the adults, always combined with repeated treatments after 15 and 30 days, and net changes to avoid reinfection from the released eggs. Other parasites have also been described, such as the blood fluke *Paradeontacylix sp*., which is a blood parasite

Figure 7b
The skin parasite *Neobenedenia girellae*

that has been observed in cultured greater amberjack in the Mediterranean (Figure 9). The proliferation inside the host circulatory system could produce obstruction of blood flow, resulting in ischemia and necrosis, and gill destruction when the eggs hatch. *Penella sp.*is one of the largest copepod parasites of fish, typically from swordfish (*Xiphias gladius*) and marine mammals. This parasite gets imbedded inside the skin of greater amberjack, nevertheless, it is not considered a problem for greater amberjack culture.

Epitheliocystis is a bacterial disease caused by *Chlamydia, Endozoicomonas spp. and Ichthyocystis spp*. The presence of multiple cysts in the gills cause respiration problems in the fish and high mortality. New tools have been developed for the early diagnosis of the disease, with molecular PCR probes.

A Health Manual for greater amberjack describing different pathologies has been produced (https://www.diversifyfish.eu/amberjack-workshop.html) and is freely available in the project's website, and can be used immediately by the industry in order to improve their stock management. The manual describes the general incidence and potential treatments and some tools for identification of *Epitheliocystis, Zeuxapta seriolae,* the blood fluke *Paradeontacylix sp*. and the copepod *Penella sp*. The description of new or emerging pathologies for greater amberjack is of special importance for a rapid diagnosis and selection of the most efficient treatment to reduce mortalities in farms.

SOCIOECONOMICS RESEARCH

In addition to the biological and production research, DIVERSIFY also included market research concerning greater amberjack and its potential for Europe. The impact of this market research goes beyond marketing of this new fish species and provides the key insights on how any fish product can be marketed with added-value, what consumer segments are important, how the product should be priced, and where costs could or should be reduced to improve the margins. Therefore, there is a practical use of the research done within DIVERSIFY project for all aquaculture companies.

Market research has identified two cross-cultural consumer segments of "involved traditional", "involved innovators" across the top fish markets in Europe (*i.e.* France, Germany, Italy, Spain, and the UK) comprising of consumers that could be more interested in adopting new DIVERSIFY fish species and greater amberjack in particular (Reinders et al., 2016). The market segmentation has further shown that the future

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aquaculture production lays in the hand of the consumers who are more dependent on and involved in ethical and sustainability issues.

The market segmentation further allowed opportunity to co-create new product concepts from new DIVERSIFY fish species at the cross-border European level. The co-creation was undertaken with consumers from the same selected market segments mentioned above (Banovic et al., 2016). The key points for aquaculture companies when developing products from new fish species, such as greater amberjack, is that these products should reflect convenience, packaging must be appropriate with accompaniments and preparation suggestions, as well as related to environmental and health claims. These are important factors that should be met in order to proliferate success and create competitive advantage in the market. Finally, it was found that consumers would ignore the product if it is not relevant to a specific consumption problem, for instance convenience, and if the product is not superior to which consumer is presently consuming. Hence, marketing campaigns that merely promote current and traditional products are expected to flop, as the deficit of these products is what makes consumer to shift to other products.

The co-created product ideas were screened out and developed into product concepts and prototypes. From the selected concepts a few showed promising future if developed with greater amberjack. One (*i.e.* fresh fish steak) was selected for the greater amberjack product prototype involving lower levels of processing (Figure 10a and b). The physical prototype was selected based on the market potential, the consumer value perceptions, physicochemical characteristics of raw material, the technical properties of the products and the process, and the availability of similar products in the market. The undertaken research showed that product from greater amberjack was in all cases and across all investigated countries the best-perceived and -preferred product over all the other products developed from meagre, pikeperch, and grey mullet, always providing alignment with consumer expectations and consumption experience. Furthermore, it has been found that the products with a lower degree of processing and those characterized by the distinctive fish sensory properties, as the product from greater amberjack, were those products that had higher consumer acceptance. Products with higher degree of processing were more accepted by the consumers who do not like fish because of its taste, odor, as well as the presence of bones. This shows that the presence of different processed product alternatives could be a good solution to be able to cover more consumer segments.

The developed product concept from greater amberjack was further tested for optimal labelling attribute combination on packaging and price range. The experiments were undertaken in the same selected countries and with the same product from greater amberjack developed into the previously tested prototype. Based on this study it was concluded that country of origin and price are the attributes that drive the product acceptance, followed by quality certification (*i.e.* Aquaculture Stewardship Council - ASC label), while nutrition and health claims had a varying effect dependent on the country. The use of ASC label as the marketing signal to consumers that the

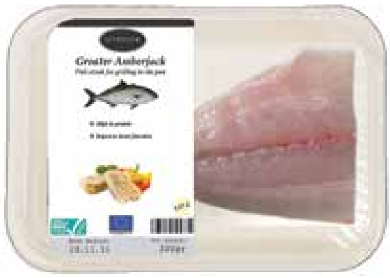

Figure 10a/b. Greater amberjack fillets

product is coming from a controlled, certified and responsible aquaculture actually increases the likelihood of consumers adopting this product. On the other hand, the use of nutrition and health claims actually assist European consumers to make more informed choices aligned with their preferences and stimulate health-related behaviour. However, nutrition and health claims are needed to be customized based on the target country. This research has also pointed to different segments of people how are nutrition conscious, ethnocentric, price conscious and eco-conscious, further suggesting possible targeted marketing campaigns that could be designed and used to further facilitate adoption of new fish species and greater amberjack in particular. Willingness to pay has also been estimated for the product from greater amberjack across investigated countries showing how the product should be priced.

The results from the virtual online market test also showed good acceptance of greater amberjack and its product in the same markets. This is related to two findings. First, the percentage of first time buyers of greater amberjack product was above 10%. Even if one assumes that not every one of these first time buyers might like the flavor of the new fish, it does inform that the new product has the serious potential on the market. Second, even those consumers that had not selected product from greater amberjack in the online market test, after receiving additional information decided to switch, with this number being above 11%. This finding additionally supports the fact that the product from greater amberjack would be well received by the market, chiefly as consumers had been met with alternative new species. Finally, when the numbers of people that directly or indirectly purchased greater amberjack have been aggregated, a total acceptance rate of 1/4 was estimated with slight variations depending on the country (*i.e.* southern versus northern countries).

The evaluation of communication effectiveness around new DIVERSIFY fish species and developed product mock-ups from greater amberjack in changing consumers' attitudes and behaviour showed good overall acceptance. Specifically, in the same selected countries it has been shown that there is a clear need for the aquaculture companies to endorse new products through marketing communication efforts and involve consumers that they can establish an superb source of sustainable food. This study has further shown that product from greater amberjack should not be promoted using nonspecific communication messages that cannot stand competition. These marketing communication efforts should instead promote distinctive production process by aggregating their prominence as traceable, healthy, but also flavorsome options for modern diets.

Based on the above DIVERSIFY results, greater amberjack shows very promising market prospects, given its superior sensory characteristics, good consumer acceptance, and price margins. Nevertheless, its introduction would have a larger impact if done country by country instead of general pan-European level. This should be seriously taken into account due to the differences in important consumers' buying motives and factors in each market. The developing outlooks per country vary, as in some countries early adopters easily try new fish species, while in other countries consumers' need extra marketing efforts. In all investigated countries, introduction of the new products with a reference to already familiar products advances consumer acceptance. Thus, the production of products from greater amberjack at an industrial scale is a feasible task if raw materials of good quality are used, as sensory properties are decisive factor for consumers, especially in new fish species. Additionally, good production practices should be applied with proper traceability, as this further influences overall product acceptability. The above factors are necessary and adequate conditions for achieving high quality and economically satisfactory products.

CONCLUSIONS

DIVERSIFY has achieved all its objectives in the greater amberjack research. Firstly, methods are now available to control the reproduction of greater amberjack reared under different conditions in the Mediterranean Sea and in the eastern Atlantic Ocean. This is a fundamental step towards the large-scale aquaculture production of this species. Secondly, using eggs from controlled spawning, a large number of artificially propagated juveniles were produced and already within the time frame of DIVERSIFY, a number of commercial operations in Greece –both partners of DIVERSIFY and non-partners—have begun pilot production of greater amberjack using eggs and juveniles produced by the project. As a result, the first harvesting of food-size fish begun already before the end of the project (Dec 2017) and in the Canary Islands this species has already been included in the Regional Plan for the Management of Aquaculture (PROAC), approved on July 9, 2018. It is apparent that it is now feasible for the aquaculture industry to consider the inclusion of greater amberjack in farmed species, and start developing the market in Europe and abroad.

Furthermore, through it socioeconomics and business research, DIVERSIFY is expected to have also a significant impact on removing bottlenecks in markets and consumer's perception and preferences, resulting in increases in the EU consumption of aquaculture products. Such an integrated combination of biological, technological and socioeconomic activities will lead to a reduction in the dependence of the EU on imports from third countries.

DIVERSIFY
New species for EU aquaculture

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