

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

**D**IVERSIFY 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 (GnRH<sub>a</sub>) 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 GnRH<sub>a</sub> 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.

### 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<sup>-1</sup>. 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 2:** Greater amberjack breeders maintained in sea cages in Greece and induced to spawn using GnRHa delivery systems (photo by HCMR)



**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



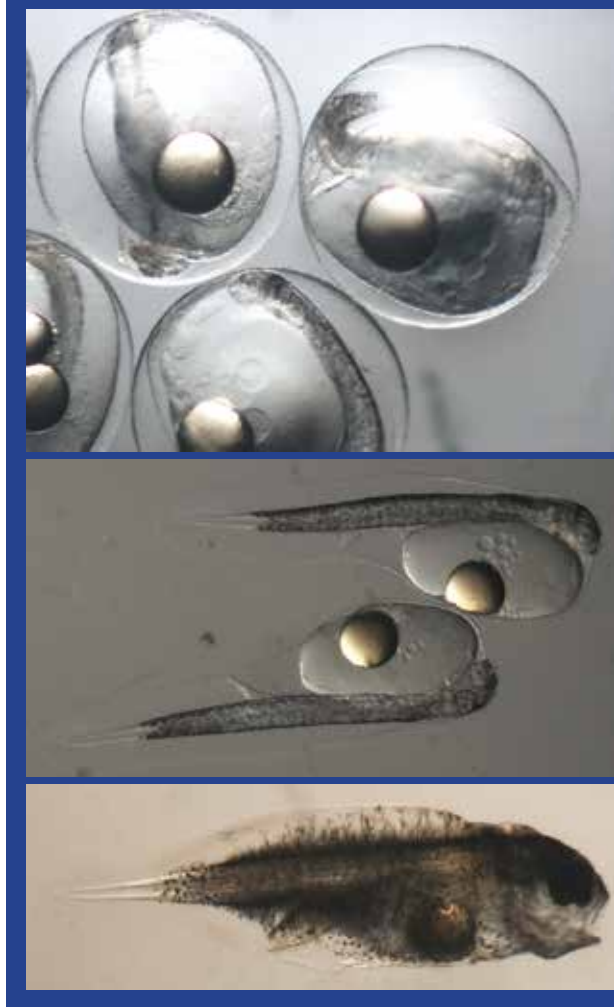
**Figure 4:** Greater amberjack juveniles from HCMR, sent to a number of commercial sea cage sites for on-growing trials (photo by HCMR)



**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.

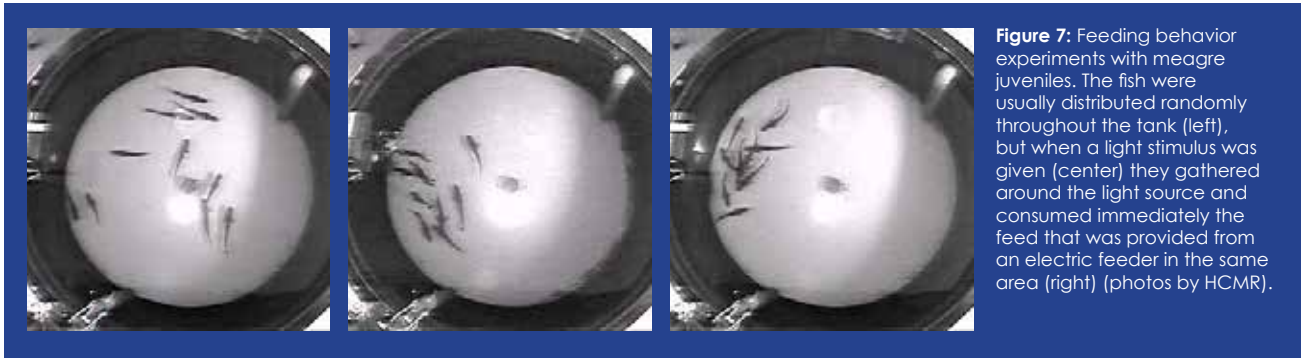
Finally in grey mullet, the first study that has been completed related to the definition of an optimal weaning diet. It was shown that fishmeal (FM) substitution did not affect any of the performance and condition parameters analyzed and that weaning wild grey mullet fry (which are zooplanktivorous) 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 damsela* 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 seriola* was the most prevalent and important parasitic pathogen.

Apart from *Zeuxapta seriola*, 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.

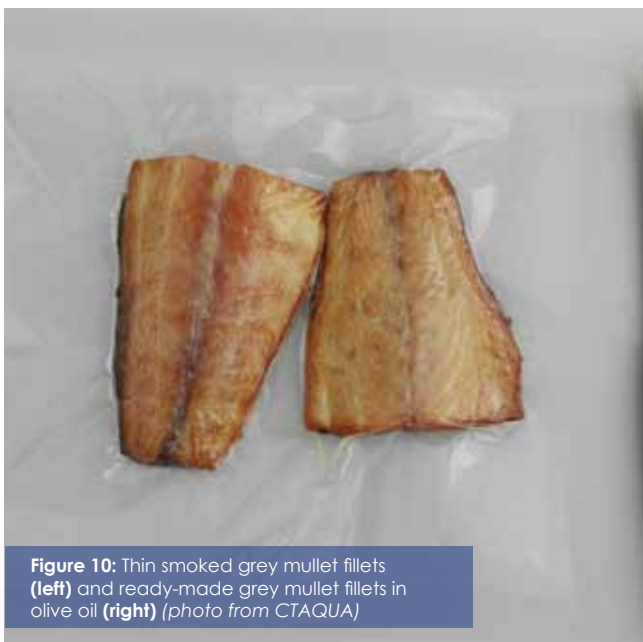
This growth can only be realized at the expense of other protein sources, since the protein market has been stabilized in the last few years. The southern countries eat more fresh whole fish, while northern countries prefer processed fish.

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>)

DIVERSIFY project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY).



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# DIVERSIFY NEW AQUACULTURE SPECIES

## Consumers' perception of new fish products

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**C**onsumers are the last step in the production chain and those who ultimately decide on the success or failure of a new product launched into the market. It is therefore essential to understand the factors affecting consumer behaviour and the key aspects driving decision-making and product purchasing.

Sensory properties have been identified as one of the main determinants of food selection and consumption. However, sensory perception per se might be strongly affected by other aspects such as individual characteristics (e.g. attitudes or expectations) and environmental factors (context, origin, brand name, price, etc.). In the same vein, expected quality seems to be one of the most important factors in consumers' intention to purchase food.

It is evident that quality cues are used to infer expected fish quality attributes at the point of sale. These cues can be grouped into intrinsic (colour, odour, eyes shape, brightness) and extrinsic (price, origin, quality labels), and their role in developing expectations depends on the type of fish or fish products and on the context (circumstances in which the product and individuals will interact) in which the product will be used or consumed.

In addition, it is worth making a clear distinction between experiential quality attributes such as convenience, freshness or sensory characteristics that will be experienced and ascertained at the time of consumption, and credence quality attributes, such as healthiness or naturalness, that cannot be experienced directly even after frequent consumption. Both quality attribute types can generate individual expectations, but only quality attributes experienced directly can be assessed, confirmed or disconfirmed.

To enhance consumer perception (both expected and experienced) of fish and fish products, additional information provided at the point of purchase through communication (i.e. on the product label/package), may play an important role in reducing uncertainty in the formation of quality expectations. In fact, detailed information seems to be one of the most effective ways to facilitate more appropriate expectations and to improve enjoyment.

In the framework of the DIVERSIFY project, twelve products

from new aquaculture fish species have been developed and tested from a technological, physical/chemical, microbiological and sensory perspective:

- (1) Frozen fish fillets (meagre, *Argyrosomus regius*) with different recipes,
- (2) Fish (meagre) burgers shaped as fish,
- (3) Ready to eat meal - salad with fish (meagre),
- (4) Fresh fish fillet (pikeperch, *Sanders lucioperca*) with different "healthy" seasoning and marinades,
- (5) Ready-made fish (pikeperch) tartar with additional soy sauce,
- (6) Fish (pikeperch) spreads/pâté,
- (7) Thin smoked fillets (grey mullet, *Mugil cephalus*),
- (8) Ready-made fish fillets (grey mullet) in olive oil,
- (9) Fresh fish fillet (grey mullet) with different "healthy" seasoning and marinades,
- (10) Frozen fish fillet (greater amberjack, *Seriola dumerili*) that is seasoned or marinated,
- (11) Ready-made fish (greater amberjack) tartar with additional soy sauce
- (12) Fresh fish steak (greater amberjack) for grilling in the pan.

These products were selected from a pool of 41 concepts 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 (France, Germany, Italy, Spain and UK), thus focusing on both experiential and credence quality attributes. One hundred participants were recruited in each of the five selected countries according to the following criteria: regular fish consumers (farmed and wild), evenly distributed by age and gender and without any food allergy or food intolerance. Purchase probability (willingness to buy) was also evaluated in order to estimate those aspects having a major impact on the individuals' buying intention.

Whilst extrinsic properties were evaluated for the twelve developed new products, for practical reasons intrinsic attributes were only assessed for 6 of them ((2) Fish burgers shaped as fish, (3) Ready to eat meal - salad with fish -, (6) Fish spreads/pâté, (7) Thin smoked fillets, (8) Ready-made fish fillets in olive oil and

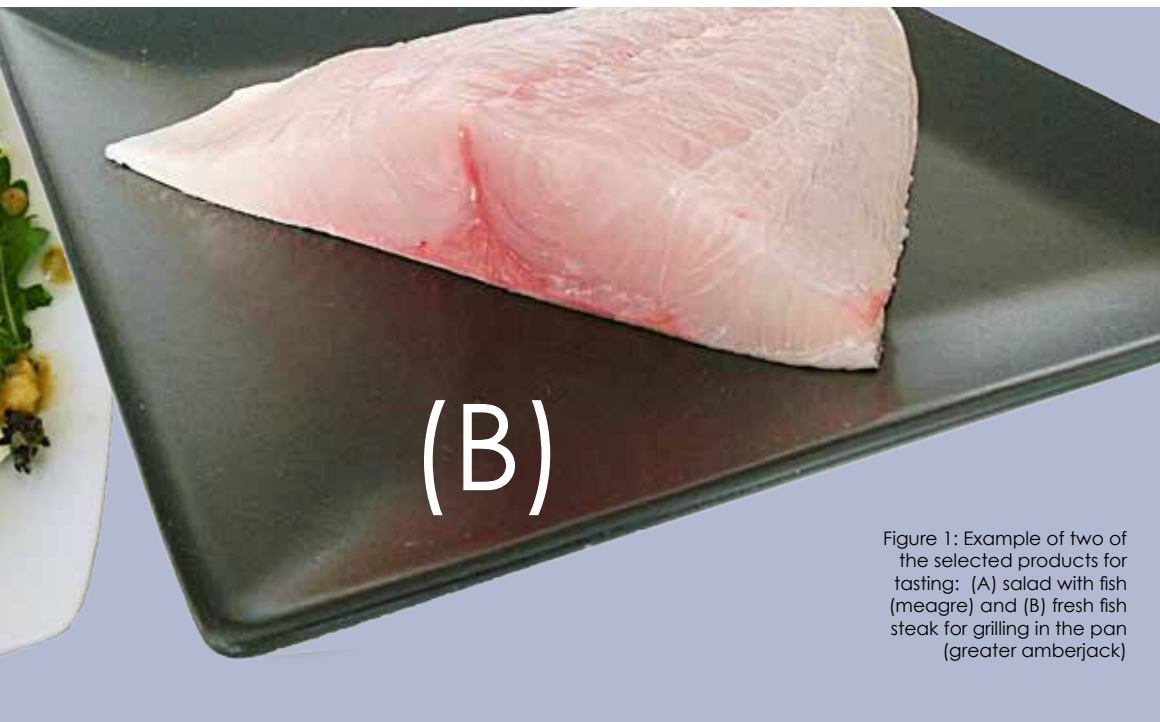


Figure 1: Example of two of the selected products for tasting: (A) salad with fish (meagre) and (B) fresh fish steak for grilling in the pan (greater amberjack)

Probably, products having a higher degree of processing would be more appropriate for consumers who do not like fish because of its taste, presence of bones, odour, etc. In these cases, the existence of different processed alternatives could be a good solution for those individuals looking for a more convenient and less “fishy” product. In general, and despite of the lower expected liking for the processed products all of them were perceived positively.

(12) Fresh fish steak for grilling in the pan. Figure 1 shows two of the selected products for tasting.

Products with a lower degree of processing were those who generated higher expected acceptance. Consumers involved in the present study were selected based on their regular consumption of fish (wild or farmed). This recruitment procedure could explain the higher preference for those products having the genuine sensory properties of fish, without any interference.

Hamburger and fish pâté were the two products that were worst perceived regarding the presence of additives and naturalness. Grilled fillet was in all cases the best-perceived product in agreement with its higher expected acceptance. The most important parameter affecting liking expectations was the expected taste of the product.

Health, nutritional and well-being related issues were relevant as well in order to increase individuals’ expectations, but to a lower extent. These findings seem to indicate that, in general,



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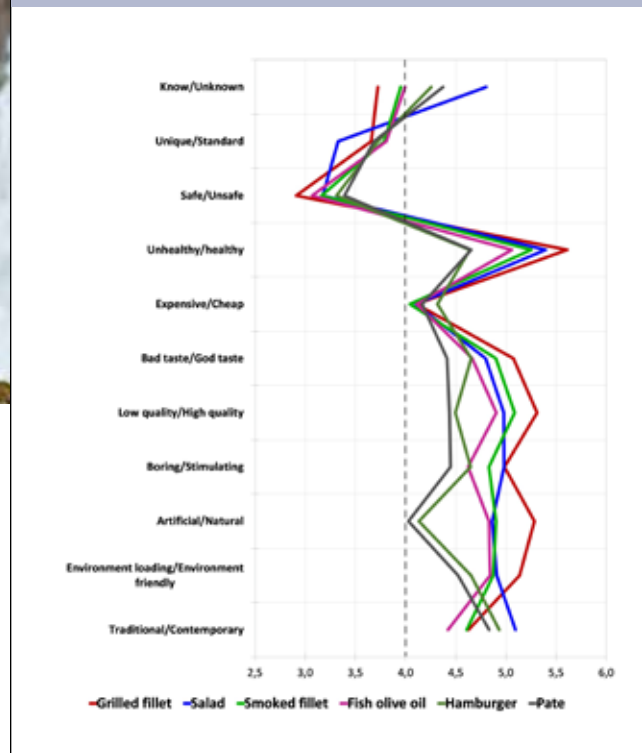
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Figure 2. Semantic profiles obtained for the pooled data set (5 countries, n=500).



consumers are unwilling to sacrifice taste by an improvement in health or functional properties. In a general sense, the perception of these products was similar across countries.

Once the product was blind tested, the most preferred product was the grilled fillet and the least appreciated the fish pâté in agreement with the previously reported expected liking. The same pattern was observed in all the studied countries with the sole exception of Spain, where the least preferred product was the fish salad.

The acceptability results obtained confirm those previously reported regarding consumers' expectations, and also seems to indicate a tendency to prefer the low processed fish products; although, fish hamburgers were the product that most improved their valuation compared to their expected liking. Based on the segmentation analysis carried out, all of the selected fish products assessed in the present study seem to have a specific niche within the European market.

Even though the different products were perceived similarly in the different locations regarding the acceptability ratings, they were described in a clearly different way when dealing with the main intangible dimensions that might define them (taste, convenience, environmental impact, etc.)

Figure 2 shows the results obtained by means of a semantic differential scale designed to measure the connotative meaning of objects. In this scale respondents from the five countries were asked to choose where his or her opinion about each product lies between two polar adjectives, for example: 1 being "Safe", 7 being "Unsafe".

Generally speaking, the sensory dimension seems to have an important contribution to the overall acceptance of the product and to its purchase probability. Importantly, the stimulating character of the product also seems to play an important role.

On the contrary, it is worth mentioning the always-irrelevant environmentally friendly characteristic of the products. The most plausible explanation for this finding is that most of the different products assessed included in their description "sustainably produced" or "produced in an environmentally sustainable way", which could have minimized the perceived differences between products. In any case, noticeable differences between products regarding environmental friendliness were

observed, especially in the UK and Germany.

These results open a new framework of research aimed to understand the rationale behind the observed differences between countries and how they can be exploited to better design and commercialize the new products already developed. Such information as this will be essential in order to build different business models that aim to develop launching strategies for the different tested new products in different markets.

This 5-year-long project (2013-2018) has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY).

The consortium includes 37 partners from 12 European countries –including 9 SMEs, 2 Large Enterprises, 5 professional associations and 1 Consumer NGO- and is coordinated by the Hellenic Center for Marine Research, Greece.

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