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Advances in greater amberjack research

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Advances in greater amberjack *(Seriola dumerili)* research: the DIVERSIFY project

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The greater amberjack (*Seriola dumerili*) is a species with high potential for the EU aquaculture due to its fast growth (6 kg in 2.5 years), excellent flesh quality and global market. Its farming in the Mediterranean region started in the 1990s with wild-caught juveniles, but the production is still negligible, as several bottlenecks exist for its industrial production. These include the absence of reliable reproduction, limited availability of juveniles, lack of knowledge on the nutrient requirements and pathology of the species. The EU FP7-funded DIVERSIFY project (www.diversifyfish.eu) examines the major aspects of greater amberjack aquaculture in order to overcome these bottlenecks and develop appropriate rearing methods for commercial production. This article provides some highlights from the first 2 years of the project.

Reproduction & Genetics

The research activities on greater amberjack reproduction have focused on three aspects. Firstly, on the identification of the reproductive dysfunctions in greater amberjack reared in captivity compared to fish in the wild, in terms of sex steroid plasma levels, histological and nutritional assessment of gonad maturation, the vitellogenic process (liver vitellogenin synthesis and oocyte yolk accumulation), as well as male germ cell proliferation and apoptosis. Secondly, on the development of a spawning induction protocol and an egg collection method for wild-caught greater amberjack maintained in land-based tanks and cages in the Mediterranean Sea and in the eastern Atlantic Ocean. Finally, work has focused on the development of an optimized spawning induction protocol for a greater amberjack broodstock born in captivity (F1 generation) in the eastern Atlantic.

Sex steroid plasma levels and histological evaluation of ovaries and testes underlined that gametogenesis in some captive-reared greater amberjack broodstocks is unreliable and may be seriously impaired (**Fig. 1**). Although liver vitellogenin gene expression was lower in captive fish, the capacity of the oocytes to accumulate yolk did not seem to be affected compared to wild fish. In the males, an early decrease of germ cell proliferation was observed and spermatogenesis ceased completely during the natural spawning period in captivity. In addition, captive male germ cells appeared to be affected by

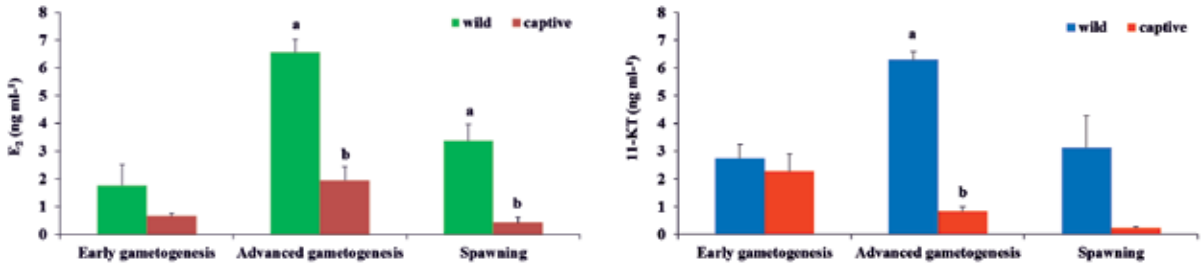

Figure 1. Plasma levels of 17β-Estradiol (E_2) in female and 11-ketotestosterone (11-ββ) in male wild and captive greater amberjack during the reproductive season. Asterisks indicate significant differences between wild and captive fish (ANOVA, $P < 0.05$), both in males and females.

an unnaturally high rate of apoptosis. It is hypothesized that the reproductive dysfunctions observed in captive-reared greater amberjack might result from the combination of captivity-induced stress, the lack of appropriate 'natural' spawning conditions, as well as nutritional deficiencies.

Experiments to induce maturation and spawning in the Mediterranean greater amberjack broodstocks by means of gonadotropin-releasing hormone agonist (GnRHa) implants, showed difficulties in obtaining eggs from broodstocks maintained in tanks. On the contrary, encouraging results toward the development of methods for reliable egg production have been obtained from fish maintained in cages (**Fig. 2**). Good quality of sperm during the spawning period, better development of the ovaries and better response to hormonal treatment with good fertilization of eggs was obtained when fish were maintained in sea cages during the year and either allowed to spawn in the cage or transferred to land-based tanks for spawning and egg collection. Unfortunately, egg collection in sea cages was either inefficient or negligible, and more effort is needed to develop appropriate egg collection methods for sea cages. However, the alternative method to transfer the fish to tanks for spawning after GnRHa induction was very effective, leading to the collection of ~22 million eggs from a small number of broodfish (n=6).

Experiments carried out in the eastern Atlantic Ocean (Canary Islands) demonstrated that wild-caught fish adapt readily to the captive environment and are capable of undergoing complete reproductive maturation, and are spawning spontaneously without the need of any hormonal therapies. Also, for the first time F1 generation greater amberjack at the final stages of vitellogenesis and at spermiation, were induced to spawn with treatment with GnRHa implants (**Fig. 3**). The fish underwent repeated spawnings for a period of 3 months, with a total production of about 15 million eggs. A major difference observed between the Mediterranean and Canary Islands broodstocks was the timing and duration of the reproductive season. Spawning started earlier (May) and lasted much longer (September) in both broodstocks in the Canary Islands, whereas in the Mediterranean the spawning season was limited to June and early July. These differences are probably due to differences in photoperiod and water temperature fluctuations, although genetic differences between the stocks have not been ruled out yet.

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Figure 2. Induction of spawning in a sea cage-reared broodstock in Greece (left), after evaluation of oocyte maturation stage (right). Some fish were transferred to land-based tanks for spawning after GnRHa treatment, but the majority of the fish were allowed to spawn in the cage (bottom).

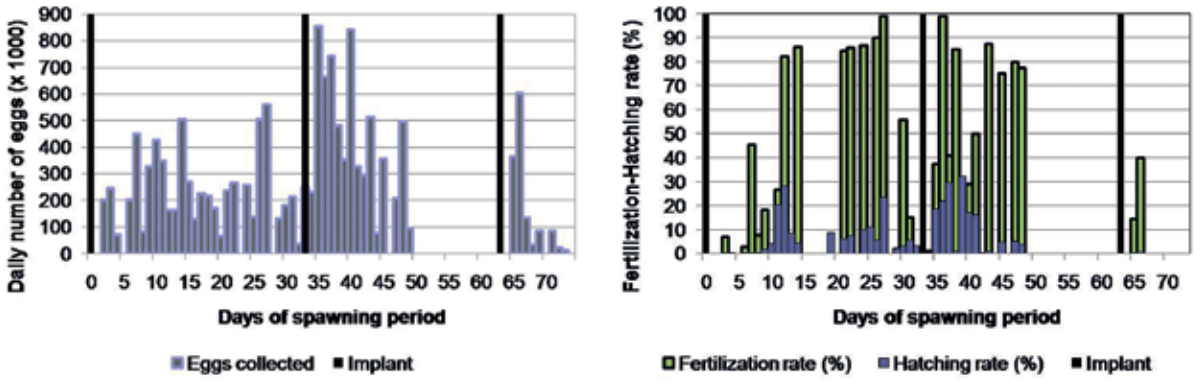

Figure 3. Induction of spawning in an F1 greater amberjack broodstock in Tenerife, Canary Islands (Spain) after three consecutive treatments with GnRHa implants.

Nutrition

The scarce knowledge on greater amberjack larval nutritional requirements leads to low larval survival and performance, and poor juvenile quality. Under this perspective, the overall objective of three feeding experiments performed by FCPCT and IEO/ULL was to determine the optimum levels of long-chain poly-unsaturated fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA), eicosapentaeonic acid (EPA) and arachidonic acid (ARA), as well as the combination of LC-PUFA and carotenoids in enrichment products for rotifers and *Artemia*. Trials were performed using different lipid and antioxidant sources, and evaluations included their effects on survival, growth, welfare, stress resistance/tolerance, bone development and tissue composition of the larvae. The results suggested that *Artemia* enriched with DHA at levels of 1.5 g 100 g $^{-1}$ dry weight and a 1.6 DHA/EPA ratio is sufficient to promote fast growth, whereas increased levels were associated with skull anomalies (**Fig. 4**). Skeletal elements such as the maxilla or mandible that develop from a cartilaginous precursor would be more sensitive to oxidative risks and, therefore, to dietary DHA elevation. Results also suggested that essential fatty acid requirements during rotifer and *Artemia* feeding are similar to those reported for larvae of other marine fish species. Requirements of greater amberjack larvae for DHA were higher than those found in other marine fish species and similar to those for other fast growing species, such as the yellowtail (*Seriola quinqueradiata*) or striped jack (*Pseudocaranx dentex*), whose larvae require 1.3–2.6 g and 1.6–2.2 g DHA 100 g^{-1}, respectively.

In addition, an enrichment protocol for rotifers was developed, containing 10 ppm of astaxanthin-based carotenoids, and a DHA-rich marine lecithin (LC-60) supplemented slightly with ARA (E1-10). Its use for just 3 h resulted in rotifers with high contents of DHA in the polar lipids, and a 2.7 DHA/EPA, resembling the composition of greater amberjack eggs. Feeding trials showed also improved larval performance (**Fig. 5**), and we expect that the data obtained will be used to design well-balanced enrichment products and weaning diets for this species.

Husbandry

The specific husbandry requirements for the rearing of greater amberjack are studied at different developmental stages to define appropriate and efficient practices. Preliminary results studying light conditions (photophase, intensity and background color) during the larval stages showed a beneficial effect of the long photophase on growth and survival of the larvae. Rearing trials comparing intensive and semi-intensive conditions are also implemented, and analysis is underway relating the ontogenetic changes of the digestive system with the expression of somatotropic axis genes. Results until now showed that intensive rearing conditions favor amylase, alkaline protease and pepsin activities in 30 days post hatching (dph) larvae, while in earlier stages (12 dph) amylase activity was also higher, in contrast to alkaline protease and lipase activities (**Fig. 6**).

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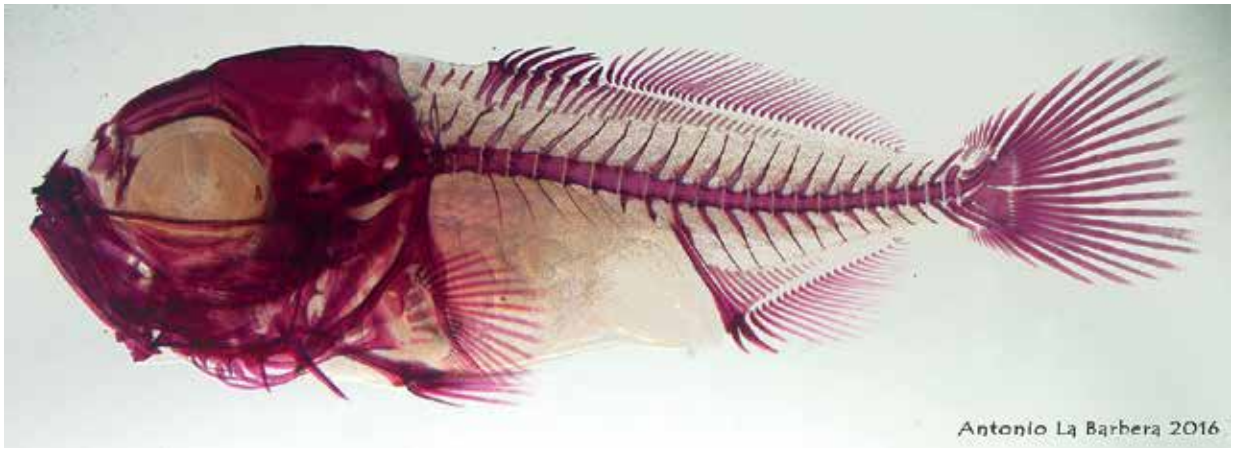

Figure 4. Greater amberjack juvenile with cranial malformation from the study of the effect of dietary Artemia DHA content.

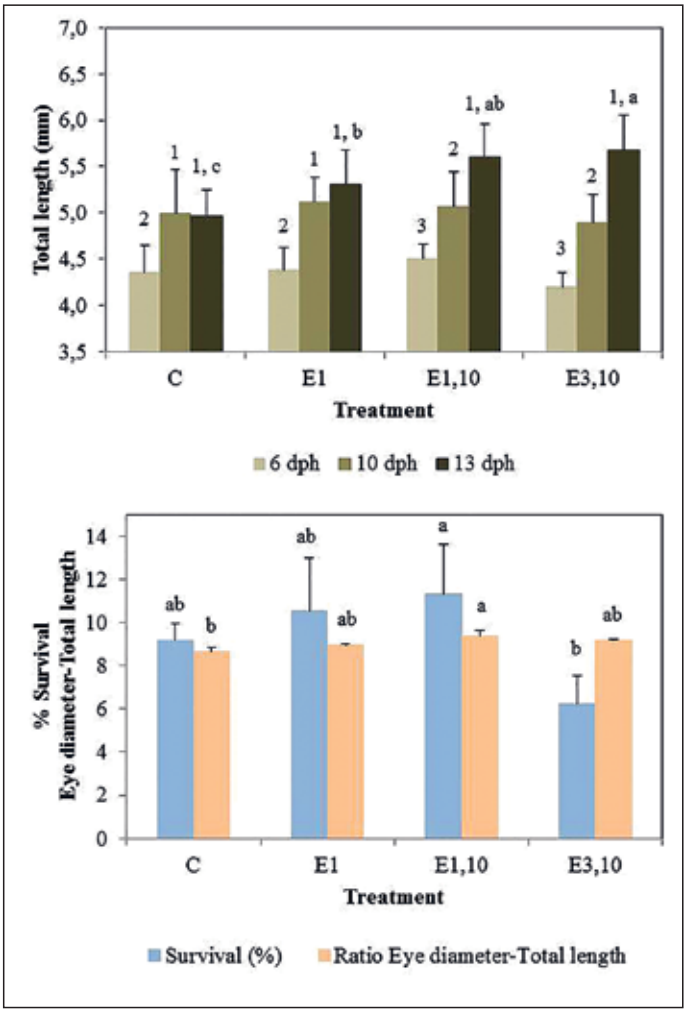

Figure 5. Effect of rotifer enrichment protocols on greater amberjack larval performance (a) Evolution of total length, (b) survival and eye diameter-total length ratio at the end of the experiment. Different numbers indicate significant differences along the feeding period, different letters indicate significant differences among dietary treatments. C, commercial enrichment; E1, LC-60 based emulsion; E3, triacylglycerol (TAG) DHA-rich emulsion; 10, 10ppm astaxanthin-based carotenoid supplementation.

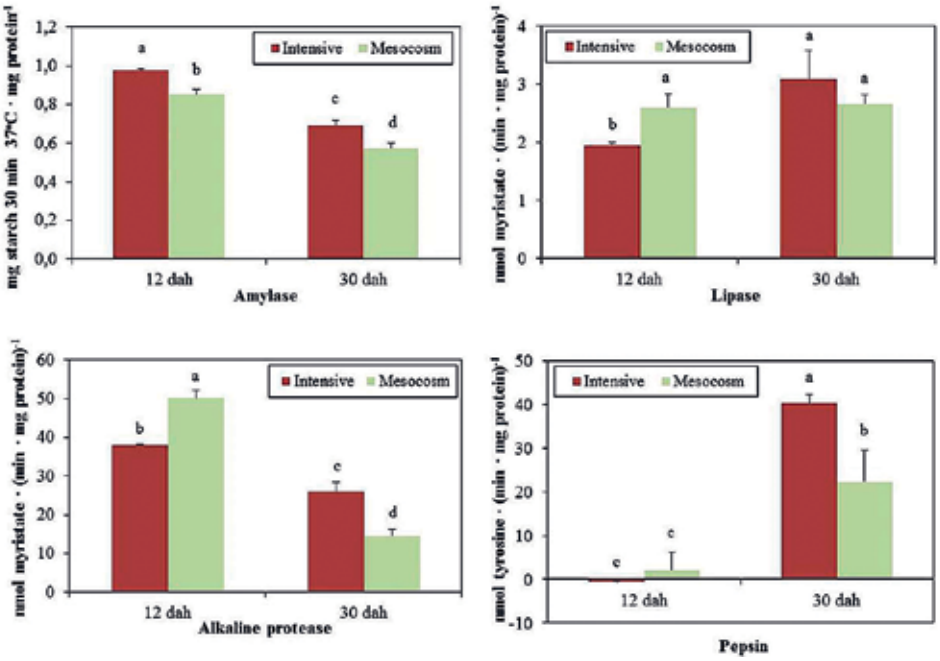

Figure 6. Comparison of digestive enzyme activities in greater amberjack larvae reared using intensive and Mesocosm protocols.

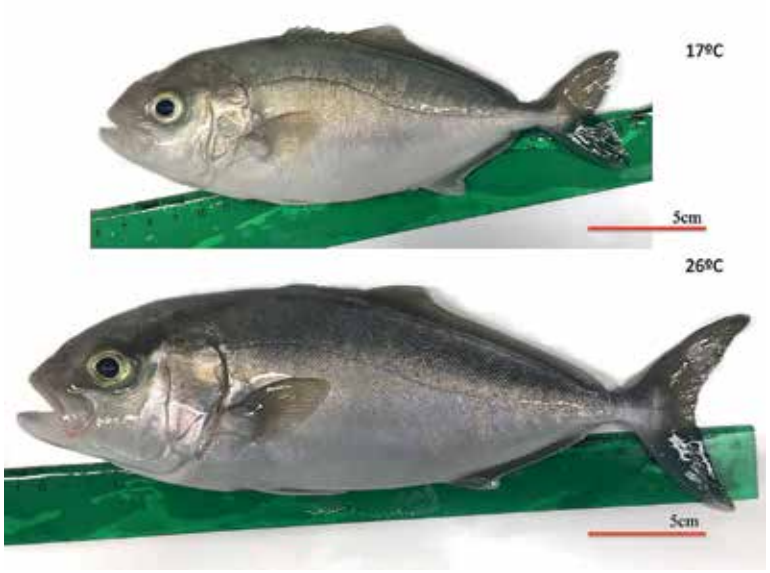

Figure 7. Greater amberjack reared at 17°C (above) and 26°C (below) showing the effect of rearing temperature on body morphology.

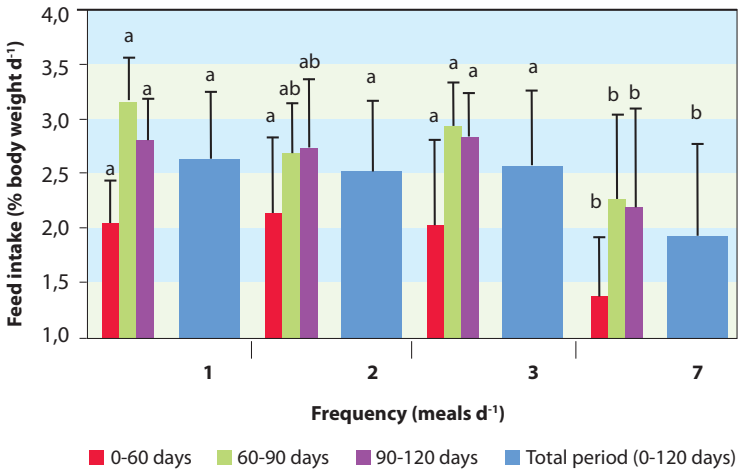

Figure 8. Feed intake (% of body weight d^{-1}) of greater amberjack juveniles at various feeding frequencies, at different times during the experiment (0-60 days, 60-90 days, 90-120 days and the overall effect). Different letters indicate significant differences (ANOVA, $P < 0.05$) for each period.

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A study has been implemented examining the effect of stocking density during on-growing (initial densities of 0.2, 0.3 and 0.5 kg m^{-3} reaching 3.5, 5.7 and 7.4 kg m^{-3} after 4 months) on growth. The first results with juveniles (5 g) showed significantly lower specific growth rates (SGR) and condition index at high-density conditions. A second trial is currently under way with 200 g individuals, looking at feed intake, immune and welfare conditions. Regarding temperature tolerance, we have evaluated the performance of juveniles at 17, 22 and 26°C, with the latter being the most effective for growth and feed utilization. Furthermore, the body morphology of the individuals was affected by the temperature, with 26°C resulting in a better, more elongated body shape (**Fig. 7**).

The effects of different feeding rhythms on growth performance and welfare status were also examined. A trial was implemented with juveniles (~200 g) fed 1, 2, 3 and 7 meals day^{-1} for 4 months. Fish fed 1 meal day^{-1} showed the lowest SGR, condition index and hepatosomatic index, while those fed 7 meals day^{-1} showed the lowest feed intake (% of body weight day^{-1}) suggesting greater feed efficiency (**Fig. 8**). Although some monthly differences in mucus immunological, hematological and biochemical parameters, as well as oxidative stress enzymes in several tissues were observed, at the end of the study there were no statistical differences suggesting that greater amberjack juveniles are able to adapt to the different feeding frequencies under the particular culture conditions.

Health

Over the past 2 years, we have been studying the disease issues that impact production of greater amberjack, by monitoring several populations of cage-cultured fish for their health status. In Greece the main pathological problems have been caused by opportunistic bacteria and parasitic infections, especially during the transitional period between the hatchery and on-growing at the open sea. The most important bacterial infections were caused by *Vibrio harveyi*, which causes typical vibriosis (**Fig. 9**) and may result in high mortality, especially when water temperature rises above 20°C. The monogenean parasite *Zeuxapta seriolae* (**Fig. 10**) was the most prevalent and important parasitic pathogen. It is transmitted to cultured fish from wild populations and since its life cycle is direct (not requiring an intermediate

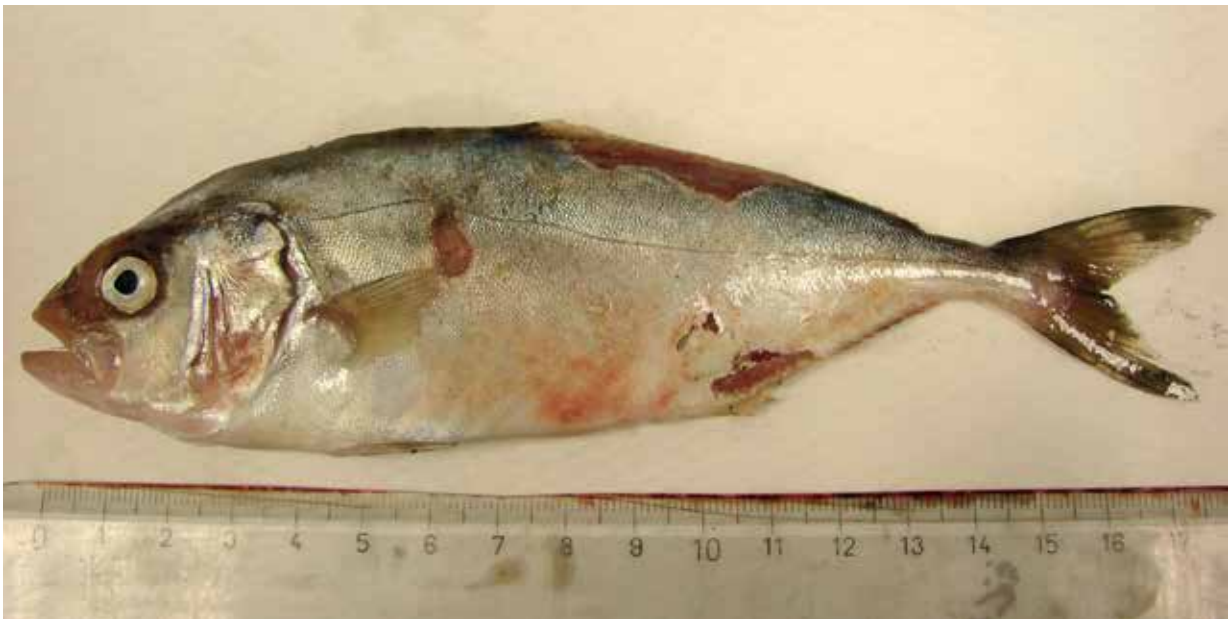

Figure 9. Juvenile greater amberjack infected by *Vibrio harveyi*.

host), it can propagate rapidly, reaching enormously high numbers on the host fish. The parasite is attached on the gills and feeds on blood, causing severe anaemia. Currently there are no adequate registered therapeutics for this parasite and we are investigating several experimental drugs that can be used to mitigate its impact on greater amberjack aquaculture.

Figure 10. The polyopisthocotylean monogenean parasite *Zeuxapta seriolae*.

Apart from *Zeuxapta seriolae*, we have also identified the blood fluke *Paradeontacylix* sp. to be present in greater amberjack reared in Greece. This digenean parasite resides within the blood vessels of the fish and releases its eggs into the blood stream. The eggs and the encysted metacercariae obstruct the gill capillaries, causing severe inflammation and damage of the gill tissue (**Fig. 11**). There is scarce information on the biology of this parasite and almost nothing is known about its life cycle. We are currently investigating possible alternative or intermediate hosts, and we are trying to locate the source of infection. In parallel, we are aiming to develop tools to treat this parasitic disease. The skin fluke *Neobenedenia spp.* is a monogenean parasite that is especially important for aquaculture due to his broad host range and the damage that it causes to cultured greater amberjack (**Fig. 11**). The parasite is well distributed in temperate waters around the world and it caused mortalities to one of our stocks in the Canary Islands. In Japan, infection prevalence rates of 70% have been reported for reared amberjack. Once infected, fish scratch the nets and tanks in order to remove the parasite, producing wounds that cause important injuries on the skin, leading to secondary infections, immunosuppression and, in most cases, the death of the fish.

For this reason strategies for reducing skin fluke incidence are being examined. For example, ways to promote the fish mucosal immune system, particularly skin mucus production (quality and quantity) are being studied since this is the host's first defensive barrier against this parasite. Thus, histopathological analyses for evaluating the derived-skin mucosa wounds, mor-

Figure 11. Numerous visible white nodules on the gills of greater amberjack caused by the eggs of *Paradeontacylix spp* (left) blocking the capillaries. Greater amberjack infected by the skin fluke ***Neobenedenia spp*** (right).

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phometric studies for determining skin mucus quantity and an evaluation of the host immunological status are being conducted. The morphological and hydrodynamic differences between cranial and dorsal regions suggest that the cranial region is an easier place for the attachment of the parasite. *N. girellae* attachment induces epidermis disorganization, increase of goblet cells and massive migration of immune cells (mononuclear lymphocytic type) around the site of parasite attachment. The attachment of *N. girellae* in greater amberjack causes a dermatitis that can be the site for opportunistic pathogens to occur. The incidence of *Neobenedenia* seems to be related to fish size and temperature, with outbreaks of parasites observed in animals larger than 100 g and temperatures above 20°C. These studies are being completed with biochemical and immunohistochemical analysis and examination of the expression of immune and mucus production related genes.

New product development

The technical characteristics and muscle composition of greater amberjack were studied in two different size groups (**Fig. 12**), in order to define both the range of these quality characteristics and the effect of fish size on them (**Table 1**).

Figure 12: Farmed greater amberjack during yield measurements and filleting.

	Group A (small fish)	Group B (big fish)
Size (fish weight in Kg)	1.19±0.19	13.00±1.62
Dressing yield (% of body weight)	92.8±0.79	94.7±0.39
Filleting yield (% of body weight)	50.5±2.89	
Visceral loss (% of body weight)	5.60±0.71	2.89±0.83
Fillet composition (%)		
Protein	22.9±1.29	20.5±0.56
Fat	3.87±0.93	12.3±0.11
Moisture	71.03±1.07	65.5±0.55
Ash	1.35±0.49	1.31±0.04

Table 1. Somatic yield and fillet composition of farmed greater amberjack

Figure 13. Sample preparation and testing of great amberjack (taste panel, IRTA – Spain).

groups was the much higher fillet fat contents in big fish. The sensory characteristics of the species' fillet have been examined analytically with descriptive sensory analysis by trained panelists (**Fig. 13**). In summary, the great amberjack fillet exhibits homogenous color, laminar structure, high juiciness and acid and butter flavors, while its texture is characterized by high teeth adherence and chewiness but of medium hardness (when compared to other fish species). Based on the technical and sensory characteristics of the species and out of a list of 43 different products of variable process, three products have been chosen to be generated as prototypes: frozen fish fillet that is seasoned or marinated (product 1), ready-made fish tartar with additional soy sauce (product 2) and fresh fish steak for grilling in the pan (product 3). One of these products will be selected for consumer acceptance in the near future within the DIVERSIFY project.

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 38 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.

Co-funded by the Seventh Framework Programme of the European Union

Further information may be obtained from the project site at "www.diversifyfish.eu".