1. *Publishable summary*

Improvement of feeds and feeding efficiency for seabass in cage farms in the Mediterranean.

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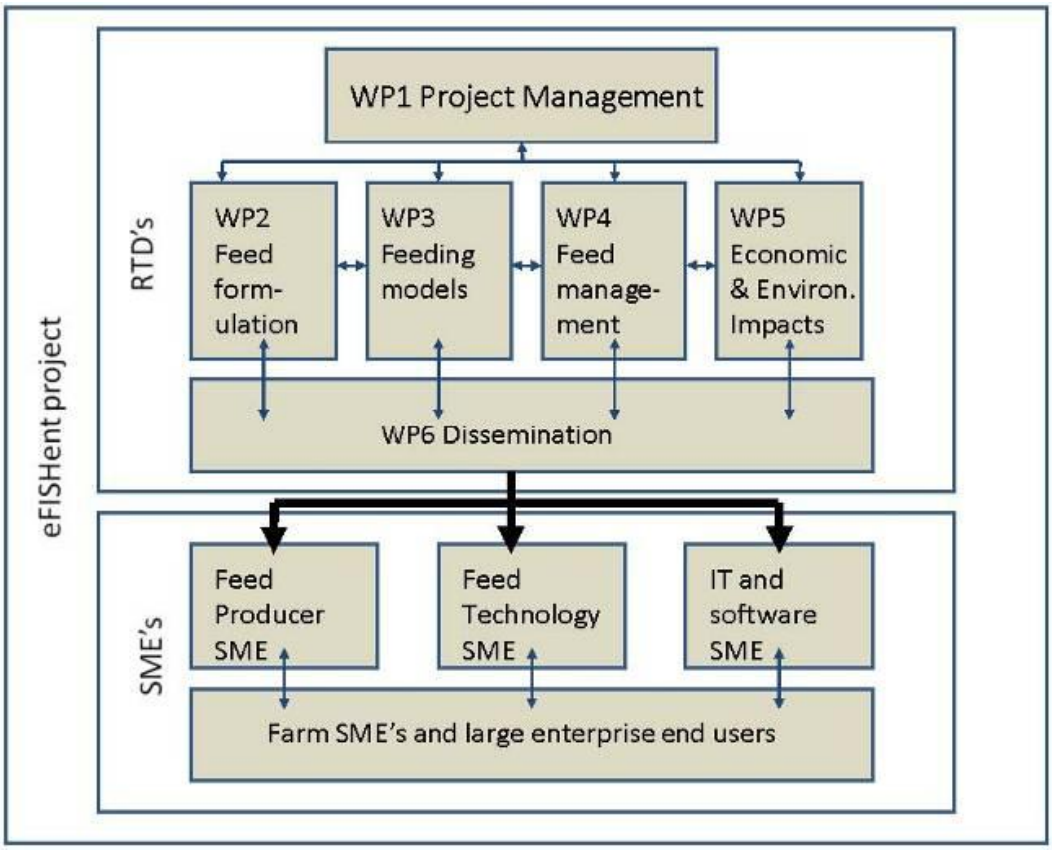
The main objective of this study is to optimise feed management methods and allow the European sea bass farmer to significantly reduce production FCR and thus feeding costs. At the same time nutritional and technical improvements will allow improvements in fish growth, labour costs reduction and minimise the environmental impacts of fish farming. Currently, one-third of the world's fish catch is used to produce fishmeal and fish oil, and in 2004, the aquaculture industry used 87% of the world's fish oil and 53% of the world's fishmeal. Improved production FCR will reduce the overall demand for raw materials for fish feed production.

Figure 1. Relationships between the Work Packages and role of the partners in the project



WP 1. Project coordination and management

Objectives

- To coordinate the overall development of the project, to assess the accomplishment of tasks allocated to each participant, to discuss the results obtained during different phases of the project.
- To submit internal reports that will make the bases of the periodical reports to be submitted once a year to the REA.
- To fix deadlines for submission of manuscripts for publication, and for submission of results for the elaboration of Period Reports.
- Development of an Exploitation plan, exploitation of results, including the implementation of new rearing methods and feed recipes.
- Handling of relevant IPR issues.

Among the management activities were the monitoring of project progress, deliverables and results, ensuring that the milestones for each phase were achieved. It has kept minutes of all the project meetings and circulated these to the EU and project partners. The partners have formally met 5 times (once pre submission) to discuss planning and achievement of progress. Partners have also met informally with RTD partners visiting SME facilities and during conferences and exhibitions.

Task 1.1 Consortium meetings and coordination of the project

Akvaplan-niva has undertaken the coordination of the overall progress of the project. Among the management activities were the monitoring of project progress, deliverables and results, ensuring that the milestones for each phase were achieved. It has kept minutes of all the project meetings and circulated these to the EU and project partners.

The partners have formally met 5 times to discuss planning, achievement of progress and solve any problems.

- 07 June 2010, Brussels, Belgium
- 28 – 30 November 2010, Athens, Greece
- 25 - 27 May, 2011, Bergen, Norway
- 3 - 4 October, 2011, Brussels, Belgium
- 15 - 7 October 2012, in Izmir, Turkey

Minutes of the meetings were prepared and circulated to all partners and EU project officers. The Coordinator made regular follow up activities to the RTDs for the Project deliverables and ensured that milestones have been met. Partners have also met informally between RTDs and SMEs and during conferences and exhibitions and have communicated regularly through SKYPE and email. Coordination was undertaken by an assigned staff at Company headquarters in Tromso.

Partners have also met informally with RTD partners visiting SME facilities and during conferences and exhibitions.

Task 1.2 Exploitation of results
Draft exploitation and dissemination plan was prepared at Month 9 as a deliverable and the final exploitation and dissemination plan was prepared at the end of the project.

Task 1.3 Handling of IPR matters
A brief description of how the IPR matters would be handled was prepared for the Consortium agreement.

Information was given on background and foreground knowledge in the Attachments to the Consortium Agreement.

- Attachment 1. Background Knowledge
- Attachment 2. Foreground knowledge
- Attachment 3. Access to foreground knowledge Role of the partners:

WP 2. Sea bass nutritional requirements *and* feed formulation

Objectives

- Survey on the nutritional requirements, the commercial feeding strategies and the available feed technology in sea bass culture and diet formulation for lab and production scale experiments.
- Determination of growth potential and energy composition of sea bass under different commercial production environments.
- Comparison of different methods to measure energy flow in fish and determination of energy flow in sea bass for diets with different energy densities.

- Determination of optimum energy density for growth and feed utilization of sea bass.
- Determination of optimum protein to energy levels, seasonal feeds and feeding strategies for commercial feeding practices.
- Creation of models for the prediction of fish performance and cost efficiency of production of sea bass using different feed formulations at different fish ages and water temperatures.

The scope of this work package was to fine tune the European sea bass diets' composition in order to achieve cost efficient industrial production. To achieve this goal we need to reach in depth understanding of the qualitative and quantitative needs of this fish species at different life stages, environmental and physiological conditions. In reality feed producers will fabricate fish diets according to raw material quality, availability and price, not necessarily in that order, unless they are in hold of good arguments for acting otherwise. There is a big but not uniform available data on the effects of each commercially used raw material on different fish species performance. However, it was neither in the time frame nor scope of this project to identify specific raw material effects and qualities on European sea bass performance. We therefore needed to use all experience we had, published or not in order to use in this project's fish experiments commercially relevant feed formulations of highly predictable quality and performance.

The eFISHent partners joined their efforts in the first part of the project to collect all available production and research data regarding nutrition and production technology of European sea bass in order to be able to formulate well balanced feeds for the different experiments and to choose the most appropriate feeding regimes to be followed. A scientific review on the nutritional requirements of European sea bass and an extensive database including commercial sea bass production results was produced and updated throughout the life time of the project. We determined the maintenance energy requirement of E. sea bass at different temperatures and ages. Following, the utilisation efficiency of dietary protein and energy was determined using two methods, of which the metabolic chamber was proven the easiest and most precise. Best European sea bass growth for body weight sizes from 10 to 200 g was obtained with feeds of 45/16 and 60/12 crude protein/lipid. Best feed utilization was observed for the 45/16 diet. Protein utilization efficiency decreased as dietary protein increased indicating higher nitrogen loss to the environment with higher protein diets and *vice versa*.

The fish whole body composition and immune status of the fish was not influenced by the different experimental treatments. Last we demonstrated that the fish farmer should expect an economic loss from reducing feed cost by using oils (plant or fish oil) in the expense of dietary protein, even at small increment changes. Reduced feed intake rates and growth were observed by slight increases in the dietary lipids and best growth was observed in E. sea bass of 5-25 g body weight receiving daily 8.3 g protein per kg fish body weight.

WP 3. Seasonal feeds and feeding strategies for sea bass

The purpose of this workpackage was to determine, under laboratory conditions, rational feeding protocols based on the biology and behaviour of Atlantic European Sea bass *Dicentrarchus labrax* of varying size and under differing environmental conditions. It also included laboratory scale testing of the diets formulated in WP2, and examining the effect

of FCR (Feed Conversion Rate) on diet palatability and pellet size. A nutritional study on reducing fish maturation was also performed.

Objectives

The elucidation of natural feed intake quantities, feeding frequency patterns and pellet size preferences in bass

- To evaluate if specifically designed winter feeds and winter feeding protocols can be used to improve overall cycle FCR
- To evaluate if feed intake and FCR might be improved by adding specific attractants to the winter and summer diets
- To develop new suggested multivariate feeding tables and protocols, also following the results from WP2, for commercial scale validation in WP4

Summary of progress and significant results

We demonstrated the significant economic benefit potential from using appropriate dietary formulations and oxygenation in cages where E. sea bass is reared at low water oxygen saturation levels. Though higher protein and lower lipid diets proved in several eFISHent trials superior in terms of E. sea bass performance, this result was inverted at low oxygen levels, where fish fed a lower protein and higher fat diet had best FCR and growth.

In the winter period, FCR was improved significantly by fasting E. sea bass every other or every third day with no reduction in growth. Moreover, by using sustainable 0% fish oil diets rich in low n-6 plant oils (such as palm oil, olive oil and salmon by-product oil), gonadal growth and expression of genes coding for maturation hormones was reduced.

Both at low and high water temperatures E. sea bass is able to growth with low to very low FCR by use of well balanced and nutritionally complete diets, even when only 10% fish meal is used in the diet. No negative histopathological effects were observed in the fish fed the high plant diets.

WP 4. Optimising production scale feed management

Objectives

- evaluate and benchmark current feed management protocols in relation to feeding efficiency and growth
- investigate the optimal timing and frequency of feed delivery in relation production performance and welfare, and examine how this changes with time of year and each SME's labour and budget constraints through CBA (cost benefit analysis)
- develop a series of customised feed management strategies for each SME based upon various demand feeding and feed monitoring technologies

Summary of progress

The primary objectives of eFISHent WP4 was to deliver operational research to help commercial sea bass aquaculturists reduce their on-farm feed conversion ratios (FCRs) and thus improve production efficiency, reduce feed waste and reduce feed costs. Specifically, the WP4 partners aimed to help our commercial SME partner, Özsu Balik Ltd, improve their

operational FCR and feeding efficiency. These findings can then be rolled out amongst other sea bass producers in the Mediterranean region.

The main findings of the WP4 benchmarking study was to show the relationship between water temperature and fish size in relation to feeding efficiency (FCR) and growth performance (SGR). Commercial FCRs are generally higher both in Greece and Turkey during the winter months when water temperatures are low. FCRs also increase as the fish get bigger. The experimental phase of WP4 demonstrated that increasing daily feeding frequency from 1-meal per day to 2-meals per day on commercial farms during winter offers no benefits in terms of FCR and SGR performance. Feeding fish every other during early winter may also be a cost-effective feeding regime for commercial sea bass farmers during early winter. The feasibility study that investigated whether there is a need or current market for linking feeding and oxygenation systems for Mediterranean cage aquaculture suggests farmers in Turkey are currently prioritising updating their feeding systems and will take the step of possibly upgrading their oxygenation systems when they can find a cost-effective and robust solution to dealing with potential oxygenation problems in cage aquaculture.

Significant results

The general aim of WP4 was to deliver operational research to help commercial sea bass aquaculturists reduce their on-farm feed conversion ratios (FCRs) and thus improve production efficiency, reduce feed waste and reduce feed costs. Specifically, the WP4 partners aimed to help our commercial SME partner, Özsu Balik Ltd, improve their operational FCR and feeding efficiency. These findings can then be rolled out amongst other sea bass producers in the Mediterranean region. To achieve the aims of improving operational on-farm FCRs, farmers need to i) understand how their feeding practices affect operational FCRs, and ii) identify ways to improve their on-farm feed management. This can be enhanced through improved dissemination of existing knowledge on best practice in feed management, and also via the identification of commercial technologies and feeding strategies that can be customised to each farmer's specific needs.

The initial aim of WP4 was to benchmark feed conversion ratios on commercial farms. Benchmarking is an emerging aquacultural management tool that allows a farmer to compare their farming practices and performance metrics within their company and against others within the same industry (e.g. Soares et al., 2011). Farmers can then identify areas for potential improvement within their current set-up and economic constraints.

The experimental aspect of WP4 involved the operational evaluation of different feeding practices such as i) the effects of different daily feeding frequencies, and ii) the effects of different lengths of feeding/re-feeding periods upon the on-farm FCR, growth performance and welfare of European sea bass held at commercial production facilities.

A feasibility study was also carried out by Nofima, ANCO, Element, NESNE Electronics, Özsu Balik and Akvaplan-niva on whether there was scope to combine feeding and oxygenation systems for cage aquaculture, to combat potentially low ambient oxygen levels that can occur during the summer months in Mediterranean sea bass farming.

To achieve these aims the benchmarking aspect of WP4 and the feasibility of combining oxygenation and feeding systems started at the beginning of the project, and the majority of experimental work commenced during Year 2 of the project.

WP5. Economic and environmental assessment and impact

The overall purpose of the project is to improve the cost effectiveness of bass and bream culture and thereby to also reduce its local and global environmental impact. The purpose of this WP is thus to quantify the potential economic and environmental benefits likely to accrue from the improved feeds and feeding methods developed. In addition it seeks to clarify the decision making process by which aquaculture companies find out about, evaluate and take decision for the purchase of new technology. This will allow companies such as the SME partner FCN to target their sales and marketing of feed control technology more effectively.

Nine farm managers in Greece (3 small, 3 medium and 3 large companies) and 11 farm managers in Turkey (3 small, 3 medium and 3 large companies) have been interviewed to analyse and assess their decision making process and behaviour.

Methodology for the assessment in reduction in local environmental impact in the vicinity of individual units and the assessment in the reduction in the wider ecological footprint using LCA was undertaken.

Significant results

Decision and Purchasing Classification

Large size aquaculture company. The company structure of large sized farms is that they operate between 6 - 18 sites producing a between 8,000 to 20,000 tonnes/yr total. The large aquaculture companies have a less bureaucratic structure than medium sized farms with farm managers needing to get approval from administration department to take decisions E 5,000 to greater than E 10,000. The company strategic planning for identification of new technology is generally that they find out from trade shows, magazines and neighbours. The decision to purchase new technology is based on economics and a need to solve problems. In addition they want to have spare parts and service available locally. The recommended marketing strategy for large aquaculture companies are that the technology providers should exhibit and have local agency with spare parts and servicing.

Medium size aquaculture company. The company structure of medium sized farms is that they operate between 2-5 sites producing a total of 2000 to 4000 tonnes/yr. The medium sized companies have a bureaucratic structure with farm managers needing to get approval from administration department to take decisions between E 5,000 and 10,000. The company strategic planning for identification of new technology is generally that they find out from trade shows and magazines. The decision to purchase new technology is based on economics and science based analysis with a need to improve quality, In addition they want good quality equipment with Guarantees. The recommended marketing strategy for medium aquaculture companies are to advertise in trade magazines, publish scientific results and exhibit locally.

Small size aquaculture company. The company structure of small farms is that they operate between 1-3 sites producing between 300 and 1000 tonnes/yr. The small companies have a flat management structure with farm managers/owner able to take decisions in excess of E 10,000. Decisions are taken at farm level. The company identify new technology by watching neighbours and wait until they see other companies using it. The decision to purchase new technology is based on economics and must reduce production cost. In addition, the equipment needs to be easy to maintain and have local servicing. The recommended marketing strategy for small aquaculture companies are to demonstrate the equipment in farming areas and exhibit locally.

Assessment in reduction in local environmental impact

The actual environmental impact was undertaken collecting and analysing data from round the test cages. Data was also collected to input into the predictive model.

Survey data collection

- Sediment trap collection 24 hours
- Bathymetry around test cages
- GPS reading of cages and sample sites
- Current speed and direction, oxygen levels – 4 days
- Salinity, temperature and oxygen profile through water column around cages
- Sediment samples 0, 10, 30 and 100 m from cages
- Oxygen level inside cage 3 days

The MERAMOD model was set up for Ozsu fish farm, Turkey (38°11' N, 26°39' E) made up of three separate cage groups. Bathymetry data for the surrounding area taken from a survey and an electronic chart were combined and contoured using the kriging algorithm in Surfer for Windows™ (cell spacing 30 m). These data along with cage position data taken from Google Earth™ were converted to UTM positional format (35S) and incorporated into MERAMOD grids.

This MERAMOD modelling study draws the following conclusions:

- all of the cage groups have adequate spacing between them, so that no overlap of deposition footprints were predicted
- all cage groups are situated in quite deep waters, which aids dispersion of wastes; similarly, advection of wastes by the measured current was also quite high, although the current record was relatively short
- the area occupied by the deposition footprint in all increased production scenarios was less than the mooring area; group 2 had the largest extent of deposition footprint; the model predictions implied that the total deposition footprint area was acceptable for all scenarios in relation to the mooring area
- the increased production scenarios for groups 2 and 3 resulted in a predicted severe impact area of around 5 % of the total footprint; no severe impact was predicted for group 1 for increased production
- however, the predicted areas of severe impact were reduced significantly in the increased production scenarios, when lower FCRs of 1.8 and 1.6:1 were used to represent more efficient diets and practices; this was particularly the case for group 3

- the severe impact predicted for cage group 3 scenarios, were a result of a few cages with high feed input (and biomass); this suggests that a more even distribution of feed input across the whole cage group, or moving around high biomass cages within the group, would limit severe impact in a particular location
- The MERAMOD model predictions also suggest that when increasing production, adding larger and well-spaced cages (rather than adding another row of cages) results in better dispersion of wastes.

Assessment in the reduction in the wider ecological footprint using LCA Resource use and greenhouse gas emissions by seabass cage culture systems

The aquaculture sector has been receiving a great deal of criticisms on its potential environmental impacts. The potential impacts from aquaculture systems often highlighted are: Impacts on water quality and sediments, GHG emissions, and high resource use (i.e. wild seed, fish in-fish out ration). Recently, there is a major interest on its potential emissions of GHG (Greenhouse Gas) which is being the international agenda on climate change mitigation as well as adaptation.

In order to identify culture systems that produce the most greenhouse gas emissions (GHGs) and prioritise better practices for the culture systems with high environmental impact, the different case studies need to be benchmarked against each other and other aquaculture technologies. This analysis uses resource use analysis to estimate the resource use and nutrient impact to the environment and Life Cycle analyses to estimate GHG emission. In this way the aquaculture culture systems that are resource heavy or have high GHG emissions can be highlighted and best practice guidelines to reduce impacts. The analysis assesses the GHG emissions and resource use per tonne of food produced by the culture of seabass in cages at the Ozsu fish farm in Turkey.

The Task evaluated the potential impacts associated with inputs and outputs by the culture of seabass in cages at the Ozsu fish farm in Turkey using some of the Life Cycle Analysis (LCA) methodology. The system boundary was at the farm-gate level, thus covering the hatchery, farm and feed production activities. However, related transports in all stages are excluded. The functional unit was set as one ton of fish/shrimp (wet weight). The impact categories of interest are: Biotic resource use (wild seed, fish-in:fish-out), Abiotic resource use (land, water, and energy), Global Warming Potential, and Eutrophication Potential. The impact methodology used was based on midpoint impact by following the CML2 Baseline 2000 method. The results of LCA study would be used to identify the hot spots where improvement can be made, including the potential options for environmental performance improvement.

- **Fish-in Fish-out Ratio (FIFO).** The FIFA ration for seabass cultured on BioMar feed with a food conversion rate of 2.03:1 is 3.18 kg wild fish to produce 1 kg of cultured seabass. The FIFO reduces to 2.43 when FCTR is improved to 1.6:1.
- **Analysis of land or sea area resource use.** Aquaculture uses land in two ways. First, aquaculture facilities occupy a defined area or space on land or in water; however, facility area accounts for only a portion of the total land or water area needed to produce an aquaculture crop. Additional ecosystem area is needed to provide

support or service functions. The land and sea utilisation is calculated to be 442.7 m2/tonne production.

- **Analysis of energy resource use.** There are many uses of energy in aquaculture including energy used for construction of facilities, production and transport of feed and feed ingredients, operation of machines and vehicles during culture and harvesting, processing, transportation, etc. In seabass production the highest use of electricity is for the production of feed, production of fry and servicing of the cages by boat with a total of 8,588 MJ per tonne of seabass produced.

The GHG emissions from seabass cage culture are mainly from the use of compound feed and production of fry. Thus, the feeding management and the optimal operation of the hatchery must be given the attention in order to reduce the GHG emissions. More importantly, the potential impacts associated with feed ingredients especially fish meal, fish oil and wheat flour should be taken into account at the feed mill.

Task 5.4 – Analysis of the cost benefit of the results of the implementation of the knowledge:

For the scope of the eFISHent fish feeding trials optimised diets were formulated for European sea bass of body weight from 5 g to 300g reared in experimental tanks or sea cages, at temperatures from under 10^{o}C and up to 25^{o}C, and oxygen levels of 3.5 ppm to 6.5ppm. Both relevant eFISHent partner SMEs, one fish farmer and one feed producer, have had early access to the best performing and sustainable feed formulations used in the eFISHent trials with documented good fish growth results and remarkably low FCR.

The cost benefit analysis shows a range of benefits (additional profit) to the farmer (see Table 3 below) from highly beneficial to no additional benefit.

Table 3. Additional profit per 1,000 tonnes of seabass produced (€) for improved feed and feeding measures.

Measure	**Additional profit per 1,000 tonnes of seabass produced (€)**
Use of camera and feed control	436,800
Use of cage oxygenation systems for a site with continuously low dissolved oxygen levels	251,400-366,800
Use of cage oxygenation systems for a site with low dissolved oxygen levels during parts of the year	145,000-220,000
Use of feed to reduce maturation	66,640
Feeding every other day in winter	60,965
Substituting dietary protein by plant versus marine oils	6,000
Feeding once per day compared to twice per day during summer	neutral

The seabass farmer gains the highest benefits by using a camera and feed control system in the feed boat. The cost of the investment is paid back within approximately one month. This is followed by the use of oxygenation systems at farm sites which have low dissolved oxygen levels (this dose not apply to the farm managed by Ozsu). There are some additional benefits from the use of specialised feed to reduce maturation during the second winter, the feeding strategy of feeding every other day during winter and the substitution of plant protein and oils. There is no significant benefit from feeding only once per day during summer.

WP 6. Dissemination and outreach

The work in this work package was led by Akvaplan-niva, with considerable input from the other partners. All RTD partners played an active role in developing the overall dissemination strategy for the eFISHent project. A close interaction is foreseen with the work in WP5, which also is a crosscutting horizontal activity drawing together the technical, economic and social factors relating to the best practices in feeding management of sea bass farming.

A project web site (www.efishent.eu) was set up at the start of the Project in November 2010 and has been updated regularly.

A number of scientific and technical papers and articles were published or presented

- AquaNor project handout detailing objective and proposed research (APN)
- Fish farmer article (Viking)
- Izmir Offshore conference poster (APN)
- Izmir Offshore conference paper (APN)
- International Innovation article (APN)
- Pan European Article (APN)
- EAS 2011 conference in Rhodes, Greece entitled “Presentation of ‘eFISHent’ FP7 EU Project for the benefit of the SMEs: Improvement of feeds and feeding efficiency for seabass in cage farms in the Mediterranean & a review of the current status of Knowledge on nutrition and feeding of European Seabass (*Dicentrarchus labrax*)”.

Manuscripts in preparation for publishing in peer reviewed magazines:

1. Katerina Kousoulaki & Sissel Albrektsen, 2013. Review of the nutritional requirements and growth potential of European sea bass (suggested review accepted by Aquaculture Nutrition), *Manuscript*.
2. André Bogevik, Jolanda Arjona, Jim Treasurer, Tim Atack, Raja Rathore, Ivar Rønnestad & Katerina Kousoulaki, 2013. Modulation of precocious genetic maturation in male E. sea bass by marine or plant dietary oils, *Manuscript*.
3. Jolanda Arjona, Jim Treasurer, Tim Atack, Tal Prag, Eran Hadas & Katerina Kousoulaki, 2013. Interaction of dietary protein, lipids and dissolved oxygen levels in E. sea bass on-growing performance, *Manuscript*.

4. Katerina Kousoulaki, Jolanda Arjona, Tim Atack, Jim Treasurer, 2013. Modeling of cost efficiency of small gradual dietary DP/DE changes in E. sea bass on-growing performance, *Manuscript*.
5. Jolanda Arjona, Tim Atack, Jim Treasurer, Katerina Kousoulaki, 2013. Winter feeding of E. sea bass with low fish meal diets supplemented with marine hydrolysate attractants, *Manuscript*.

WP 7. Operational validation of feeding technologies and feed formulations

This workpackage set out to validate the findings based around the on-farm testing of feed technologies and diets. The validation work will be carried out on the commercial scale farm cages with the SME partner Oszu. All sampling procedures for evaluating production performance (growth, feed delivered, mortality) were carried out in tandem with Operational Welfare Indicators (OWI's) to assess whether the different feed technologies and diets are beneficial for aquaculture production in a commercial, on-farm environment.