AdriAquaNet

Enhancing Innovation and Sustainability in Adriatic Aquaculture

Deliverable WP 3.1.1

New environmentally sustainable dietary formulations for cultured marine fish to be proposed to the aquafeed-mill industry

Control sheet

This document is prepared by the working group WP3, under the coordination of the WP leader, prof. Emilio Tibaldi

Place, 30.06.2022

www.italy-croatia.eu/adriaquanet

European Regional Development Fund

Project number:	**10045161**
Project Acronym:	**AdriAquaNet**
Project Title:	**Enhancing Innovation and Sustainability in Adriatic Aquaculture**
Start of the project:	**01/01/2019**
Duration:	**42 months**
WP/activity:	**WP3, Activity 3.1.1**
Deliverable name:	**New environmentally sustainable dietary formulations for cultured marine fish to be proposed to the aquafeed-mill industry**
WP leader:	**LP (UNIUD), partners involved PP3, PP8, PP10**
Author (s):	**E.Tibaldi, G. Cardinaletti, T. Segvić Bubić, I. Cvitić**
Delivery date:	**30/06/2022**
Status:	**Final**

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

PART 1

1) **Introduction** *(objective and purpose of the deliverable)*

There is broad consensus among socio-economic analysts, scientists and industry professionals on the fact that, to face the challenge of sustainability the aquaculture industry has to move towards a new generation of fish feeds inclusive of protein sources that are more compliant with the principles of circular bioeconomy and less disputed between farmed animals and rapidly growing world population [1, 2]. In this direction, processed animal proteins (PAPs) are considered very suitable candidates as substitutes or complementary ingredients to conventional protein sources originating from fisheries (fish meals) or from agricultural crops. Amongst PAPs, poultry byproduct meal (PBM) and insect meals deserve great interest. In fact, they

i) fully complain to the principle of circular bioeconomy;
ii) possess a high nutritive value to fish;
iii) have low environmental footprint [3].

To improve sustainability of Adriatic mariculture a while further, with this deliverable we anticipated the expected protein transition of marine aquafeeds by providing the main stakeholders (aquafeed–mill industry and fish farmers) with a solid base of data and results demonstrating that a new generation of sustainable aquafeeds including PBM and insect meal specifically designed for the sea bream and the sea bass, are environmentally sound and safe.

2) **Presentation of the deliverable** *related to the previous progress report (what, when, it consists of…, photos, graphics or design,)*

<u>1-Designing novel prototype complete diets for </u>gilthead sea bream (*S. aurata L.) and* European sea bass (*D. labrax L.*)

During the first 4 months of the AAN project, LP in cooperation with PP3 designed different prototype aquafeeds for both fish species to be preliminary tested in Lab scale experiments. To face the sustainability, issue the idea was to generate original feed-formulations with minimal levels of fish meal where variable proportions of conventional vegetable protein sources were replaced by processed animal proteins such as poultry by product meal (PBM) and/or a novel PAP such as the insect meal obtained by pupae of black soldier fly-BSFM (*Hermetia illucens*). In case of gilthead sea bream two series of original complete aquafeeds were formulated (table 1).

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it

Table 1. Composition in major nutrients and origin of protein and lipid of the diets tested at lab scale by LP on S. aurata.

	Dietary treatments			
	FISH	**VEG**	**AVI**	**AVI+INS**
Diets first phase, juveniles – sub adult (4mm pellet size)				
Crude protein % as fed	45	45	45	45
Crude lipid % as fed	20	20	20	20
Source of crude protein (% total)				
Fish meals	90	10	10	10
Vegetable ingredients	10	90	50	50
PBM	-	-	40	30
BSFM	-	-	-	10
Source of crude lipid (% total)				
Fish	67	33	33	33
non-fish	33	67	67	67
Diets second phase, sub-adult up to market size (6 mm pellet size)				
Crude protein % as fed	42	42	42	42
Crude lipid % as fed	21	21	21	21
Source of crude protein (% total)				
Fish meals	90	10	10	10
Vegetable ingredients	10	90	50	50
PBM	-	-	40	30
BSFM	-	-	-	10
Source of crude lipid (% total)				
Fish	70	50	50	50
no-fish	30	50	50	50

In both diet series, aquafeeds denoted as FISH and VEG were based on fish meal/oil and vegetable protein/oils respectively as major protein and lipid sources. The diets denoted as AVI included a huge proportion (40% of total dietary crude protein) of protein from PBM in spite of vegetable proteins while in the diets AVI+INS a combination of PBM and BSFM was used to replace a same proportion of vegetable proteins (i.e. 40%). All diets were formulated to be grossly isoproteic and isolipidic and nutritionally complete. A slightly higher protein content and protein to lipid ratio was adopted in the first series for the growing phase than in the second series (finishing period).
In case of European sea bass, a series of five prototype aquafeeds were formulated with the same criteria indicated for those of sea bream (table 2). They were produced in two different pellet sizes to be compared in two sequential trials: the first one with juvenile fish (pellet size 2mm) and the second with growing sea bass (pellet size 4 mm).

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it

Table 2. Composition in major nutrients and origin of crude protein and crude lipid of the diets tested at lab scale by PP3 on D. labrax.

	Dietary treatments				
	FISH	**VEG**	**INS**	**AVI+INS**	**FISH+INS**
Crude protein % as fed	45	45	45	45	45
Crude lipid % as fed	20	20	20	20	20
Source of crude protein (% total)					
Fish meals	85	15	15	15	85
Vegetable ingredients	15	85	75	45	5
PBM	-	-	-	30	-
BSFM	-	-	10	10	10
Source of crude lipid (% total)					
Fish	67	33	33	33	67
non-fish	33	67	67	67	33

Irrespective of the fish species, all test diets were produced using commercially available ingredients and were processed by a private company (Sparos Ltd. Portugal), through an extrusion treatment like the commercial ones, resulting in dry sinking pellets:

Photo 1 prof. Tibaldi and dr. Cardinaletti at the University of Udine labs in Pagnacco, Udine, ITaly (LP) - lab-scale feeding trials with Sparos.

European Regional Development Fund
prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences
Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Lab-scale feeding trial on gilthead sea bream and sea bass comparing different dietary treatments based on the prototype aquafeeds described in tables 1 and 2.

Experimental set-up and samples collection

Sea bream. From the beginning of the second semester of the project (22 July 2019) up to the beginning of the third semester (28 January 2020) a long-lasting feeding experiment (25 weeks) with gilthead sea bream was carried out at the LP's indoor fish farming facilities to compare the diets described above over the growing out and finishing periods. The experiment used 12 groups of juveniles (average individual weight 106 ± 8 g) each consisting of 15 fish kept in fiberglass tanks (350 L volume) as a part of a recirculating aquaculture system ensuring optimal water quality to sea bream (T. 23.6 ± 0.4 C°; Salinity, 30 ± 1.8 psu; pH. 8.1± 0.1; DO. 6.3±1.5 mgL^{-1}; Ammonia-N, <0.02 mgL^{-1}. Nitrite-N, <0.1 mgL^{-1}). After 2 weeks' adaptation to the farming conditions, fish groups were randomly assigned in triplicate to one of the dietary treatments FISH, VEG, AVI and AVI+INS. The diets of the first phase were fed from August the 8th 2019 up to October the 14th 2019 when fish groups were turned on the diets of the second phase up to January the 28th 2020. Fish were fed 6 days a week to visual satiety (until the first feed item was refused) over 174 days. Feed consumption and eventual mortality in each tank were recorded daily. Fish were weighed in bulk every month and at the end of each phase when specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated. At the end of the experiment, besides growth parameters and feed efficiency data, major indices of economic and environmental sustainability (economic conversion ratio, ECR; Fish In Fish Out, FIFO; Forage Fish dependency ratio meal, FFDRm and Forage Fish dependency ratio oil, FFDRo) were also calculated per each dietary treatment. Moreover 3 fish per group were sacrificed and major dressing out yield parameters were calculated. Fillet muscle were send to PP1 for proximate and fatty acid composition analysis. Other organs (intestine) were preserved for subsequent measurement of markers of gut functions and health.

Sea bass. During the first year of the project, PP3 carried two feeding trials on juveniles and subadults seabass *Dicentrarchus labrax,* irrespectively in an indoor and outdoor water open-circuit systems at the Laboratory of Aquaculture. The trials used 600 juveniles and 550 subadults obtained from local farms. Five diets were tested including one positive (fish meal and oil-based) and one negative (rich in vegetable protein and oil) that served as controls. After acclimation. all fish were measured and weighted and randomly allocated in (1) 15 plastic 100 l tanks (40 fish per tank) with three replicates per dietary treatment <u>in case of juvenile fish</u> and (2) 10 concrete 600 l tanks (55 fish per tank) with two replicates per dietary treatment <u>in case of subadults</u>. The initial average body weight (± SD) of 9.1± 1.2 g and 149 ± 21 g did not differ significantly among tanks. The trial with juveniles started in May 2019 and lasted for 12-weeks while for subadults the trial started in July 2019 and lasted for 22-weeks with monthly feed adjustment following weighing and measuring. Fish were hand-fed the experimental diets to apparent satiation in several daily meals. The feed consumption and water parameters were measured daily.

At the end of the trial sea bass were anesthetized with 50 mg/L of MS-222 for final weighing and digital imaging while fish needed for biological samples were sacrificed with an overdose of MS-222 (100 mg/mL) to collect the different tissues for analytical purposes. The gastrointestinal tract was removed from 3 fish per each tank for later gut microbiota analysis. The digestive tract was also collected from additional 11 fish per tank and divided into 3 section parts: pyloric cecae (PC). proximal intestine (PI)

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

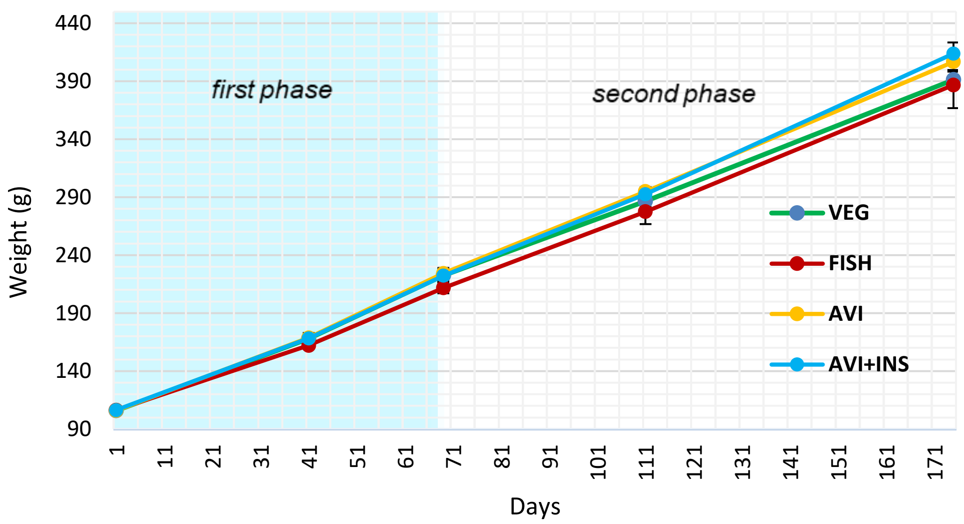
and distal intestine (DI). Tissue samples of each part from 5 fish per tank were individually stored at −20 °C until the analysis of the activity of the BBM enzymes. Samples from a further 3 fish/tank were individually stored at −80 °C for gene expression analysis while from last 3 fish/tank were preserved in a 4% phosphate-formaldehyde buffer (pH= 7.2) for histological purposes. Fish carcasses were stored at −20 °C until analysis.

Results

<u>Sea bream</u>. Growth curves, growth performance and feed conversion efficiency of sea bream subjected to the different dietary treatments are shown in figure 1 and table 3.

Fish fed diets including PBM alone (AVI) or combined with BSFM (AVI+INS) tended to outperform those given diets FISH and VEG in terms of growth and FCR in both phases. Overall, sea breams fed diet AVI+INS resulted in improved feed conversion ratio relative to those fed diets FISH and VEG (P<0.05) while diet AVI had an intermediate value.

Figure 1. Growth curve of sea bream subjected to the different dietary treatments



European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Table 3. Growth performance and Feed Conversion Ratio (FCR) of sea bream subjected to the test dietary treatments at the end of each phase and over the whole trial (Mean ± pooled std. error of triplicate groups per dietary treatment; row means not sharing same letters differ significantly: a,b; P<0.05).

	VEG	FISH	AVI	AVI+INS	sem
Initial weight g	106.3	106.3	105.9	106.3	0.167
Weight at the end phase 1	222.6a	211.7b	224.2a	223.7a	1.891
Weight at the end phase 2	391.3	386.7	406.8	413.8	30.909
SGR phase 1	1.08a	1.00b	1.09a	1.08a	0.234
SGR phase 2	0.53b	0.57a	0.56ab	0.58a	0.028
SGR overall	0.73	0.74	0.77	0.76	0.023
FCR phase 1	1.30b	1.44a	1.28b	1.29b	0.013
FCR phase 2	1.61a	1.55a	1.48ab	1.41b	0.007
FCR overall	1.48ab	1.51a	1.40ab	1.36b	0.008

As shown in Figure 2 also major sustainability indicators such as economic conversion ratio (ECR), Fish in-Fish out (FIFO), fish meal and fish oil dependency ratios (FFDR meal. oil)) were markedly improved with diets AVI and AVI+INS when compared to those calculated for fish fed diet FISH and were also marginally better than those attained with the dietary treatment VEG.

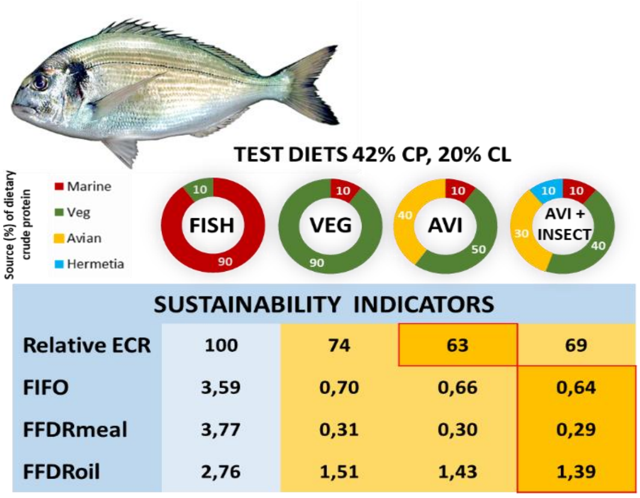


Figure 2. Effect of the dietary treatment on major sustainability indicators calculated at the end of the trial

As shown in table 4, treatments VEG and FISH resulted in extreme values of eviscerated carcass yield (P<0.05) which was similar in fish fed diets AVI and AVI+INS. Fillet yield, viscera and abdominal fat weights were little affected by dietary treatments.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

	CVEG	CFISH	AVI	AVI+INS	sem
Carcass Yield	94.4a	92.8b	93.8ab	93.2ab	2.67
Fillet yield	45.0	43.8	43.6	43.5	2.01
Viscera	2.9	3.4	2.9	3.8	0.17
Abdom. Fat	0.9	1.0	1.0	1.1	0.07

Table 4. Slaughter yield (% whole body weight), proximate composition (% fresh weight), energy content and fatty acid profile of sea bream fillet muscle (mean ± SD of six samples per dietary treatment; row means not sharing same letters differ significantly: a.b. P<0.05).

<u>*Testing prototype diets for sea bream under suboptimal water temperature.*</u>

To provide more robust evidence of the diet results exhibited by sea bream kept under nearly optimal water temperature in the lab scale trial, the experiment itself continued during the 3rd semester of the project to evaluate fish response to the prototype diets under sub-optimal culture (water temperature) conditions. As shown in Figure 3, all groups were subjected to a fasting period for 20 days while reducing water temperature from 24 to 19 °C. Fish groups were then refed to apparent satiety with the test diets over the next 135 days when water temperature was gradually increased up to 24 C°. Figure 3 shows that after the starving period and subsequent refeeding (compensatory growth phase) fish fed the different diets resulted in the already established growth ranking with diets VEG and FISH still resulting the worst in terms of SGR and FCR when compared to diets AVI and AVI+INS (P<0.05) and the same differential in growth was maintained over 135 days from the start of the second part of the trial.

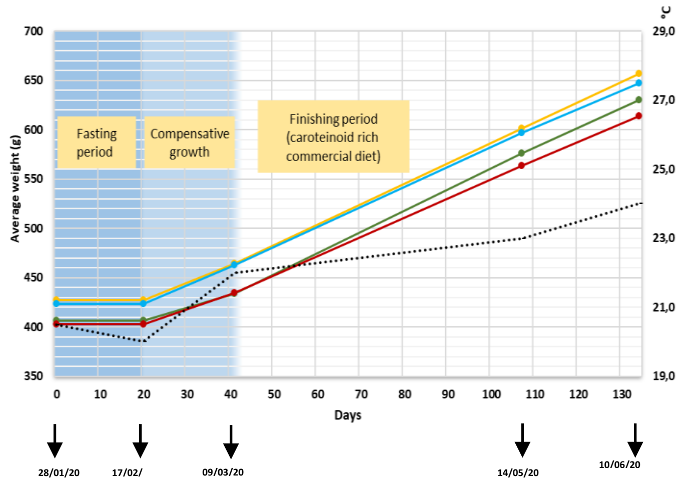


Figure 3. Growth curves and water temperature profile during the extended part of the trial with sea bream

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Sea bass.

The test diets were well accepted by the fish and all feeds were consumed without loss. In the trial with juveniles, fish fed diets FISH and FISH +INSECT, showed significantly higher specific growth rate (SGR) and lower feed conversion ratio than all other dietary groups (results not shown). In case of grower sea bass different results were found. As shown in figure 4 and 5, fish receiving the diets VEG+INSECT and AVI+INSECT resulted in marginally improved SGR and in significantly improved feed conversion ratio (FCR) in comparison to the other dietary treatments.

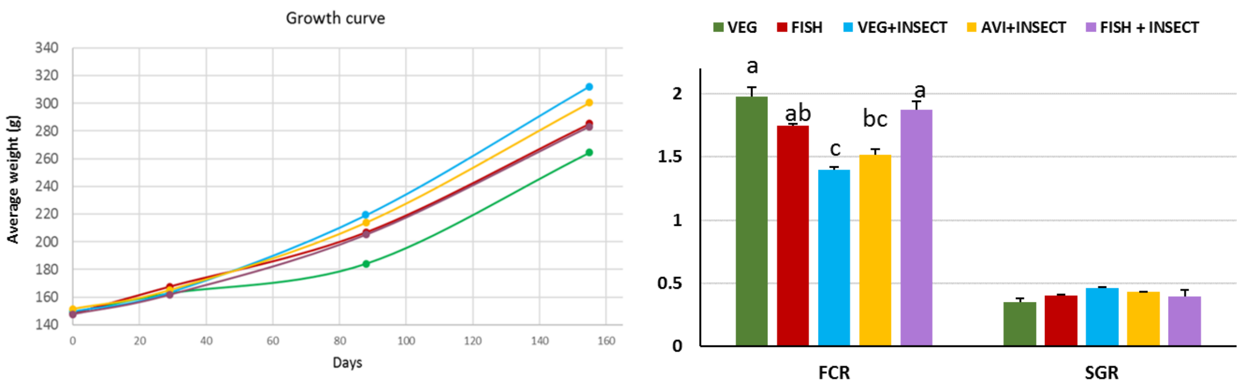
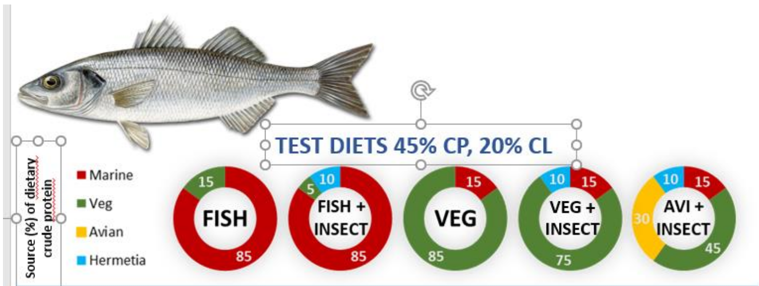


Figure 4 and 5. Growth performance and Feed Conversion Ratio (FCR) of rowersea bass subjected to the test dietary treatments at the end of the trial (mean ± std. dev. per dietary treatment; mean values not sharing same letters differ significantly : a,b, c; P<0.05).

Moreover, as shown in Figure 6, all major sustainability indicators such as economic conversion ratio (ECR), Fish in-Fish out (FIFO), fish meal and fish oil dependency ratios (FFDRmeal, oil) were also improved with diets including insect meal compared to the other ones.



SUSTAINABILITY INDICATORS					
Relative ECR	100	116	101	86	69
FIFO	4,16	4,42	1,06	0,73	0,81
FFDRmeal	4,45	4,75	0,78	0,55	0,60
FFDRoil	2,80	2,81	2,38	1,57	1,82

Figure 6. Effect of the dietary treatment on major sustainability indicators calculated at the end of the trial on grower sea bass.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Expectedly, the best preservation of PI morphology was observed in sea bass fed the CF or FH10 diets, while fish fed the CV diet exhibited significant degenerative changes in the proximal and distal intestines. However, PBM supplementation mitigated these effects and significantly improved all gut morphometric parameters in the VH10P30 group. Partial substitution of the plant mixture with insect meal alone or PBM also induced most BBM genes and activated BBM enzymes, suggesting a beneficial effect on intestinal digestive/absorption functions. Regarding intestinal microbiota, fish fed diets containing H. illucens meal (FH10, VH10, VH10P30) had the highest richness of bacterial communities and abundance of beneficial genera such as Lactobacillus and Bacillus.

Deliver novel feed formulations to feed-mill companies for farm scale trials

Based on the overall outcomes from the lab-scale trials available (i.e. growth performance, sustainability indicators, gut status), the dietary treatment **AVI+ INSECT**, combining Poultry by-product meal (PBM) and insect meal (BSFM) in low fish meal diets was found the most suitable feed formulations to be proposed to feed-mill companies and tested in farm scale experiments.

Consequently, LP designed two novel practical aquafeed formulations based on the test diets (AVI+INS) for bass and bream. Such completely formulated feeds were proposed and delivered to various feed-mill companies for being produced in large scale after minor arrangements.
The test feed formulations to be tested by Orada Adriatic in Cres (HR) on E. sea bass and by Friškina, Rogoznica (HR) on the Gilthead sea bream were produced by Skretting Italia and VRM Naturalleva, respectively. Their ingredient and proximate compositions are shown in table 6.

Table 6 Ingredient and proximate compositions (%) of test diets for farm trials

	Test feed BASS	Test feed BREAM
PBM	20,0	19,0
Insect meal BSF (Black Soldier Fly)	6,0	7,6
Soybean Meal Non-GMO	20,0	12,0
Veg protein mix	12,5	30,3
Fish meals	10,0	3,0
Squid/Krill meals	1,0	2,0
Fish oils	14,7	10,0
Veg oils	6,5	4,6
Wheat	7,7	10,4
Premixes	1,6	1,1
Crude Protein	41,1	42,1
Crude fat	26,0	20,7
Ash	7,4	7,5
Crude Fiber	3,0	2,8

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it

Feeding trials at farms

During the 3rd semester of the project, Partners PP8 and PP10, under the supervision of the scientific partners PP3, PP1, PP4 and LP set up the on farm experiments aimed at comparing the novel prototype feeds (Table 6) to the commercial ones actually used at farms and having the same gross nutrient composition as the prototype test diets.

Photo 2. Mr. Cvitić, Friškina operator (PP8) on the farm with Naturalleva feeds and dr. Oraić (PP1) preparing for the fish blood test.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Photo 3. Mr. Pežej, Ms Prkić, Friškina director and operator (PP8) on the farm with Naturalleva feeds and dr. Bubić and dr. Hrabar (PP3) and dr Manfrin (PP4) preparing for the fish blood test.

Photo 4 Dr.Hrabar with Naturalleva feeds on the Friškina farm – preparation of the feed trials.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

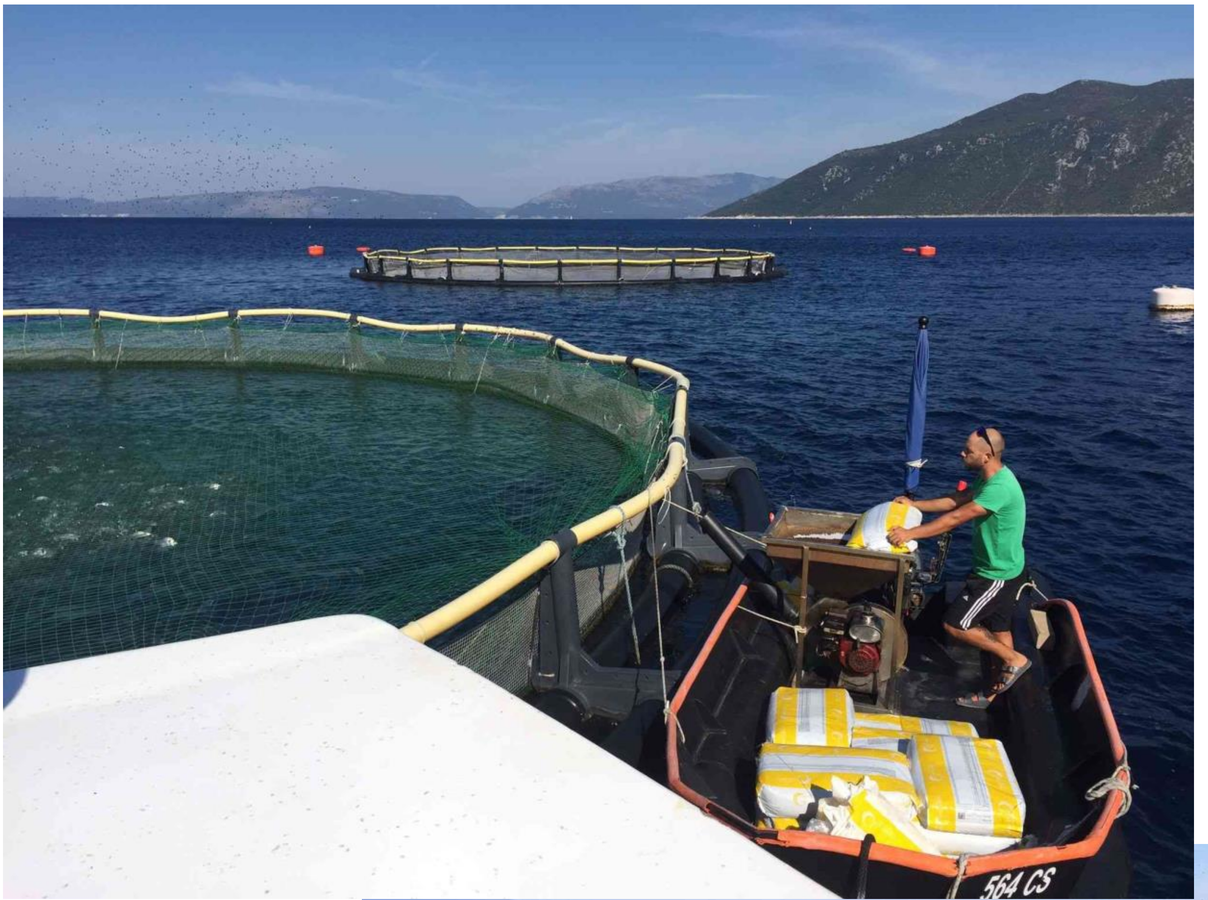


Photo 5 and 6. Aquaculture Operator and the feed trials at Orada Adriatic (PP10).

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Photo 7 and 8   Aquaculture Operator (PP10) and dr Manfrin (PP4) on the Orada Adriatic farm with Skreting feeds during the feed trials.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Photo 7. Field visit on Orada farms in 2019 with PP1, PP4 PP8 and PP10 partners.

Photo 8. Dr Zrncic (LP), dr. Balenović (PP10), dr.Krešić (PP5) at the Orada Adriatic farm. Filed visit in 2022.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Sea bream was the key species at Friškina fish farm and E. sea bass was the key species studied at Cres by Orada Adriatic.The experimental layout at Friškina (PP8) fish farm is summarised in the following scheme

Treatment	*N. of replicates (5m x 5m x 5m cages)*	*N of fish per cage*	*Initial size g*
Commercial feed	2	4,000	110-130
Test feed AAN BREAM	2	4,000	110-130

The trial started at the end of the 3rd semester and finished at the end of the 4th one. The experimental layout on sea bass at Orada Adriatic (PP10) fish farm is summarised in the following scheme

Treatment	N. of replicates (cages 22 m in diameter)	N of fish per cage	initial size g
Commercial feed	2	128,000	150
Test feed AAN BASS	2	128,000	150

The trial was started at the beginning end of the 4th semester and finished at the beginning of the 5th one. Major outcomes of the farm-scale trials are summarized in the following t**ables.**

Sea BREAM Friskina

DIET	final. Weight g	SGR	FCR	Mortality %
Comm	416	0,52	1,9	7,9
AAN	447	0,57	1,7	7,4
	P<0,11	*P<0,12*	*P<0,08*	*NS*

Sea BASS Orada Adriatic

DIET	final. Weight g	SGR	FCR	Mortality %
Comm	395	0,25	2,0	1,5
AAN	409	0,26	1,9	2,4
	P<0,11	*NS*	*NS*	*NS*

Sea BREAM Friskina

	Condition Factor	Carcass Yield%	Fillet Yield%	Abdominal Fat %
Comm	1,90	91,4	45,3	1,3a
AAN	1,84	91,1	44,3	0,9b
	NS	*NS*	*NS*	*P<0,05*

Sea BASS Orada Adriatic

	Condition Factor	Carcass Yield%	Fillet Yield%	Abdominal Fat %
Comm	1,07	88,3	49,2	2,8
AAN	1,11	88,6	51,2	2,4
	NS	*NS*	*NS*	*NS*

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it

Sea BREAM Friskina			
DIET	FIFO Fish In –Fish Out	FFDRm	FFDRo
Comm	**1.11**	**1.02**	**1.89**
AAN	**0.77**	**0.37**	**1.25**
	☺	☺	☺

Sea BASS Orada Adriatic			
DIET	FIFO Fish In–Fish Out	FFDRm	FFDRo
Comm	**1.16**	**0.82**	**2.76**
AAN	**0.82**	**0.57**	**1.44**
	☺	☺	☺

As shown in the tables above, feeding sea bream and sea bass the AAN test diets resulted in slightly better growth performance and feed conversion ratio relative to the commercial feeds. Major commercial quality traits were unaffected by the feed used apart from a slight beneficial reduction of adiposity with the AAN feeds. All sustainable indicators such as FIFO and FFDRm/o were markedly improved when fish were fed the prototype novel diets compared to the commercial ones.

4. Conclusion of the outcome

From the results of both pilot and farm-scale trials there is consistent evidence that:

- novel low- fish meal aquafeeds for sea bream and sea bass, inclusive of PBM and Hermetia meal, ensure growth performance, feed conversion ratios, commercial yields similar or better than the currently used commercial preparations.
- novel low- fish meal aquafeeds for sea bream and sea bass, inclusive of PBM and Hermetia meal, are safe to the fish
- *novel low- fish meal aquafeeds for sea bream and sea bass, inclusive of PBM and Hermetia meal, are more sustainable and ecofriendly than the safe to the the currently used commercial preparations.*

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it

3) *List of references with links where possible*

- [1] Tacon A.G.J. (2020). Trends in global aquaculture and aquafeed production. Rev. Fish Sci Aquac 28:43–56. https://doi.org/10.1080/

- [2] Cottrell, R.S., Blanchard, J.L., Halpern, B.S. et al. (2020). Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. Nature Food 1, 301–308. https://doi.org/10.1038/s43016-020-0078-x 23308249.2019.1649634

- [3] Maiolo, S., Parisi, G., Biondi, N., Lunelli, F., Tibaldi, E., Pastres, R. (2020). Fishmeal partial substitution within aquafeed formulations: life cycle assessment of four alternative protein sources. Int. J. Life Cycle Assess. 25, 1455–1471. https://doi.org/10.1007/s11367-020-01759-z.

- Ivana Lepen Pleić, Ivana Bušelić, Maria Messina, Jerko Hrabar, Luka Žuvić, Igor Talijančić, Iva Žužul, Tina Pavelin, Ivana Anđelić, Jelka Pleadin, Jasna Puizina, Leon Grubišić, Emilio Tibaldi, Tanja Šegvić-Bubić (2022). A plant-based diet supplemented with Hermetia illucens alone or in combination with poultry by-product meal: one step closer to sustainable aquafeeds for European seabass. Journal of Animal Science and Biotechnology volume 13,
  Article number: 77 (2022) https://jasbsci.biomedcentral.com/articles/10.1186/s40104-022-00725-z

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it

- **PART 2**

A. CONTRIBUTION TO EUSAIR

Please provide a description of the project contribution to the EUSAIR in terms of synergy with the Strategy's pillars and alignment of implemented project's activities with the Action Plans and labelled projects.

The project directly involved researchers from University and public Institute, fish farms and hatcheries, enterprises (SMEs being part of the aquaculture business chain such as companies for feed producing, recycling wastes, fish food transforming), and different type of stakeholders (experts, general public, productive associations, policy) from Italy and Croatia in order to improve the competitiveness of the mariculture sector Adriatic Sea. The results of task 3.1.1 will ensure important positive impacts on innovation, economic development, job creation, and environmental sustainability. The project approach and outcomes can be transferred to other territories of the EUSAIR macro region, thus multiplying the positive effects of project outputs. In particular, new fish feeds and feeding strategies that have been set up in lab and subsequently implemented in commercial farms, can cut down eutrophication emissions and improve fish health status and consequently guarantee healthy fish products as consumers' demand. Moreover, tailored feeding protocols that have been developed and tested on sea bass/bream commercial farms proposed in Adriatic mariculture, allow an innovative food management of farmed fish, will contribute to reduce the marine pollution and maintain the marine biodiversity, as required by EUSAIR action plan that identifies aquaculture as a key sector in the blue economy of Italy, Croatia and Greece, having the potentiality to play a pivotal role in the entire area.

B. CONTRIBUTION TO HORIZONTAL PRINCIPLES

Please provide a description of the project contribution to the horizontal principles of equality between men and women, non-discrimination and sustainable development.

The project engaged technical and administrative staff based on personal characteristics, complying with the equal opportunities and without discriminations, such as gender, race, nationality, ethnic origin, religion or belief, disability, age or sexual orientation. The employment relationship was based on the principle of equal opportunity and fair treatment, including type of contract, wages and benefits, working conditions and terms of employment, access to training, promotion, and termination of employment as for any other Italian or Croatian staff hired. The staff and external services involved were formed without any kind if discriminations based on personal characteristics, genre, age, belief, race, nationality, ethnic, religion and belief, sexual orientation, etc. The project activities and outcomes can be considered as contribution to sustainable development and within the task 3.1.1 in particularly, in terms of environmental, economic and social sustainability. New efficient technologies for the management of fish farm and the outcomes produced support fish farmers in acquiring new tools to improve the impact and environmental footprint of fish product and production process. This has an impact on waste effluents and the related new technologies taht were tested and improved, in order to reduce the environmental impact of sea bass/bream farms. The application of these implemented technologies in sea bass/bream farming in the Adriatic area will also ensure a better productivity and profitability of fish farm, providing permanent employment opportunities and increase the sustainability of the aquaculture sector.

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

C. COMMUNICATION ACTIVITIES

Please refer to the Final Communication Report template and provide a summary on the main achievements trying also to identify which were the most successful communication tools in reaching general public/decision makers/other target groups.

All the activities performed to reach the present DL have been documented with photos and videos taken by LP, PP1, PP3, PP4, PP8 and PP10 communication specialists. The material has been uploaded on the Intranet website of the project. Some of the materials was used to produce this report (see above), and to produce communication materials. The aforementioned activities have been presented at the training events held in Padua, Ostuni, Pordenone, Zadar and online. During the final conference in Zadar (3 June 2022) and Udine (20 June 2022) a summary of the most important results have been presented by LP, PP1, PP3, PP4, PP8 and PP10 staff. Numerous reports, meetings, brochures, training courses, conferences, a website and a YouTube channel have been produced to communicate the results.

D. NATURA 2000

Please describe, if it is the case, measures foreseen and implemented by the project:

a) In case the project involved Natura 2000 sites, describe what measure the project envisaged and implemented to avoid any negative impact: No Natura 2000 sites are included in the areas where the project activities have been carried out; therefore, no measures have been envisaged and implemented during the project in order to avoid negative impacts.
b) In case the project had a positive effect on Natura 2000 sites, please describe which measure the project has foreseen and implemented in order to reach a direct or indirect positive impact: No Natura 2000 sites are included in the areas where the project activities have been carried out; therefore, no measures have been envisaged and implemented during the project in order to avoid negative impacts.

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

E. TYPES OF ACTIONS ADDRESSED (as defined in the Cooperation Programme)

These are our primary objective's types of actions, that we addressed by the Project:

Specific Objectives	***Types of action***	***the most relevant one within the SO addressed by your project***
1.1 Enhance the framework conditions for innovation in the relevant sectors of the blue economy within the cooperation area	*Joint projects and actions aimed at creating platforms, networks and at supporting exchange of good practices in order to enhance the knowledge transfer and capitalization of achieved results in the field of blue economy*	***X***
	Actions aimed at cluster cooperation, joint pilot initiatives in order to boost the creation of marketable innovative processes and products, in the field of blue economy	***X***

F. TYPES OF OUTPUTS PRODUCED

Specify the types of outputs generated by your activity that are reported here and provide a brief description

Output typology	**Description**
Trainings	9 training courses in Italy and Croatia regarding new environmentally sustainable dietary formulations for cultured marine fish have been performed during the project.
Monitoring systems	N.A.
SMEs clusters	Potential collaboration and exchange of work and resources among enterprises involved in the aquaculture business chain such as fish farms and companies for aquafeeds producing and waste recycling were established. The innovative techniques and systems implemented during the project within the task 3.1.1 are already applied on SMEs involved and they can be applied in other Italian and/or Croatian fish farms and facilities. The cross border production chain that involves Italian hatcheries, which grow sea bass and sea bream fingerlings and juveniles, and Croatian on-growing sea cages-based farms, which than exported the fish to the Italian market, was implemented thanks to the project training courses and events.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

New networks	Through the project, very important was the teamwork with API – ASSOCIAZIONE PISCICOLTORI ITALIANI (ITALIAN Pisciculturers' association) that gather more than 300 Smes and that collaborated with the project in different ways – from dissemination to event organisation, from training lectures to elaboration of Protocols on common policy for aquaculture and cooperation perspectives for public authorities and Recommendations on quality indicators of farmed sea bass and sea bream. On the other hand, MARICULTURE CLUSTER - KLASTER MARIKULTURA (PP7) partnership was fundamental for the project as the cluster currently gathers around 80 legal entities, and this community has a total of more than 800 employees divided in 3 Groups; - Group of tuna growers, president Kristijan Zanki, Sardina d.o.o. Posts - Group of white fish breeders, president Dragan Pezelj, Friškina d.o.o. Split (PP8) - Shellfish Breeders Group, President Antun Pavlović, Stone Shellfish Association
Platforms	The model for cage monitoring and managing, created by Bluefarm within WP3 is at disposal for SMEs involved in the project for the next five years free of charge. This model can be further explored and implemented also on European level.
Adaptation plan	N.A.
Building renovation	N.A.
Others (please specify)	N.A.

G. TYPOLOGY OF IMPACTS

Please indicate what type of impact(s) your project has had. You can choose more than one answer. For each tangible impact selected, please provide a concrete example from your project, where possible supported by quantitative information.

TANGIBLE IMPACTS

Tangible impacts	**Example/ quantitative information**
Improved access to services	N.A.
Cost savings	Novel acquafeeds are very promising in terms of FCR, fillets quality and environmental impact, but high relative economic ratio and can be used and proposed on the farms. However, on long term can ensure the reduction of costs for fish production.
Time savings	

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it

Reduced energy consumption	
Reduced environmental impact	The application of the innovative and smart techniques and systems, which were developed and implemented during the project within the task 3.1.1, in sea bass/bream intensive farms in the Adriatic area will effectively limit the pollutant emissions and dispersions from fish cages as well as will ensure an adequate treating of wastewater, contributing to reduce the marine pollution and maintain the marine biodiversity.
(Man-made, natural) risk reduction	Application of novel acquafeeds on commercial farms demonstrated to be of high value and impact on fish health and simultaneously provide safe products to consumers. This limits the risk of costs and production reduction.
Business development	The application of the novel feeds in combination with innovative and smart techniques and systems for pollutant reduction, waste management, and energy saving, which were developed and implemented during the project within the WP3 in sea bass/bream intensive farms in the Adriatic area will ensure a better productivity and more eco-compatible productions that will be more appreciated by the consumers, increasing the profitability of the mariculture sector.
Job creation	The application of the innovative and smart techniques and systems developed and implemented during the project within the task 3.1.1. in sea bass/bream intensive farms in the Adriatic area and the consequent higher productivity and profitability of the aquaculture sector can provide permanent employment opportunities to costal populations of both sides of the Adriatic sea.
Improved competitiveness	The increased productivity and profitability of the aquaculture sector in the Adriatic area through the application of the innovative and smart techniques and systems for pollutant reduction, waste management, and energy saving, which were developed and implemented during the project within the task 3.2.1., in sea bass/bream intensive farms in the Adriatic area will ensure an increased competitiveness of SMEs on regional and international markets.
Other tangible impacts (specify)	N.A.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

INTANGIBLE IMPACTS

Intangible impacts	Example/quantitative information
Building institutional capacity	New protocols elaborated within the WP3 were presented to several institution and public authorities (Ministries, counties, regions) and help to build institution capacity and sensibility on fish farm industry and fish production in relation also to the sea habitat and marine sustainability.
Raising awareness	The project has stimulated the attention of fish farmers regarding the issues of new environmentally sustainable dietary formulations for farmed fish and the application of energy saving and reduction technologies and the farm environmental impacts through the study of new techniques for the production of renewable energy and the treatment of wastewater from hatcheries and sea cages, so to improve the sustainability of Mediterranean aquaculture.
Changing attitudes and behaviour	The project provides to fish farmers new feeds and new techniques for the production of renewable energy and the treatment of wastewater to be applied in hatcheries and sea plants, so to improve the sustainability of Mediterranean aquaculture and consequently the competitiveness of sector.
Influencing policies	The project outcomes can produce impact on EU regulations regarding marine aquaculture. The European Commission wants to help develop the EU aquaculture sector that ensures the supply of nutritious, healthy and tasty food with a low environmental and climate footprint, creates economic opportunities and jobs, and becomes a global reference for sustainability and quality. Its policy aims specifically to • building resilience and competitiveness • ensuring the participation of the sector in the green transition • ensuring social acceptance and consumer information on EU aquaculture activities and products • increasing knowledge and innovation in the EU aquaculture sector Through the strategic guidelines for a more sustainable and competitive EU aquaculture **for the period 2021-2030**, the Commission provides a common vision for EU countries, the aquaculture sector and other stakeholders to develop the sector in a way that contributes directly to the European Green Deal and the Farm to Fork Strategy. EU countries have reviewed their national strategic **plans** to promote aquaculture to take into consideration that vision. The future project capitalisation among the partners will intensify the impact on the EU guidelines in relation to aquaculture in Mediterranean area.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it
www.italy-croatia.eu/adriaquanet

Improving social cohesion	N.A.
Leveraging synergies	N.A.

European Regional Development Fund

prof. Marco GALEOTTI DVM, Dipl. E.C.V.P.
University of Udine
Department of Agricultural, Food, Environmental and Animal Sciences

Via Sondrio, 2, Udine, 33100, Italy
+39 0432-558594
marco.galeotti@uniud.it