



SUMMER SCHOOL

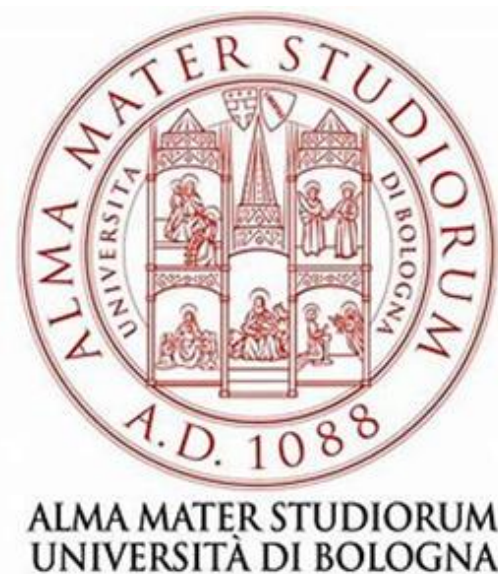
**FROM AUGUST 30TH
TO SEPTEMBER 3RD**

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773330 (GAIN)



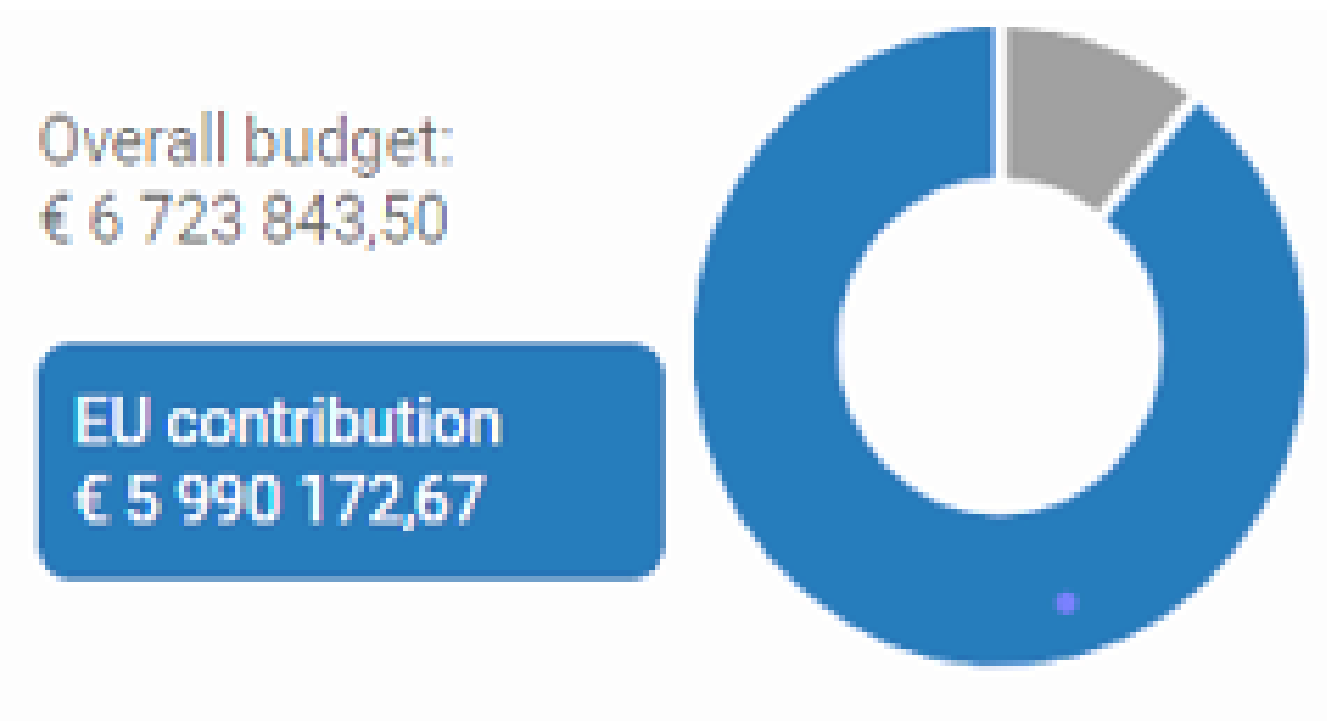
New technologies tools and strategies for a sustainable, resilient and innovative European Aquaculture (H2020 NewTechAqua)

Project overview and preliminary results from WP1: feeds and feed strategies



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Programme and funding scheme

- H2020-EU.3.2.3.2. - Developing competitive and environmentally-friendly European aquaculture
- IA-Innovation action

Call for proposal and topic

- H2020-BG-2019-1
- DT-BG-04-2018-2019 - Sustainable European aquaculture 4.0: nutrition and breeding

Grant agreement ID 862658

- Lenght 4 years
- Start date 1° January 2020
- End date 31° December 2023

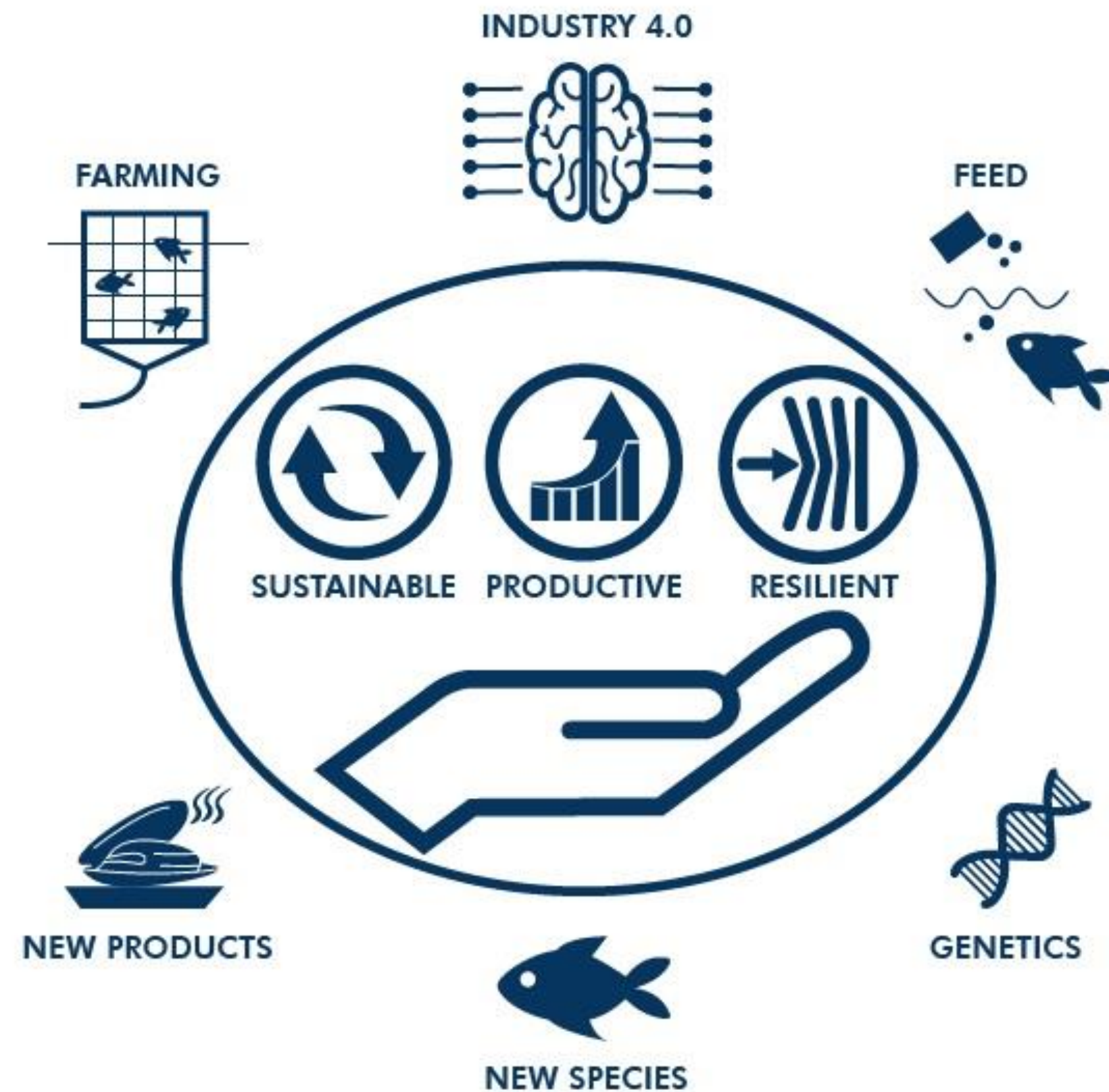
Coordinated by

- ALMA MATER STUDIORUM – UNIVERSITA DI BOLOGNA (Italy)

The NTA consortium



11 <u>Research & Development</u> 			12 Industries 			3 <u>Exploitation & Dissemination</u> 		
Part n.		Name	Part n.		Name	Part n.		Name
1 ©		UNIBO	7		AIA	14		FEAP
2		UNIVE	8		ABSA	15		CIHEAM
3		IRTA	9		CROMARIS	17		GIE
4		HCMR	10		MOWI			
5		UNIBA	11		IRIDA			
6		NOFIMA	12		RARA			
18		IFREMER	13		AQUANETIX			
19		SYSAAF	16		VIGNETO			
21		DFMR	20		ARGO			
22		ULPGC	23		TSIC			
25		CNR	24		GMF			
			26		EMAR			

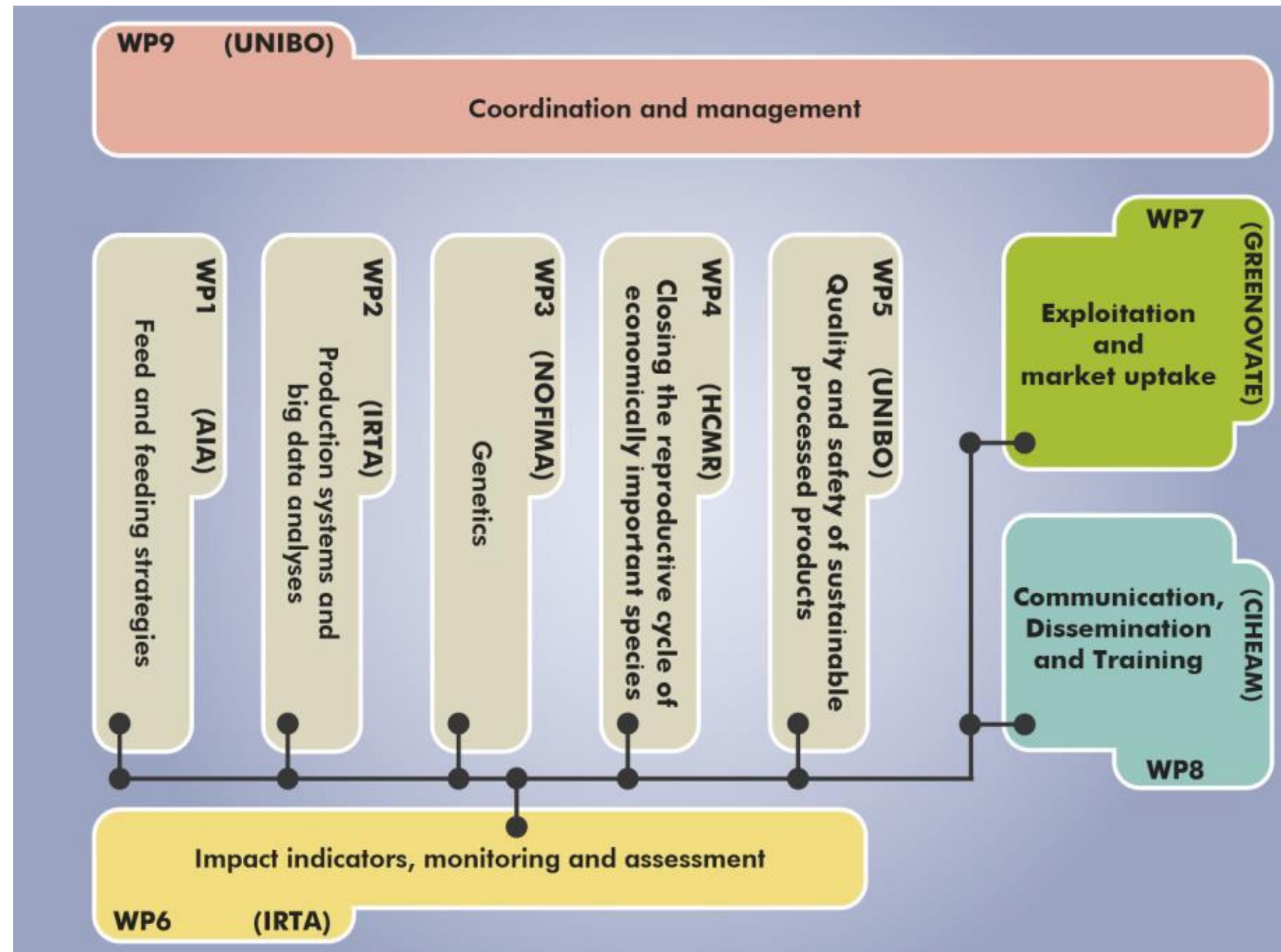


INTEGRATED SOLUTIONS FOR A NEW AQUACULTURE

Objective: to expand and diversify aquaculture production of finfish, molluscs and microalgae by developing and validating technologically-advanced, resilient and sustainable applications

- 4 commercially most **important finfish** species (salmon, trout, seabass and seabream)
- 4 economically **emerging new species** (amberjack, meagre, sole and mullet)
- 2 **molluscs** (oyster and mussels)
- 3 **microalgae** species

NewTechAqua Work Packages



Some relevant solutions under evaluation

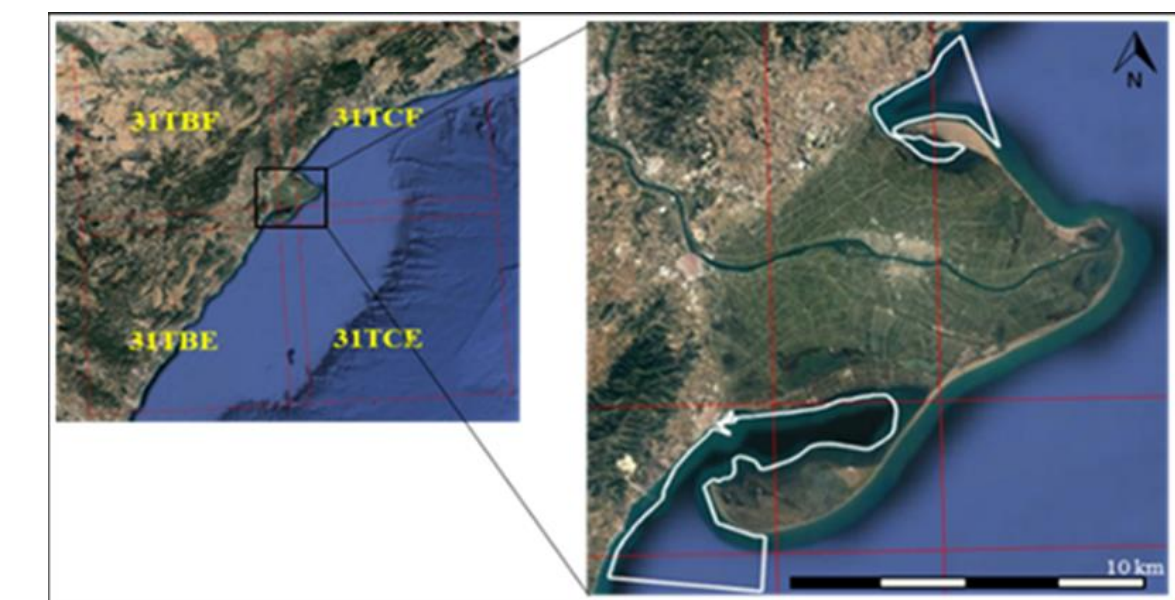
Three innovative sets of **aquafeeds**, each targeting a specific issue (**pro-health, organic, zero waste**)

Monitoring systems will aggregate and combine spatiotemporal information (**Big data**) in dynamic complex statistical and **Artificial Intelligence** models for disease prediction and health management

Decreasing the environmental loads of land based farming and increasing profitability by optimizing feed and oxygen supply: thus achieving the main goals of ecological intensification of aquafarming. Under the emerging framework of Precision Fish Farming

Welfare indicators, and microbiome analyses (NGS) will be used to evaluate the impact of different rearing systems (**RAS, biofloc technology, aquaponics, ELOXIRAS**) on fish condition

Satellite imagery by the development and validation of **biosensors** for supporting shellfish industry



Some relevant solutions under evaluation

Innovative breeding programmes to improve performance, robustness and quality of farmed fish, mollusc and microalgae, using different genomics methods.

Enhanced know-how of **the reproductive physiology** and on the reproductive dysfunctions of three emerging species: **greater amberjack, meagre and Senegalese sole** under rearing conditions.

Development of **innovative high-quality seafood** products and of tailored sustainable techniques for **valorisation of by-products** through the preparation of functional ingredients.



WP1: Feeds and feed efficiency

Main Objectives

- Improve fish health reducing *Sparicotyle chrysophrii* infections in seabream through natural functional diets
- Promote marine by-product valorization employing innovative free-catch fishmeal diet for seabass
- Develop organic low fishmeal aquafeeds in conventional and emerging species
- Define microbiome application to enhance health of farmed species in relation to feeding regimes



Tasks and subtasks of WP1

Task 1.1 Pro-health feed to reduce Sparicotyle in seabream

Subtask 1.1.1. Identification and selection of natural dietary compounds with antiparasitic effects

Subtask 1.1.2. Field cage trial to control Sparicotyle chrysophrii outbreaks

Task 1.2. Zero-waste feed by using marine by-products from fishery and aquaculture

Subtask 1.2.1. Dose-response trial to develop zero-waste diet for European seabass

Subtask: 1.2.2. Validation trial in sea cage farm

Task 1.3. Refining organic feeds for trout, seabream and meagre: merging sustainability with an affordable cost

Subtask 1.3.1. Development of new organic feed for trout, seabream and meagre

Subtask: 1.3.2. Validation trial of new organic diet in seabream

Task 1.4 Microbiome application to determine and improve fish health

Task 1.1 Pro-health feed to reduce Sparicotyle in seabream

Nowadays Mediterranean farmed gilthead sea bream (*Sparus aurata*) production and health is threatened by the hematophagous gill parasite *Sparicotyle chrysophrii* (Sitjà-Bobadilla et al., 2010).

Infection by *S. chrysophrii* can cause gill lesions, anemia, lethargy, anorexia, growth retardation and increase of FCR by a factor of >0.4 (Rigos et al., 2016), mortality at high prevalence and intensity (Vagianou et al., 2006), although severe pathology can occur also at low infection intensity (Mahmoud et al., 2014).

Antiparasitic bath immersion treatments have been applied in affected cages but they represent critical issues for the sustainability of modern aquaculture and the most used compounds (e.g. formalin) are not authorized in several countries

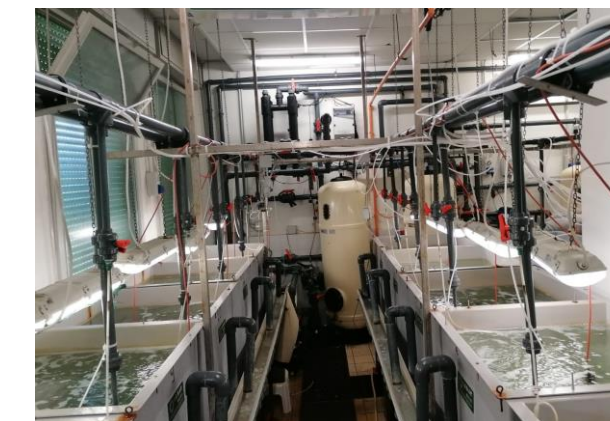
At this regard, recently the attention has been focused on feed additives and natural substances



In detail, the specific objectives are as it follows:

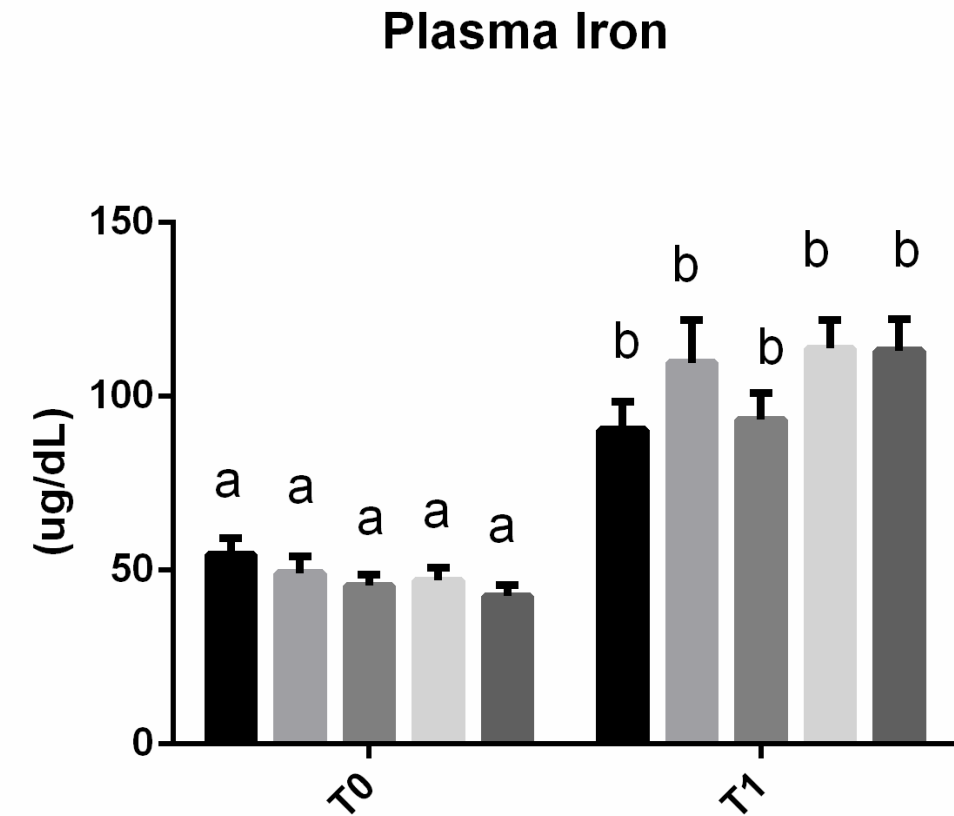
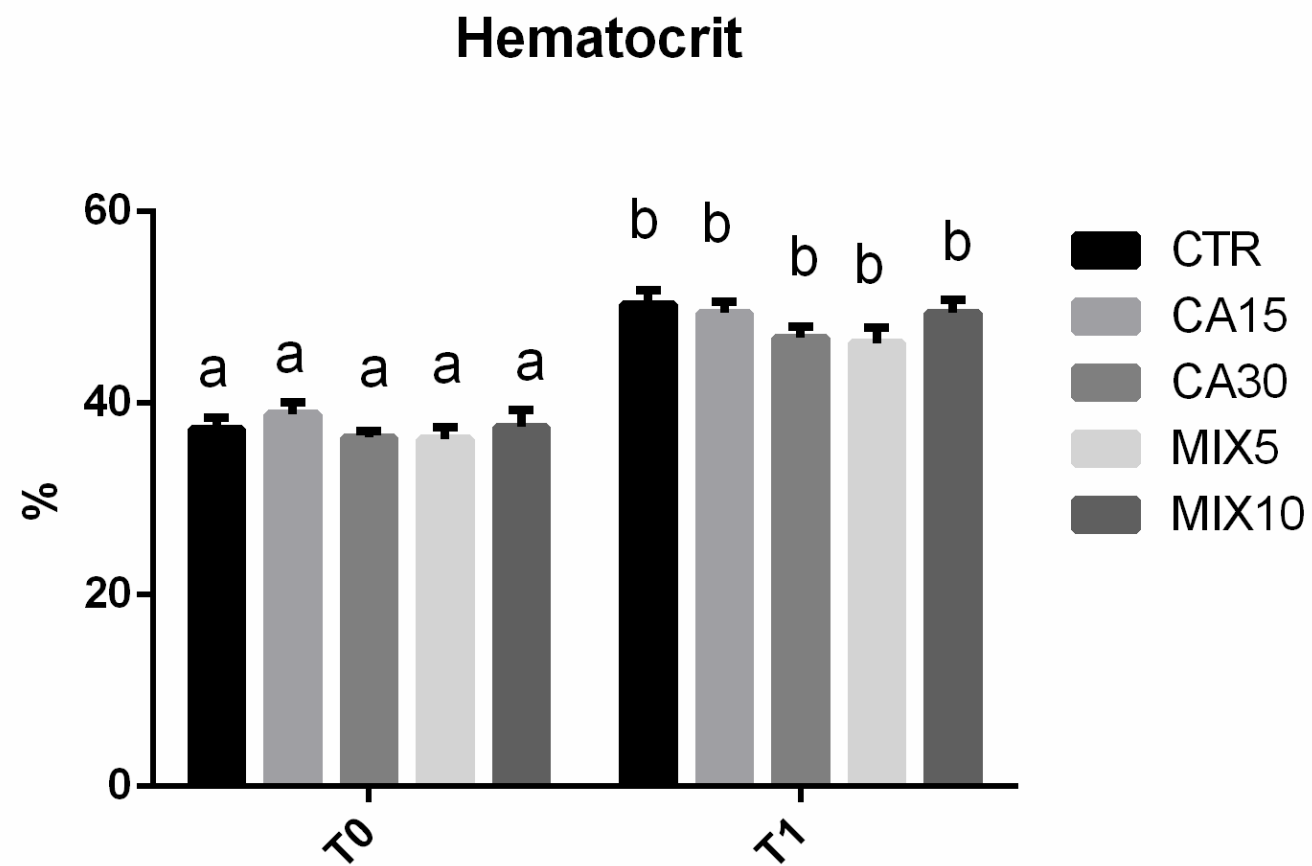
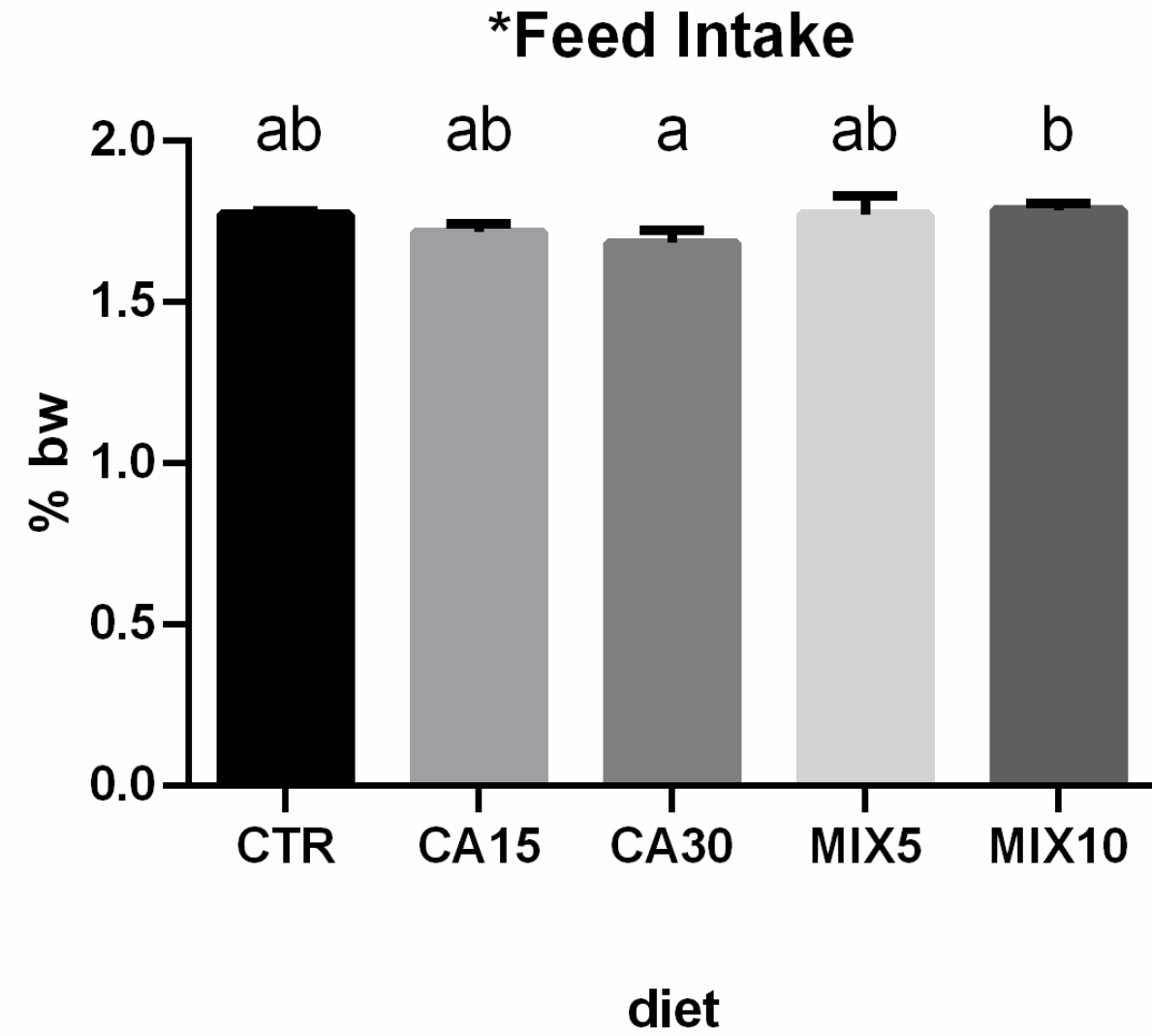
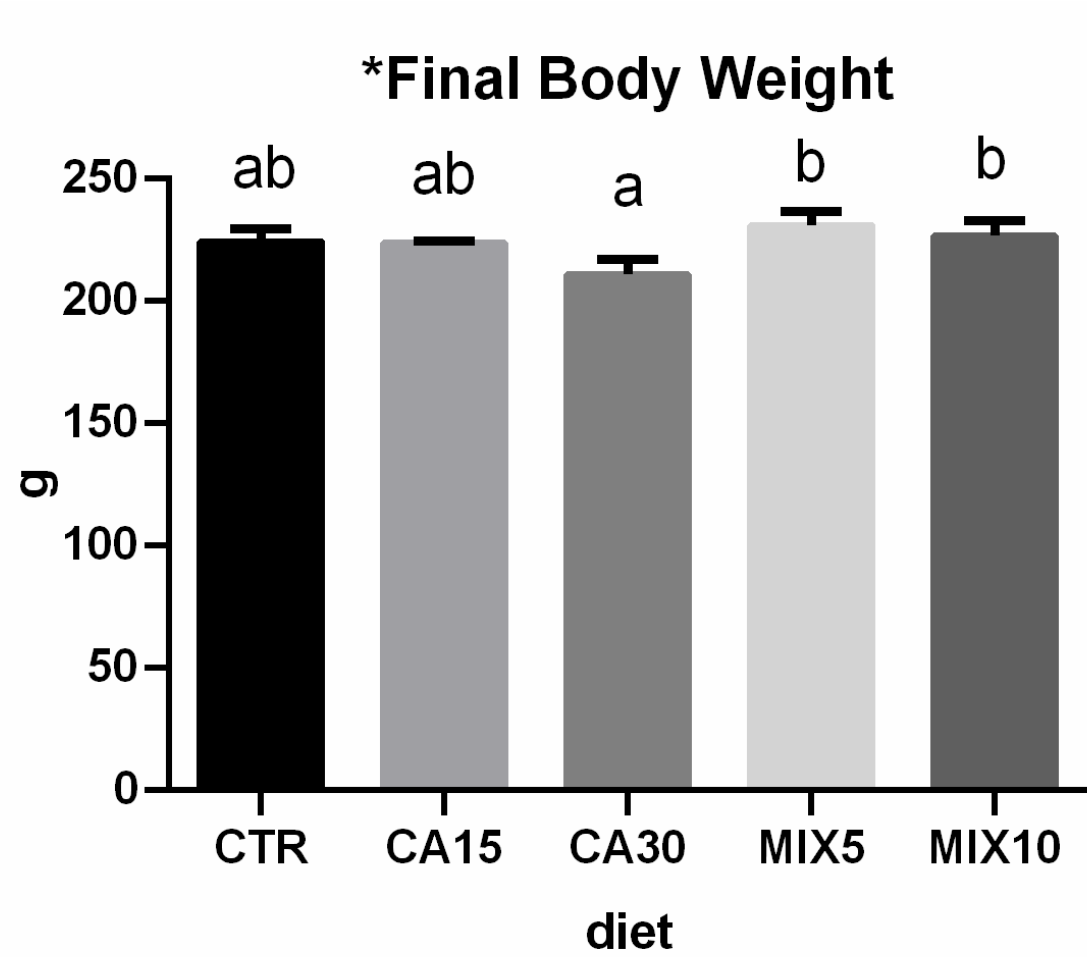
- Select natural compounds with known anthelmintic effects to be included in formulated diets for seabream
- Test a dose response trial at laboratory scale to estimate the effect of chosen natural compounds/blend at certain dietary level on gills and overall fish health
- Define microbiome application to enhance health of gilthead seabream in relation to functional feeds application
- Choose the most promising compound and dietary dose level to be tested at pilot scale in a commercial off-shore fish farm.

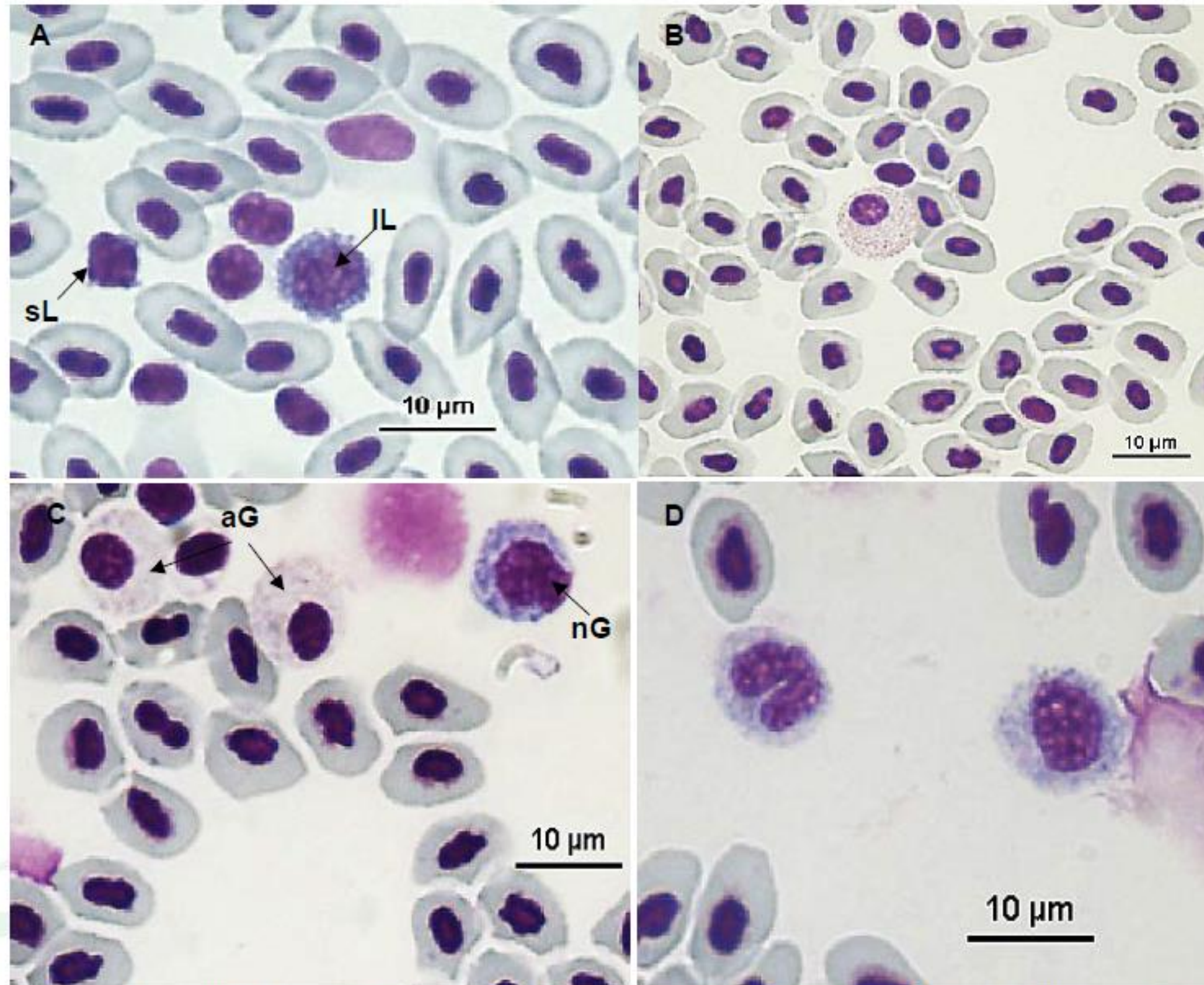
Diet name	Compound/s	Dose (g/kg)	Tank allocation
CTR	without	0	12 – 11 – 1
CA15	Caprylic acid	15	3 – 15 – 13
CA30	Caprylic acid	30	7 – 9 – 14
MIX5	Caprylic acid, Fe, Zn, vit B	5	8 – 6 – 5
MIX10	Caprylic acid, Fe, Zn, vit B	10	10 – 2 – 4



Preliminary results

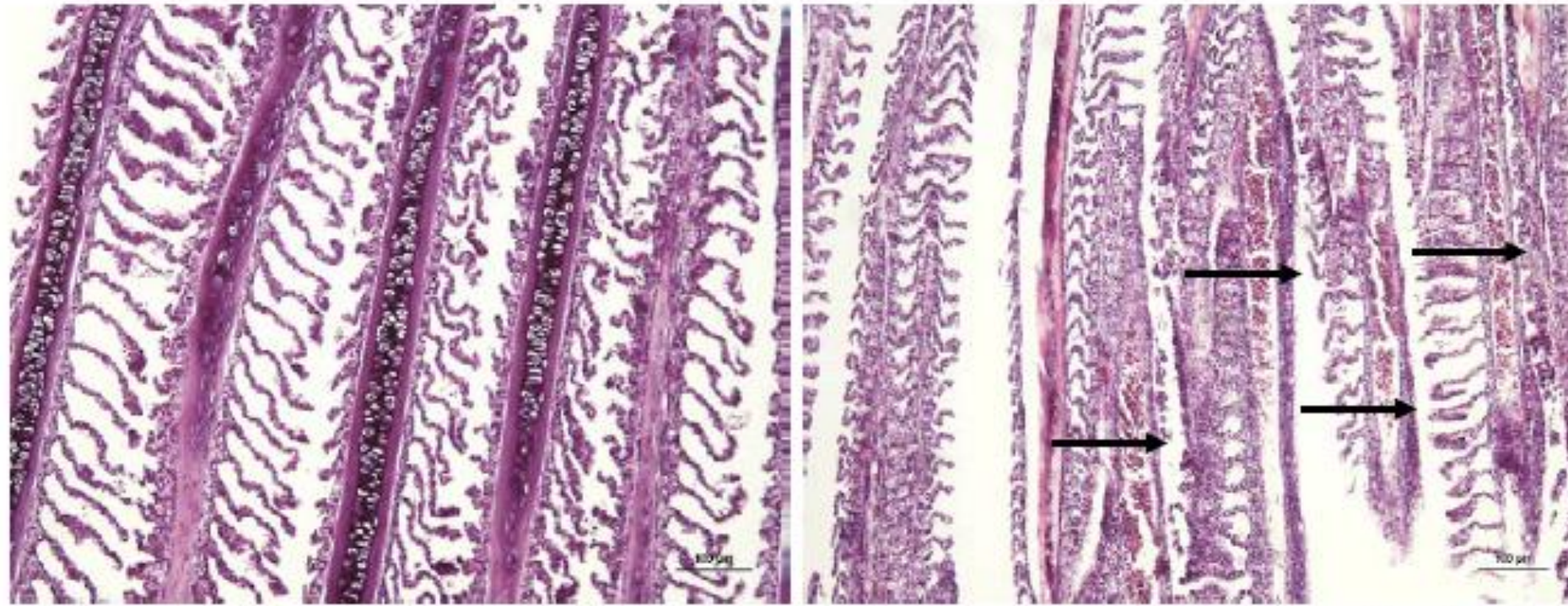
- MIX5, MIX10 showed higher final weight than CA30
- Hematocrit and plasma Fe increased for all diets at the end of the trial
- MIX5 and MIX10 showed a stronger increment in plasma Fe compared to the other diets



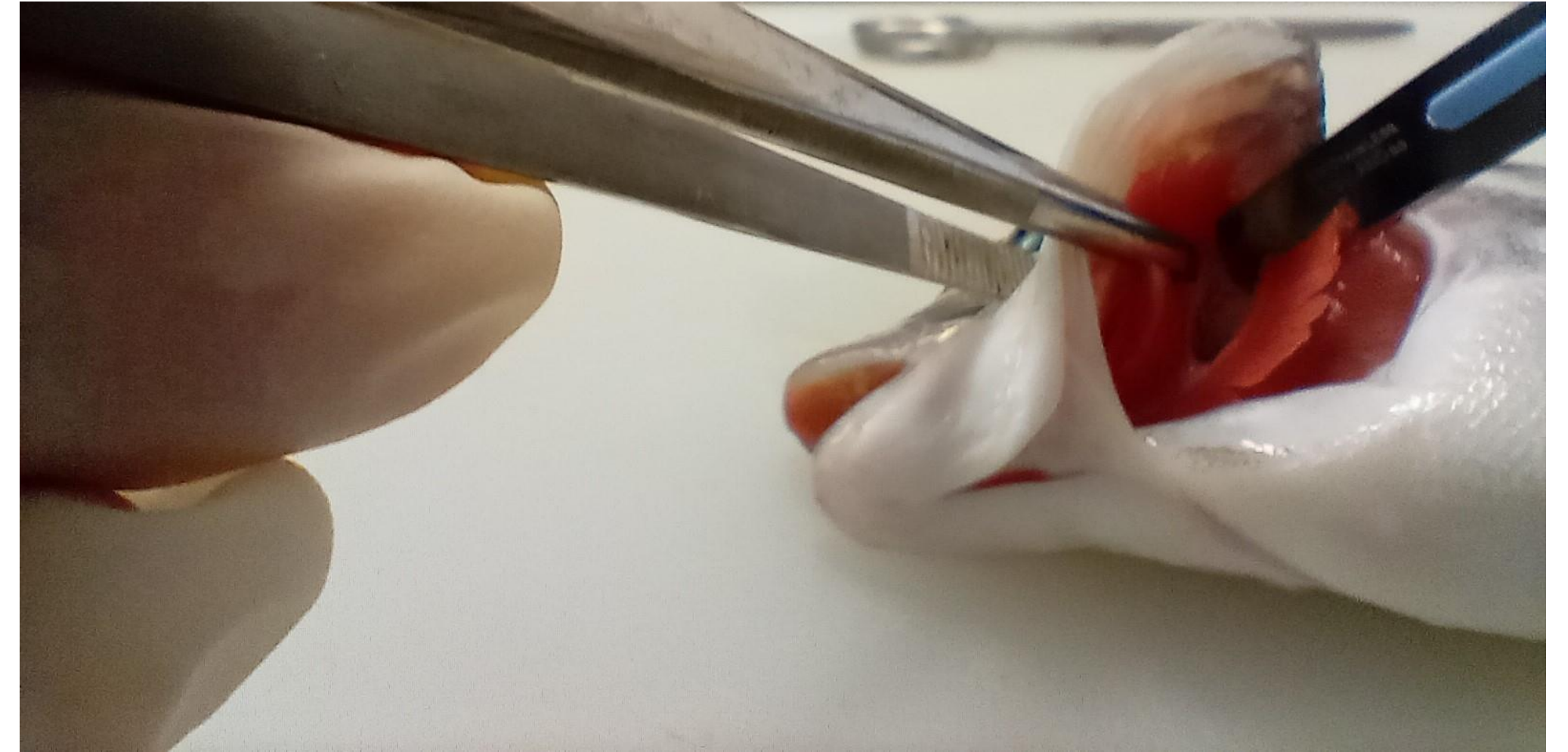
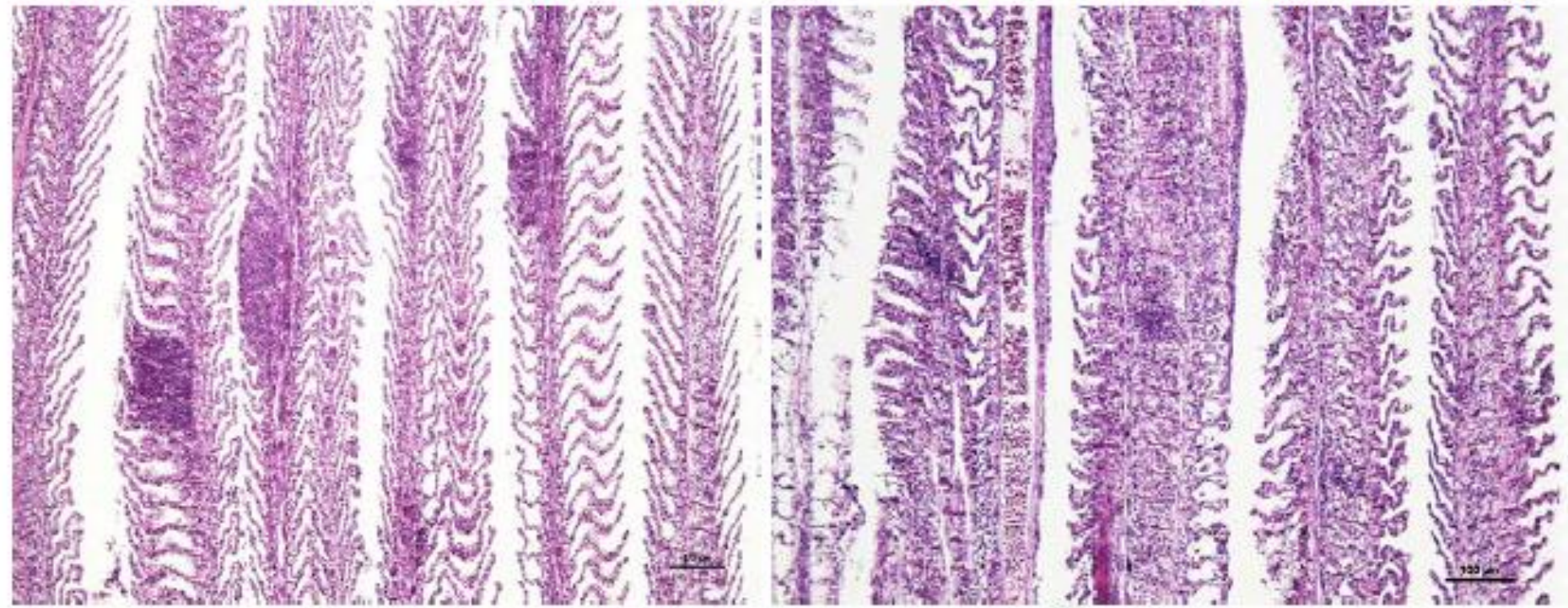


Blood white cells: A) lymphocytes (sL=small lymphocyte; IL=large lymphocyte); B) acidophil granulocyte (eosinophil); C) granulocytes (aG=acidophilic granulocyte; nG=neutrophilic granulocyte); D) monocytes





Gills: no evidence of lesions (on the left); congestion of the filaments (arrow) (on the right);



- Pro-health feed has been selected, formulated, produced and shipped to Cromaris (one control diet and two functional feeds)
- Trial in duplicate sea cages started in February and lasts 10 months
- Protocol has been defined on samplings activities to evaluate parasite incidence, gill microbiome, growth and environmental parameters



Task 1.2. Zero-waste feed by using marine by-products from fishery and aquaculture

In recent years the fishmeal content in aquafeed has progressively declined globally for the major farmed species, with decreasing ratio (FI:FO) higher for carnivorous fish species such as Mediterranean species.

In species such as European seabass and seabream fishmeal has been mainly replaced by the inclusion of relevant amount of plant ingredient such as soy products (Bonvini et al 2018a, b).

However, plant ingredients too may pose contrasts in sustainability and resource efficiency, as these resources are valid food for human consumption and may affect fish performance, fish health and fillet composition when included at high dietary level.

Recently, a growing quantity of fish by-products sourced from both wild caught and farmed fish estimates that 25% of fishmeal worldwide originates from fish by-products, with an even greater opportunity to gather raw materials for fishmeal from the estimated 60 million t of by-products produced around the globe from fisheries and aquaculture (Ytrestøyl et al., 2015).



WP1-Task 1.2

Subtask 1.2.1 Dose-response trial to develop zero-waste diet for European seabass

- Raw materials have been identified (Veronesi-Unibo)
- Feed has been formulated, produced and shipped to Unibo (Veronesi-Unibo)

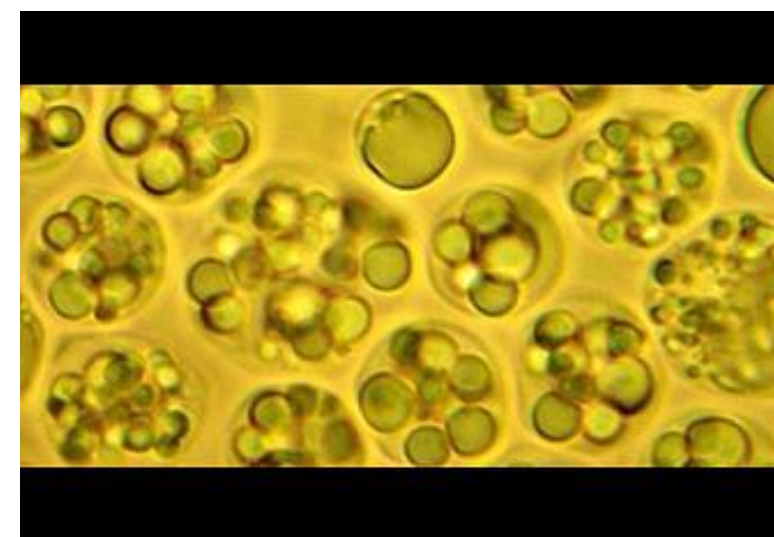
Zero-waste raw material selected

- Fish meal Trimming
- Fish oil Trimming
- Shrimp meal
- Microalgae
- Functional Yeast



Total replaced ingredients

- Fish oil by-catch
- Fish meal by-catch
- Soybean meal
- Soyprotein concentrate



Composition		C.	OFM100FO	OFMFO	OFMFO-50SP	OFMFO-OSP
MARINE	Fish Meal PRIME	250	-	-	-	-
	Fish Meal TRIMMING	-	250	250	250	250
	Fish Oil EXTRA	146	142.5	-	-	-
	Fish Oil TRIMMING	-	-	142.5	125	125
	KRILL M.	25	-	-	-	-
	SHRIMP M.	-	25	25	85	85
VEGETAL	Wheat M.	145	143	144	140	182
	SOY M.	192	192	192	96	-
	SPC	80	80	80	40	-
	GLUTEN Corn	40	40	40	100	155
	GLUTEN Wheat	-	26	25	27.5	95
	SUNFLOWER	50	40	40	50	50
	GERM OF GUAR	60	50	50	50	-
Functional Yeasts		-	-	-	-	20
Innovative Microalgae <i>Schizochytrium</i>		-	-	-	25	25
Fixed Integration	Premix	6.2	6.2	6.2	6.2	6.2
	Antiox	1	1	1	1	1
	a.a.	4.8	4.3	4.3	4.3	5.8 ¹ ₀
		1000	1000	1000	1000	1000



- Number of tanks 15
- Number of treatments 5 (1 control diet and 4 experimental diets)
- Number of replicates 3
- Number of fish per tank 50
- Initial body weight (g) ~60

	<i>Experimental diets</i>					
	<i>C</i>	<i>OFM100FO</i>	<i>OFMFO</i>	<i>OFMFO-50SP</i>	<i>OFMFO-0SP</i>	<i>P</i> va
IBW	75.95±1.06	75.79±1.80	76.38±0.79	75.84±2.13	75.83±1.45	0.98
FBW	247.19±4.75 ^b	239.51±9.89 ^b	221.31±5.89 ^a	222.24±5.85 ^a	217.39±9.24 ^a	0.00
SGR (% day ⁻¹)	0.99 ± 0.01 ^b	0.97± 0.02 ^c	0.89 ± 0.04 ^a	0.90 ± 0.02 ^a	0.88 ± 0.02 ^a	0.00
FI (g feed fish ⁻¹)	1.19 ± 0.02 ^{ab}	1.22 ± 0.03 ^{ab}	1.24 ± 0.03 ^b	1.22 ± 0.02 ^{ab}	1.16 ± 0.04 ^a	0.02
FCR	1.34 ± 0.02 ^a	1.40 ± 0.05 ^{ab}	1.50 ± 0.04 ^b	1.48 ± 0.05 ^b	1.43 ± 0.05 ^{ab}	0.00
Weight gain (g)	171.24 ± 3.97 ^b	163.72±8.10 ^c	144.93±5.57 ^a	146.40±7.89 ^a	141.57±7.84 ^a	0.00
Survival %	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	1
VSI	11.88 ^a ± 0.59	12.71 ^{ab} ± 0.47	12.93 ^{ab} ± 0.74	12.86 ^{ab} ± 0.24	13.70 ^b ± 0.42	0.02
HSI	1.93 ^a ± 0.64	2.14 ^a ± 0.18	1.97 ^a ± 0.12	2.35 ^c ± 0.06	2.82 ^b ± 0.22	<0.001

In particular, when only wild FM was totally replaced by fisheries by-product no differences were recorded, while the combine replacement of wild FM and FO resulted in a performance reduction. Interestingly, the further replacement of soy products by alternative plant proteins in the free wild fish diet did not result in a decline of performance.

Task 1.3. Refining organic feeds for trout, seabream and meagre: merging sustainability with an affordable cost



According to the EUMOFA study published in 2017, the trend in EU organic fish production shows a considerable increase for the major species in recent years: +24% for salmon, +25% for seabass/seabream and + 50% for rainbow trout.

One of the main problems for organic aquaculture development, apart from the provision of organic juveniles, is the supply of certified organic feeds and its cost, the organic feed being almost 50% more expensive than the feed used in conventional production.

Oraqua project (FP7-KBBE 2013) suggested the need for additional types of raw materials and reconsidered the sourcing of plant feed ingredients taking into account not only the amino acid profile but also carbohydrate and fatty acids content.

COMMISSION REGULATION (EC) No 710/2009

Specific rules on feeds for carnivorous aquaculture animals

1. Feed for carnivorous aquaculture animals shall be sourced with the following priorities: (a) organic feed products of aquaculture origin; (b) fish meal and fish oil from organic aquaculture trimmings; (c) fish meal and fish oil and ingredients of fish origin derived from trimmings of fish already caught for human consumption in sustainable fisheries; (d) organic feed materials of plant origin and of animal origin as listed in Annex V and the restriction laid down therein are complied with.
- 2. The feed ration may comprise a maximum of 60 % organic plant products.**
3. Astaxanthin derived primarily from organic sources, such as organic crustacean shells may be used in the feed ration for salmon and trout within the limit of their physiological needs. If organic sources are not available natural sources of astaxanthin (such as Phaffia yeast) may be used.

Ingredients (%)	Rainbow Trout			Gilthead Sea Bream		
	CTRL	PEA 12.1%	PEA 24%	CTRL	PEA 8.5%	PEA 19%
FM from timmings	47.5	33.0	19.0	51.3	38.5	25.6
Organic soybean meal	24.0	24.0	24.0	25.2	25.2	25.2
FO from trimmings	14.3	15.0	15.6	8.7	9.4	10.0
Organic wheat	13.7	12.1	12.4	14.4	11.0	12.8
Organic pea protein	-	10.0	21.5	-	8.5	19.0
Organic yeast	-	5.0	5.0	-	5.0	5.0
Seaweed	-	-	-	-	2.0	2.0
Organic premix (vit + min)	0.25	0.25	0.25	0.25	0.25	0.25
Choline 50%	0.15	0.15	0.15	0.15	0.15	0.15
Natural antioxidant	0.03	0.03	0.03	0.03	0.03	0.03
Monosodium phosphate	-	0.7	1.95	-	-	-
FM/FO	47.5/14.3	33.0/15.0	19.0/15.6	51.3/8.7	38.5/9.4	25.6/10.0

Lab tests on trout, seabream and meagre



Rainbow trout		SGR		RGR		FCR		IHS		IVS		Fillet yield				
		Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD			
CTRL	A	1.81	0.115	a	68.48	2.362	a	1.31	0.12	b	1.37	0.18	12. 1.65	b	25.29	1.77
Guisante 10%	B	1.75	0.025	a	67.34	0.526	a	1.18	0.03	c	1.43	0.32	13. 2.26	b	25.89	1.89
Guisante 21,5%	C	1.62	0.071	b	64.57	1.637	b	1.98	0.14	a	1.65	0.32	16. 1.57	a	25.07	2.17
ANOVA		P<0.001		<0.001		<0.001		0.107		<0.001		0.659				

Sea Bream		SGR			RGR			FCR		HSI		VSI		Fillet yield	
		Av	SD		Av	SD		Av	SD	Av	SD	Av	SD	Av	SD
CTRL	A	0.68	0.05	a	53.08	4.34	a	2.58	0.26	2.13	1.26	8.44	4.13	29.63	12.96
PEA 8.5%	B	0.62	0.05	b	47.45	4.68	b	2.79	0.34	2.74	1.25	10.75	5.22	32.90	15.64
PEA 19%	C	0.59	0.00	b	45.35	0.42	b	3.05	0.82	2.51	1.50	10.74	5.40	32.67	15.60
ANOVA		P<0.001			<0.001			0.19		0.623		0.532		0.873	

A preliminary conclusion might be that the inclusion of organic pea protein at high levels (19% in the case of seabream and 21.5% in rainbow trout) resulted in a lower growth rate of the fish and in the case of trout a higher viscerosomatic index. The inclusion of low level of organic pea protein did not show these effects, being the growth observed in trout the same as in the control group fed the control .

Conclusions

Most of the innovative formulations in relation to pro-health feeds, zero waste feeds and organic feeds have been already tested at lab level to determine the efficacy and feasibility of the new products.

The best formulations will be tested and validated at field level in the next period of the project

For the ecology transition in aquaculture, feed formulation remains a key factor to increase productivity, competitiveness and sustainability



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OUR LOCATION

<https://goo.gl/maps/bUKjkQtwZxFVNFtYA>

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