AdriAquaNet

Enhancing Innovation and Sustainability in Adriatic Aquaculture

Deliverable WP3.1.2

Technical-scientific manual

MANUAL FOR ADOPTING NEW LOW POLLUTING FEEDING PROTOCOLS FOR MARINE FISH FARMERS

Udine, 30.06.2022

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

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Introduction and presentation of the manual

This handbook is a product of the Italian-Croatian cross-border project AdriAquaNet, funded by the EU, which focuses on various topics which contributed to achieve the main objective of the project, i.e. improving the sustainability of sea bass and sea bream mariculture in its broadest sense, within the Adriatic region. This manual is a part of the first specific objective of the project (WP3.1), which involves actions to increase the environmental sustainability of fish farming by acting on aquafeeds. The handbook specifically addresses the role of next generation aquafeeds and managing practices in helping to minimize the waste load from seabass and seabream farming in sea-cage operations.

Indeed, although the contribution of mariculture to water eutrophication is generally smaller than that of other water polluting sources, nevertheless the potential negative environmental effects of nutrients generated by the incomplete use of feeds by fish cultured for food are of great public concern. This makes the control of waste load from fish farms a crucial point for their environmental sustainability particularly in the coastal locations along the Adriatic Sea, where marine fish farming coexists with other important economic industries such as tourism.

In open fish farming systems such as cage mariculture, the wasted feed, the excreted fecal materials and dissolved catabolites cannot be easily removed from the system or treated and therefore are mostly dispersed into the surrounding water or settle on the seabed around and beneath the cages. Siting, i.e. the choice of suitable marine locations to establish a sea cage plant (Porporato et al., 2020), is a process strictly recommended and regulated by EU guidelines and regional rules and represents the first preventive measure to avoid major environmental consequences from fish farming on marine ecosystems.
Nonetheless, since eutrophic waste load released from marine fish farms mostly originates from feeds and feeding practices, the EU and national directives also rely on the adoption by fish farmers and the feed-mill industry of all measures enabling prevention and minimization of nutrient release at source by **improving the aquafeed's conversion efficiency** while optimizing feeding practices and techniques (Tibaldi, 2010).

In dealing with those latter issues, the present handbook, took advantage from the results of the AAN project in which a representative array of conventional and novel aquafeeds were compared in farming sea bass and sea bream both at laboratory and farm scale. The manual offers to main stakeholders such as marine fish farmers and dedicated feed-mill nutritionists, clues to support decisions, adopt and use a next generation of sustainable, nutritious and highly performing marine aquafeeds, which also result in low eutrophication waste load to the marine environment.

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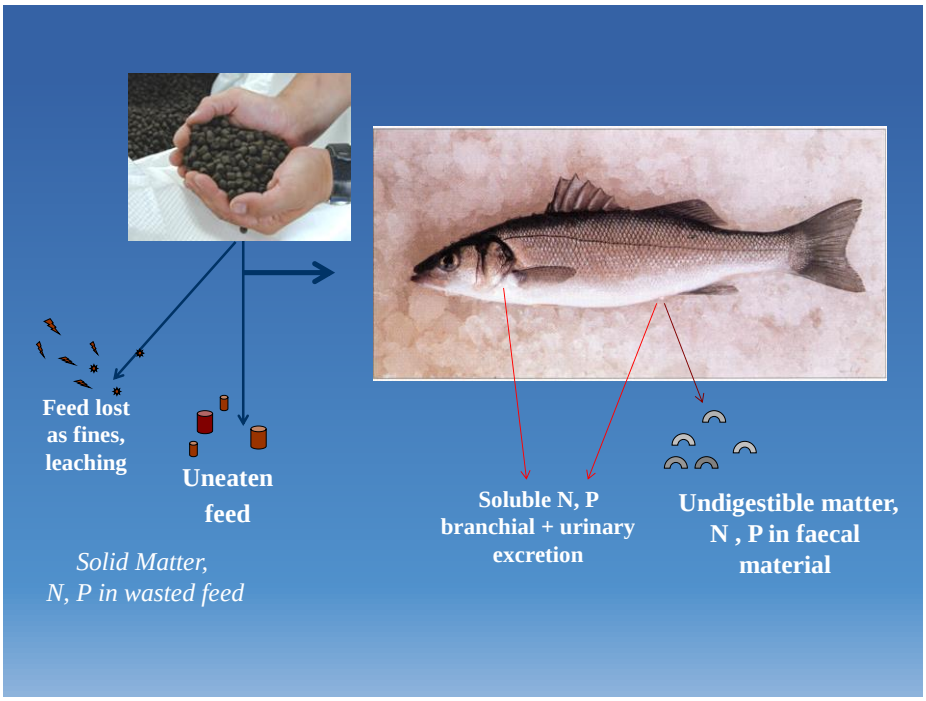
The assessment of the context

As previously mentioned, pollution generated by intensive fish farming (TW) have a prevalent feed origin (Figure below) (Cho & Bureau, 2001, Tibaldi, 2010, Tičina et. al., 2020).



It consists of solid organic and inorganic matter (TSW) and dissolved substances (DW) which include various forms of eutrophic-generating nutrients, mostly nitrogen (N) and phosphorus (P).

$$TW = TSW + DW$$

The total solid waste (TSW) consists of the faecal material (FW) produced by the incomplete digestive-absorptive utilization of gross nutrients supplied by fish feeds. In certain cases, TSW includes variable quantities of uneaten feed (TAW) due to excess feeding or poor feed palatability, but also due to inefficient distribution methods and/or sub-optimal in physical properties of feed pellets like excess buoyancy or sinking rate in water, with respect to the feeding habit of the farmed fish species and the farming condition/system. TAW also includes minor fractions of feed resulting from defects in other physical properties of aquafeeds such as excessive dustiness and/or poor pellet durability, which give rise to fines particles not ingested by the fish.

$$TSW=FW +TAW$$

The waste load of dissolved matter (DW) correspond essentially to the soluble products of fish catabolism (ammonia-ammonium ion, urea, phosphate, carbon dioxide, etc.) excreted mainly through the gills (BW) and urine (UW).

$$DW = BW + UW$$

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In the aforementioned mariculture open systems, a sound approach to obtain reliable estimates of the actual eutrophication load released by fish farms consists in computing the mass balance of nutrients. In fact, the waste load generated by feeds is spred in the surrounding environment making direct measurement of pollutant concentration and flow often unrealistic and inaccurate. This also justifies the adoption of predictive mathematical models of dispersion (Brigolin et al., 2010).
It should be noted that the same processes that generate pollution from aquafeeds also dictate the actions/guidelines to be undertaken on feed formulation/manufacturing and feeding practices to prevent or limit it. Those actions/guidelines are listed in table 1. They have been set through many dedicated congresses, symposiums and publications and will be not further discussed in this handbook.

Table 1. *Major strategies and actions to limit eutrophication waste load from cultured fish species by improving feed quality and feeding practices.*

Strategy	Intervention area	Actions/guidelines	Expected goal
Nutritional	Aquafeed formulation /composition (feed-mill)	• Select highly digestible feed ingredients	Reducing total faecal waste
		• Optimize digestible protein to energy feed ratios	Reducing soluble N waste
		• Optimize amino acid levels and dietary profile	Reducing soluble N waste
		• Optimize dietary P levels and sources	Reducing total P waste
Technology	Feed-manufacturing (feed-mill)	• improve pellet stability	Reducing TAW
		• improve pellet durability	Reducing TAW
		• reduce "fines"	Reducing TAW
		• modulate float/sinking ability as required	Reducing TAW
Feeding	Feeding practices (fish farmer)	• reliable models/tools for cost- and bio-effective feed rationing	Reducing TAW
		• reliable feeding technologies and delivery systems techniques.	Reducing TAW

As far as the nutritional strategy is concerned, the knowledge of the gross nutrient requirements of sea bass and sea bream has greatly improved over the years. Theoretical protein, energy and phosphorus dietary levels and ratios resulting in optimal growth and conversely in potentially low pollution load to the environment are well established (NRC, 2011) and adopted by feed-meal

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companies in formulating commercial aquafeeds for these marine fish species. Concurrently, modern extrusion and post-extrusion technologies used in manufacturing aquafeeds, makes physical properties of the feed pellets nearly free from major flaws such as excess fines or poor durability that could impact on feed losses. Moreover, the aforementioned manufacturing processes allow one to modulate other physical properties of feed pellets, such as sinking ability along the water column, according to the farming needs to limit feed waste.
Last but not least, also feeding practices were markedly improved over the years by fine tuning feeding ration tables through precise fish size remote control equipment coupled to feed delivery systems and software-operated technologies enabling feed losses from cages to be kept to a minimum. All this actions led to a substantial reduction in overall pollution load generated by fish farmed in cages, making mariculture a eco-friendlier industry than it was in the past. However, the growing public concern and awareness on the effects of water pollution on coastal ecosystems puts Adriatic mariculture under continuous pressure to became even more environmentally sound.

A good question to marine fish farmers and the entire supply chain is if and to what extent there is room to consolidate or even further reduce pollution from sea cages with the expected new generation of marine aquafeeds. In fact, it is widely recognized that a next generation aquafeeds for marine fish species will soon replace the conventional ones. The new formulations will include substantial levels of novel or underexploited protein-rich feed ingredients to complement plant-protein sources as alternatives to fish meals (Cottrell et al., 2020). The sustainability issue drives the interest towards raw or processed feed materials compliant with the circular bioeconomy concept and/or coming from lower trophic levels. In this context, processed animal proteins (PAPs) particularly poultry by-product meals and insect meals are attracting a growing attention among feed-mill nutritionists and fish farmers as major component of a new generation of low fish meal aquafeeds (Galkanda-Arachchige et al., 2019; Maiolo et al., 2020; Weththasinghe et al., 2022). Recent studies on sea bass and sea bream carried out within the AAN project have shown novel aquafeeds to be promising in terms of growth performance and reliance on ocean fishery resources (Pleić et al., 2022; see also AAN Deliverable WP 3.1.1, Control sheet).

This handbook will provide clues obtained through the project AAN showing next generation aquafeeds to be eco-sustainable also in terms of pollutant waste load when compared to a range of other conventional or commercial preparations for sea bass and sea bream.

New feed formulations and pollution load estimates: the AAN outcomes

A substantial part of the AAN project was devoted to compare different aquafeeds in the facilities of the University of Udine and of the Institute of Oceanography and Fisheries of Split as well as in two commercial seabream and sea bass farms located along the Croatian coast. Different aquafeed prototypes for both fish species, covering a range of possible formulations (from conventional to next generation ones) were designed and produced with extrusion technologies to be preliminary tested

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in laboratory scale experiments. To face the sustainability issue, the idea was to generate original feed-formulations with minimal levels of fish meals and conventional vegetable protein sources but inclusive of substantial proportions of processed animal proteins (PAPs) such as poultry by-product meal (PBM) and/or a novel PAP such as the insect meal obtained by partially defatted pupae of black soldier fly-BSFM (*Hermetia illucens*). Both PAPs are among the most considered novel or underexploited protein sources for a new generation of feeds for marine carnivorous fish species in terms of environmental footprint (Maiolo et al., 2020) and nutritive value to fish (Pulcini et al., 2021). For both fish species all diets were similar and optimal in terms of gross nutrient composition but differed in the proportion of main protein sources. The set of aquafeeds compared on laboratory scale included "fishy diets" coined FISH, where most protein came from fish meal/trimmings; "Veggie diets", coined VEG, where most protein came from vegetable sources; novel preparations, low in fish meal and plant proteins coined AVI and AVI+INS, where nearly 50% of protein was supplied by PBM alone or PBM+BSFM, respectively. Further details on diets composition and growth outcomes of bass and breams fed the different diets are reported in the AAN Deliverable **WP 3.1.1 New environmentally sustainable dietary formulations for cultured marine fish to be proposed to the aquafeed mill industry**, control sheet.

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Table 2 and **Figures 1a and 1b** show that, based on the mass balance of dry matter and nutrients in long lasting laboratory trials on both fish species, the novel aquafeeds AVI and AVI+INS outperformed the conventional FISH or VEG ones in terms of total solids, nitrogen and phosphorus emissions. The most striking outcome was attained by bass and breams fed the diet AVI+INS with more than halved total phosphorus waste load when compared to the FISH diet.

Table 2. *Total solid, nitrogen (N) and phosphorus(P) emission load (kg per ton of fish biomass gain) as affected by different types of aquafeeds as estimated in laboratory trials on sea bass and bream based on dry matter and nutrient budgets.*

		Aquafeeds			
		FISH	**VEG**	***AVI***	**AVI+INS**
Total solids (kg/ton fish biomass)	bass	**429**	**509**	*nd*	**372**
	bream	**422**	**345**	**318**	**322**
Total N (kg/ton fish biomass)	bass	**87**	**104**	*nd*	**70**
	bream	**74**	**72**	**67**	**63**
Total P (kg/ton fish biomass)	bass	**21**	**13**	*nd*	**9**
	bream	**15**	**8**	**8**	**7**

Figure 1a. *Laboratory trial on sea bass. Effect of different aquafeeds on total solid, nitrogen and phosphorus waste load (relative values, diet FISH=100)*

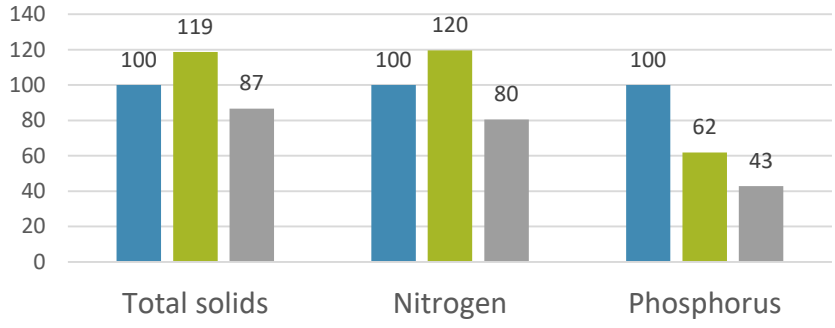

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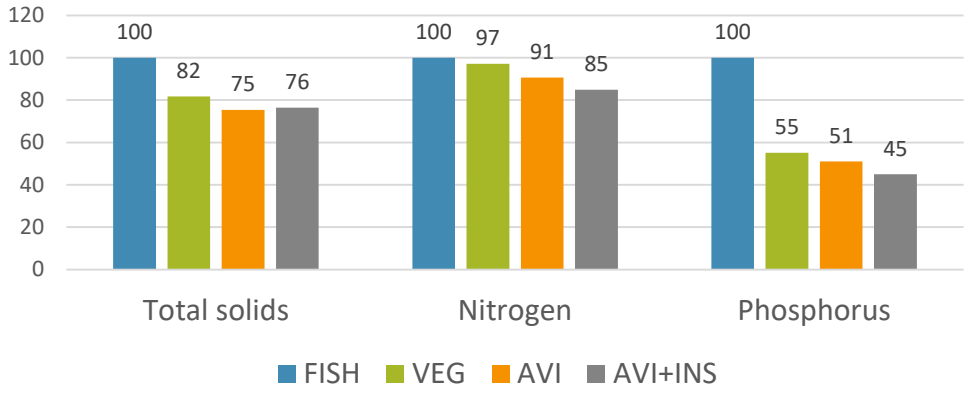
Figure 1b. *Laboratory trial on sea bream. Effect of different aquafeeds on total solid, nitrogen and phosphorus waste load (relative values, diet FISH=100)*



Apart from lab trials, to obtain a more robust picture of the impact on pollution load of novel aquafeeds for marine fish, the best next generation formulations (AVI + INS) for bass and bream were also compared to regular commercial feeds in farm-scale trials with fish farmed in cages along the Croatian coast.

Table 3. *Total solid, nitrogen (N) and phosphorus (P) emission loads (kg per ton of fish biomass gain) as affected by different types of aquafeeds as estimated in commercial farm-scale trials with sea bass and bream.*

		Aquafeeds		
		FISH	**AVI+INS**	**% Reduction in waste output**
Total solids (kg/ton fish biomass)	bass	**591**	**555**	**-6**
	bream	**529**	**410**	**-22**
Total N (kg/ton fish biomass)	bass	**97**	**84**	**-13**
	bream	**95**	**86**	**-9**
Total P (kg/ton fish biomass)	bass	**11**	**9**	**-15**
	bream	**13**	**9**	**-31**

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Table 3 shows that even in the farm-scale experiments the novel aquafeeds AVI+INS resulted again in a tendency towards reduced pollution loads from cages compared to currently used commercial aquafeeds. This was particularly clear for sea bream in terms of total solids and P waste output. Such a trend was also confirmed by simulations carried out with the software FiCIM (Brigolin et al., 2014) which has been refined within the AAN project and made available to fish farmers on-line (http://orata.bluefarmenvironment.com/individual_app.php). A major clue of the software consists in assisting fish farmers to optimize feeding practices by biomodelling fish growth curves, feed rations as well as nutrient waste load and dispersion from sea cages.

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Guidelines for adopting next generation aquafeeds in farming bass and bream in cages

- Newly formulated aquafeeds for Mediterranean fish species including PBM and BSFM do not require special guidelines for being regularly used on farm. Their digestible energy and nutrient levels are expected to be slightly improved, compared to those of commercial feeds: therefore, a parallel improvement in the operative FCR is expected. In this respect they require minor changes in the feeding levels and plans.

- A preliminary check of feed pellet behavior in water is recommended to both feed mill staff and fish farmers since newly formulated feeds could result in different pellet sinking properties relative to the currently used ones. Feed mill formulators and feed manufacturer technical staff could easily implement minor changes in feed formulation and/or in the extrusion process conditions to allow feed pellets to sink as desired. This could be required particularly when the level of insect meal (BSFM) is set at or above 8-10% by weight in feed formulations including substantial proportion of vegetable ingredients.

Conclusions and Recommendations

- The feeding trials carried out within the AdriAquaNet project to test a new generation of aquafeeds, low in fish meal content and including substantial proportion of processed animal proteins (PAPs) from poultry by-products and insect larvae, provide quite robust evidence of improved overall farming outcomes.
- As a results of better feed conversion efficiency, the adoption of these novel aquafeeds could lead to a further reduction in the dissolved and particulate nitrogen and phosphorus load from sea bass and sea bream mariculture operations, compared to conventionally formulated ones. . The improvement of FCR must therefore represent a driving force for Adriatic fish farmers, allowing their sector to become even more environmentally sound and socially acceptable.

- New feeds could easily be adopted as they do not imply major changes in feed mill and fish farm operational routines and management.

- A next generation of marine aquafeeds should then be recommended and suggested to fish farmers. This take home message, being supported by the AAN results, should also be disseminated among the entire Adriatic fish supply-value chain up to the consumers and the competent authorities as a sustainable tool for an eco-friendly Adriatic mariculture.

- The adoption of a new generation of aquafeeds by Adriatic fish farmers looks very compliant with the EU Strategic guidelines for a more sustainable and competitive aquaculture for the period 2021 to 2030 (EUROPEAN COMMISSION, Brussels, 12.5.2021, COM (2021) 236 final). In fact, they address inter-related objectives such as: i) building resilience and competitiveness; ii) participating in the

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green transition; iii) ensuring social acceptance and consumer information; iv) increasing knowledge and innovation.

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Annex 1

This Manual is a project deliverable that helps reaching and the fulfilment of the Program result **indicator 1.1R - number of EPO applications and the project specific objective nr.1 "Increase environmental sustainability of fish farming"** by providing fish farmers within the Program area with tools to improve the impact and environmental footprint of fish product and production process. Thanks to the setup of new fish feeds and feeding strategies, that cut down eutrophication emissions implemented in the farms, together with the application of photovoltaics and new technologies for the wastewater treatment chain that recovers and uses fish waste for biogas production, the sustainability of fish farming can be improved.

Novel feeds and better farming conditions can improve health and welfare of farmed fish and can ensure producers to tailor make fish quality on consumers' demand and expectation for safe and healthy food. The trials on commercial fish farms differed in environmental and operative conditions from the ones on pilot lab scale based on the scientific literature and lab test, and the excellent results obtained give now the possibility to enhance market competitiveness through improved product quality and safe label and give the companies to have an innovative feeding management.

The Manual content and application is coherent with the **EUSAIR action plan and S3 strategies** of both countries involved and it can be applied on the Mediterranean level.

The Manual has been distributed locally and to the main project stakeholders in order to be in use in everyday practice. This **contributes to the indicator CO04** (improving the productivity of the sea bass and sea bream farming as well as the improvement of the quality and marketing of the fresh and processed fish products and provide safe products to consumers). 24 enterprises of the sector were directly involved and receive support and 20 enterprises received non-financial support through learning and knowledge transfer.

Also it contributes to the indicator **CO44** and the number of the participants that joined local trainings events and the **Program indicators 1.104 and 1.101.**

These activities had a positive impact on project objectives and helped to reach the Program output indicators:

CO01 – 24 enterprises received support,
CO02 – 4 enterprises received grants,
CO04 – 20 enterprises received non-financial support,
CO42 – 7 research institutions participated in cross-border, transnational or interregional research projects
CO44 – 578 participants involved in joint local employment initiatives and joint training

Cooperation criteria and horizontal principles have been respected during the project implementation.

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Annex 2

University research experience: prof. Emilio Tibaldi and senior scientist Dr. Gloriana Cardinaletti,

Indoor aquaculture facilities of the Aquaculture & Wildlife division of the Di4A, University of Udine, Pagnacco, Italy. (authorization n. 03/2018 UT of the Italian Ministry of Health according to the D.L.vo 26/2014 on the protection of animals used for scientific purposes).

- **Laboratory trials:** 12 tanks 20 fish (sea bream) per tank initial weight of 100g
- **Trial duration:** July 2019 – July 2020
- **Role:** comparing conventional *vs* newly formulated aquafeeds. By monitoring and recording fish mortality and health status, feed intake, growth performance feed conversion and nutrient mass balance.

Results:

- fish responded well to the new feeds
- there was no mortality or signs of stress and behavioral changes due to the diet
- Newly formulated feeds outperformed the conventional Fishy and Veggie diets
- Due to better feed conversion ratio the novel feed including Processed Animal Proteins in spite of fish and vegetable meals resulted in reduced nutrient waste output.

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On the left are visible 6 units tanks for in vivo digestibility trial; each unit consists of three 60-L tanks fitted with a common drainpipe and a settling column for faecal collection (according to the Guelph system). On the right part of a marine recirculating aquaculture system (RAS) including 30 tanks each of 300-L capacity.

Details of the tanks of the RAS system. Over the top of each tank there is located a rectangular belt feeder connected to an automatic timer for programmed feed distribution.

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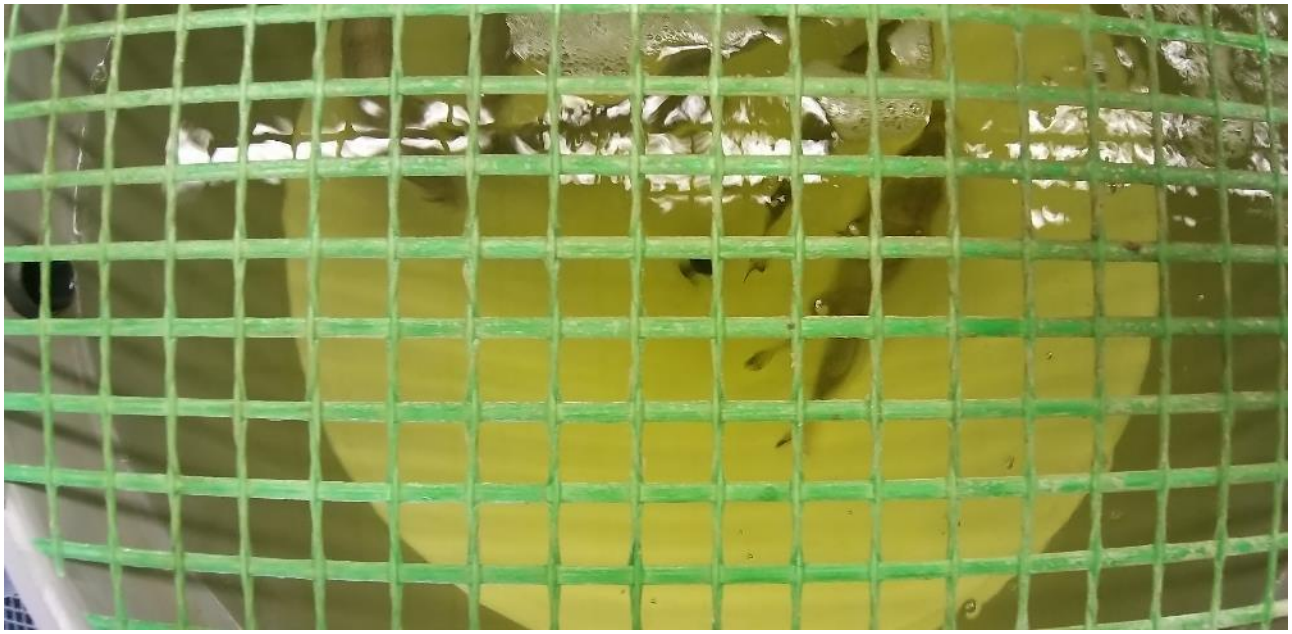

Seabream swimming at the bottom of a RAS tank

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Prof. Tibaldi and Dr. Cardinaletti in the field laboratory for routine water quality control. Scale for daily feed ration preparation before uploading pre-weighed feed amounts in the belt feeders

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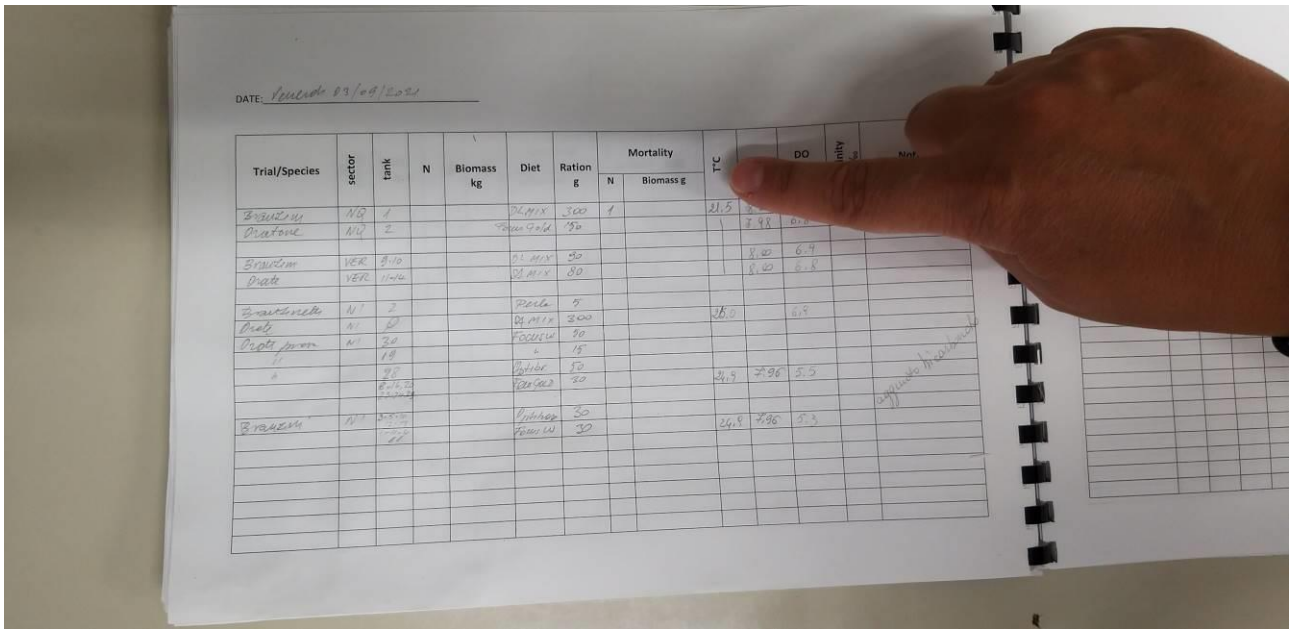
Dr. Gloriana Cardinaletti in the field laboratory for routine water quality control. Scale for daily feed ration preparation before uploading pre-weighed feed amounts in the belt feeders.



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Mechanical sand filter connected to the RAS system used to remove solid waste particle form the water.

The white cylinder in the foreground is the biological filter, on its back a protein skimmer (or foam fractionator) to remove organic compounds from water of the RAS system.

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Ozonator for disinfection of waste water to be discharged from the RAS system.

UV sterilizer lamp apparatus connected to the RAS system.

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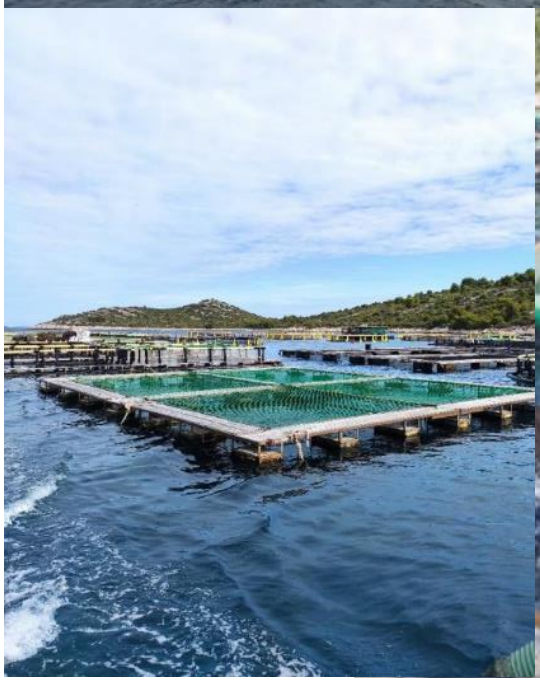
Producer's experience: Mr. Igor Cvitić, farm manager Friškina d.o.o.

- **Farm trials:** 6 cages, 4000 fish per cage, initial weight of 150 g
- **Trial duration:** May 2020 –November 2020
- **Role:** daily feed, recording fish mortality, monthly sampling to monitor growth performance, feed conversion



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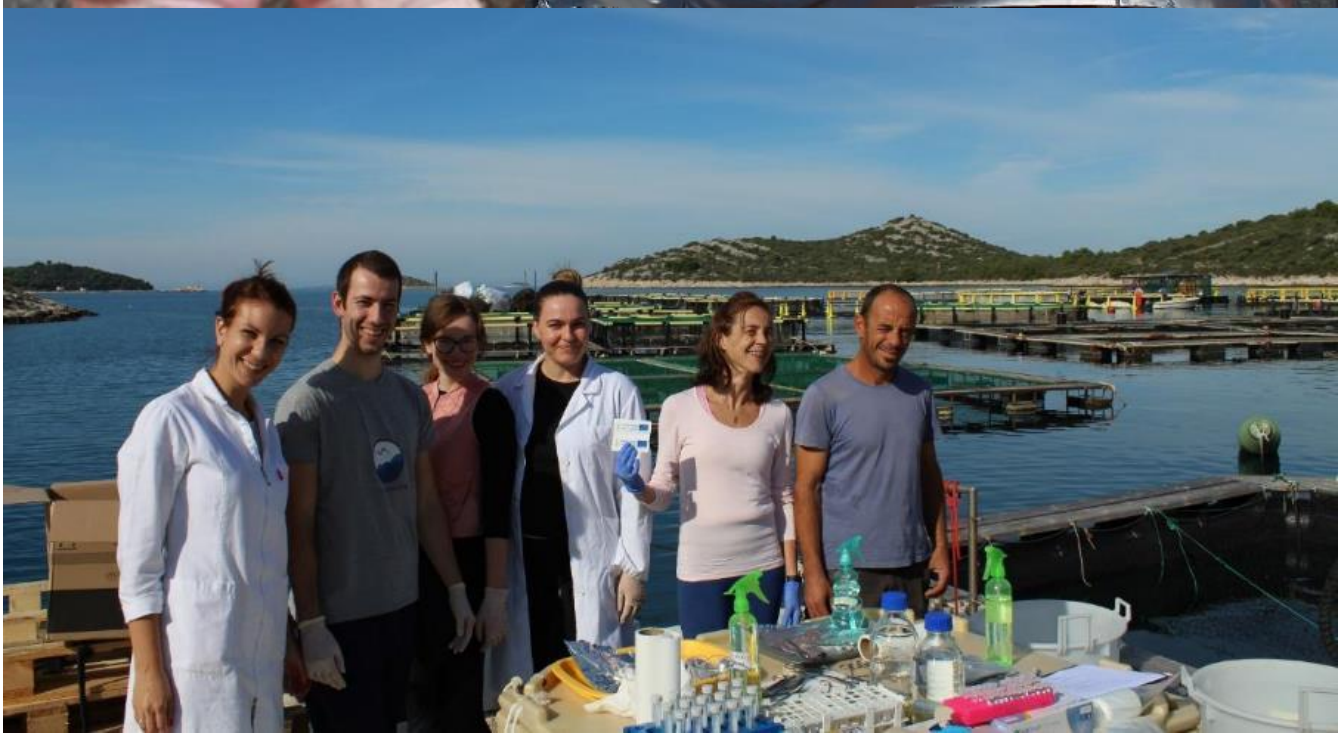
Results:

- fish responded well to the new feed
- similar physical characteristics of pellets with commercial ones
- there was no signs of stress and behavioral changes
- total biomass increase was sufficiently good
- health status as in the fish fed commercial diet – no change
- organoleptic properties very good, firm tissue, good taste



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