

## WORKSHOP REPORT

# Workshop: How outputs from EU projects can upgrade health management in the Mediterranean aquaculture

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Aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) is an essential activity in the Mediterranean basin. Several EU Horizon 2020 and regional projects are focusing on the improvement of their farming performance. This workshop aimed at displaying, sharing and discussing projects' achievements among interested colleagues engaged to work with fish diseases. The most innovative research outputs aiming to improve the European mariculture were presented during this workshop, including updates from EU Horizon 2020 projects MedAID, PerformFISH, ParaFishControl, FutureEUAqua and the Italy-Croatia Interreg project AdriAquaNet.

## Introduction

Mediterranean marine aquaculture has grown exponentially during the last decades of the 20<sup>th</sup> century, though with a slower pace over the past two decades (Muniesa et al. 2020). Despite this growth, there is a need for the improvement of competitiveness and sustainability throughout the whole value chain. Among all important areas, health management – preventing and controlling diseases, is one of the most essential pillars in building a prosperous future aquaculture industry (Vendramin et al. 2016). EU invests lots of resources for boosting the European aquaculture industry including the Mediterranean aquaculture. It gathers a variety of cultured species, feed, genetics, health management, production systems, economy, consumer's perception, product quality, competitiveness, and everything that fits under the umbrella of circular economy and sustainability. The applicability of the outcomes from different projects on health management is therefore essential for the industry and authorities to improve and move forward.

This workshop was set to discuss how can we upgrade health management based on the different project results and how can we make better use of their results to decrease diseases and to improve health and welfare. It united five projects to present some of their results on health management for further inspiration and discussion. These projects are Horizon 2020 projects,

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including [MedAID](#) (Mediterranean Integrated Aquaculture Development); [PerformFISH](#) (Integrating Innovative Approaches for Competitive and Sustainable Performance across the Mediterranean Aquaculture Value Chain); [FutureEUAqua](#) (Future growth in sustainable, resilient and climate-friendly organic and conventional European aquaculture); [ParaFishControl](#) (Advanced Tools and Research Strategies for Parasite Control in European farmed fish). Updates were also given about the Italy-Croatia financed project [AdriAquaNet](#) (Enhancing Innovation and Sustainability in Adriatic Aquaculture), a regional project aiming at the improvement of marine aquaculture in the Adriatic Sea. Each of the selected projects presented during this workshop highlighted some of the most innovative outputs, aiming to improve the European mariculture.

### **A systematic approach for quantifying biosecurity measures**

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Diseases are the major constraints in aquaculture, and biosecurity is critical for a sustainable development of aquaculture. This work emphasizes how biosecurity measures and their relative importance can be quantified and documented objectively. The Norwegian Veterinary Institute worked with partner institutes from Croatia, Egypt, France, Greece, Italy, Spain, Tunisia, and Turkey. We estimated biosecurity risk associated with disease introduction and pathogens spreading into seabass and seabream farms, also to identify the control measures to manage these risks. Eighty-eight farms from 8 different countries surrounding the Mediterranean basin participated. The system approaches internal and external biosecurity in a general manner, focusing on transmission routes shared by numerous different types of infectious agents. As the result of this network, a system for aquaculture biosecurity evaluation has been developed (Tavorntpanich et al. 2020). This quantitative system helps to identify gaps and weaknesses in the biosecurity plan, assists farmers to allocate resources and tailoring the biosecurity programme to fit the risk profile of their farms. If the system is applied in a region, it also helps to compare a specific farm with an average of the biosecurity scores obtained by neighbouring farms, so that the owners can benchmark their biosecurity and evaluate the risk profile of the region. This benchmarking may give owners impelling reasons to improve their farm biosecurity. The system can be modified to fit various farm production characteristics (e.g., RAS), different exposures (e.g., antibiotics), and for different disease agents. This system is developed to be a farmer self-assessment tool, with a user-friendly automated dashboard containing the functionalities. Farmers interested in an objective evaluation of farm or regional biosecurity can have secure access to their information.

## **Experimental testing of a VLP-based vaccine against Viral Nervous Necrosis in European sea bass (*Dicentrarchus labrax*)**

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Viral Nervous Necrosis (VNN) is a devastating disease of many marine species, including seabass and seabream (Le Breton et al. 1997). Infected fish show a characteristic spiralling swimming pattern, hyperinflation of the swim bladder with loss of buoyancy control, and darkening of the skin. Affected fish usually die, or become chronically infected with the virus. Many aquaculture farms in the Mediterranean experience recurrent economic problems with VNN caused by a *Betanodavirus* known as Viral Encephalopathy and Retinopathy Virus (VERV) or Nervous Necrosis Virus (NNV) (Chérif et al. 2009). The virus particle contains only two proteins: the Capsid Protein (CP), and the RNA-dependent RNA polymerase. CP is the main structural component and may auto-assemble into Virus-Like Particles (VLPs) even in the absence of the RNA polymerase and the viral genome. Being antigenically similar to the virus, non-infectious and relatively easy to produce, VLP-based vaccines possess great potential in the fight against infectious diseases. A prototype VLP vaccine against NNV was developed in a previous EU funded project, TargetFish. The MedAID project in collaboration with the biotech company W42 focused on the characterization and experimental *in vivo* testing of this vaccine.

Vaccination-challenge trials with seabass were performed to gain knowledge on the safety, efficacy, correlates of protection, duration of immunity, and characterization of the protective immune mechanisms. After a single intraperitoneal dose of VLP-based vaccine, significant protection against experimental challenge with live RGNNV (Red-spotted grouper nervous necrosis virus) was demonstrated (Barsøe et al. 2021). Formulation with oil adjuvant had no beneficial effect. VLP vaccinated sea bass had up to 26 times higher chance of surviving, compared to non-vaccinated controls. The protection persisted at least until 7.5 months after vaccination in fish kept at 20°C. Doses of 20-40 µg VLP/fish provided the strongest protection and, the vaccinated fish mounted a dose-dependent response of neutralizing antibodies, detectable already after 15 days [corresponding to 300 degree days (dd)], peaking around 30-45 days after vaccination, and persisting at least for 8.5 months (5000 dd). A geometric mean titre (GMT) above 200 in a vaccinated group was induced by a single dose of 20 µg VLP/fish, and seemed to be correlated with herd protection and RPS values around 70. This correlate of protection may be used as a tool to evaluate the protection level on a vaccinated

farm or as batch control in vaccine development. The VLP vaccine did not influence the growth or survival of the vaccinated fish, why it seems to be a safe and potent prophylactic tool against VNN in sea bass. Immune gene regulation in response to vaccination suggested that the high efficacy was related to the ability of the vaccine to induce both innate and adaptive immune mechanisms in a balanced manner without the need for an adjuvant.

## The PerformFish approach

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The PerformFish project was designed to achieve tangible, practical and robust results and methodologies to be directly implemented in the EU Mediterranean finfish farming industry. Amongst the areas tackled, health and welfare is the most relevant and is mainly approached in WP3, entitled 'Boosting Fish Health at all Lifecycle Stages', but also in other transversal WPs. WP3 is and devoted to the evaluation of the most relevant factors related to fish health to provide the industry with efficient diagnostic, prevention and treatment tools to improve KPI's. It is composed of 5 different tasks, covering epidemiology & health risk assessment, diagnostic techniques and challenges, vaccines and vaccination, medicines, biocides and bioactive substances and welfare evaluation. The evolution of the diseases and mainly the health risks associated with specific diseases for Mediterranean fish farming in the specific facilities (broodstock, hatchery, nursery, on-growing) were addressed. These were evaluated using different assessment techniques, with results summarised in deliverable documents entitled: '[Epidemiological status of Mediterranean farmed fish](http://www.performfish.eu/wp-content/uploads/2021/02/D3_2_approved.pdf)' ([www.performfish.eu/wp-content/uploads/2021/02/D3\\_2\\_approved.pdf](http://www.performfish.eu/wp-content/uploads/2021/02/D3_2_approved.pdf)) and '[Prophylactic practices for the Mediterranean farmed fish](http://www.performfish.eu/wp-content/uploads/2021/02/D3_3_approved.pdf)' ([www.performfish.eu/wp-content/uploads/2021/02/D3\\_3\\_approved.pdf](http://www.performfish.eu/wp-content/uploads/2021/02/D3_3_approved.pdf)) Diagnostics was done in close cooperation with the sister project MedAid to get a complete database of specialised laboratories working in Mediterranean finfish pathogens and disease diagnostics (Zrnčić et al. 2021). Other specific achievements in the scope of [diagnostics](http://www.performfish.eu/wp-content/uploads/2021/02/D3_1_approved.pdf) ([www.performfish.eu/wp-content/uploads/2021/02/D3\\_1\\_approved.pdf](http://www.performfish.eu/wp-content/uploads/2021/02/D3_1_approved.pdf)) are the development of a rapid identification method for NNV variants; methodological variations in the microbiological test procedures to get faster and more accurate data on Minimum Inhibitory Concentration (MIC); new data on MIC for several antimicrobial substances; new profiles for the assessment of *Vibrio anguillarum* (Kazazić, Topić Popović, Strunjak-Perović, Babić, et al. 2019) and *Photobacterium damsela* subs *piscicida* with Maldi-Tof MS (Kazazić, Topić Popović, Strunjak-Perović, Florio, et al. 2019); advances in the quickness and accuracy diagnostic of enteric diseases in seabream caused by *Cryptosporidium* and *Enterospora nucleophila*; and the setup of biosensors for the early detection

of *V. anguillarum*. The consortium has also produced a relevant review document entitled ‘Challenge methods for the Mediterranean farmed fish’. Vaccines and vaccination was addressed in WP3 and improved *Betanodavirus* (bath) vaccine, a trivalent IP vaccine (*Betanodavirus* + *P. damselae piscicida* + *V. anguillarum*) and an improved vaccine against Photobacteriosis has been developed in WP3 and are currently under test. Antibacterial and antiparasitic medicines and their rational and sustainable use in Mediterranean farming has been also covered with specific pharmacokinetic studies in seabream and in seabass in molecules that required further attention such as the antibacterials doxycycline, lincomycin and spectinomycin and the antiparasitic mebendazole and toltrazuril. The results of these studies and broader review and recommendations for efficient and sustainable use has been implemented in the deliverable ‘Best therapeutics practices for the Mediterranean farmed fish’ and also in related published papers (Rigos et al. 2020). Biocides and new bioactive substances were also explored in this task. The efficacy of different biocides (Virkon, peracetic acid, and hydrogen peroxide) and methods of use against different pathogens (*Betanodavirus*, *P. damselae piscicida*, and different *Vibrio* species) were assessed in and the results are detailed in the deliverable on prophylactic practices. The benefits of stimulation of the immune system by the use of krill meal or a more explorative and complete study of the potential use of bioactive extracts of the macro-algae *Padina pavonica* were developed. A great effort was done in the development of a robust system for sea bass and sea bream welfare assessment. This is based on a systematic assessment of the current scientific and technical knowledge on areas related to the welfare of these two species, definition and selection of the most suitable OWIs according to experts, and to current farming practices, data collection and final use validation in farms. The methodology used is summarised in a deliverable and the system will be presented to the public in the next months. A particular effort was made in the dissemination of the results achieved during the project and in the promotion of methods towards more efficient and sustainable health management. PerformFish regularly organises open education and training initiatives for the different professionals working on health and welfare in the Mediterranean farming and members of the consortium have participated in different events related to this topic. All the produced scientific and technical papers and documents as well the specific deliverables can be easily identified and downloaded from the PerformFish website in the section ‘results’.

### **Novel natural compounds as potential therapeutics in aquaculture**

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The supplementation of fish feed with biologically active natural compounds is frequently proposed to boost fish growth and immunity and counteract infectious agents. Within the Italian-Croatian interregional partnership, AdriAquaNet, we assessed the efficiency of eco-friendly compounds with potential antibiotic/antiparasitic/immunostimulant properties to improve the

health status of European sea bass and gilthead sea bream reared in Adriatic farms. We carried out *in vitro* and *in vivo* screening of a potentially applicable wide panel of compounds derived from marine organisms (MNPs) and medicinal plants, including antimicrobial peptides (AMPs) and probiotic supernatants (postbiotics). Strains of bacterial pathogens *V. anguillarum* and *P. damsela piscicida* served as targets to assess the antimicrobial potency of active candidates. The gilthead seabream SAF-1 cell line and primary cultures of sea bass HK leukocytes were used to evaluate the potential *in vitro* cytotoxicity of active substances. The significant antimicrobial activity was displayed by 7 out of 130 tested MNPs originating from the sponge *Crambe crambe* and the mollusc *Gastropteron meckeli*. One out of 14 tested AMPs, Cecropin A-Mellitin CA (1-7) M(2-9) resulted active against target bacterial strains. Both 7 MNPs and the AMP gave MIC values comparable or lower than those of the reference antibiotic, oxytetracycline, with promising results especially against *P. damsela piscicida* strain which showed resistance to oxytetracycline. An autologous probiotic strain *Bacillus subtilis* has been added to the gilthead sea bream's diet up to 9 weeks and increased serum peroxidase activity, whereas the other humoral parameters were unaffected.

These preliminary observations of *in vitro* tests will provide the necessary scientific base for transfer into *in vivo* small scale and field trials. The proposal of a lab/*in vivo* model to test the biological properties of natural compounds will be useful in aquaculture applications but also transferrable to human/animal medicine. Still, the choice of the compounds panel should be screened to favour those more easily (less costly) purifiable and approvable by current legislation.

### **Novel feed formulation supplemented with autochthonous *Bacillus* sp. positively altered host-microbiome**

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With the main goal to improve fish health and sustainability of Adriatic aquaculture, a four-year-long R&I initiative granted within Italian-Croatian interregional partnership, AdriAquaNet, focused also on strategies to combat antimicrobial resistance. After developing and laboratory-scale testing of a functional feed that incorporates cost-effective, sustainable and non-fish protein source (insect) and a novel probiotic isolate, the concept has been validated within a technology-relevant environment over a six-month trial at a commercial facility. A probiotic strain of *Bacillus* sp. isolated from the autochthonous microflora of cultured fish was incorporated in the functional feed after assessing its immunomodulatory, antimicrobial and biochemical properties. Over a 6-month feeding trial at the commercial facility, gilthead seabream (*Sparus aurata*) of ~121 g were separated into three cages in

duplicate, and fed standard commercial (control), novel alternative feed and novel alternative feed supplemented with the probiotic (functional feed). For the latter, a switch-on/off regime was employed, i.e., the probiotic feed was alternated for two consecutive weeks with the new alternative feed. Two weeks, one and 6 months after the trial started, samples were collected for intestinal microbiota analyses (V4 region of 16S rRNA; sequenced by Illumina NovaSeq), immunity status (sera for total IgM, respiratory burst, complement), gene expression analyses (44 targets related to intestinal function and integrity, nutrient transport, and immune response) and histology, including standard growth parameters. At the end of the trial, no difference in growth performance was found between fish fed three different feeds, which encourages the application of alternative protein sources. Analyses of intestinal microbiota metagenomics encompassing 85.5% sequences within Bacteria and Archaea, showed significant differences among treatments and sampling time points. The change in community was noticeable already one month after the trial started, although predominant genera were generally not affected by novel feed formulations. In conclusion, both novel feed formulations positively altered host intestinal microbiota even though the effect was partially influenced by environmental conditions. Correlations of intestinal microbiota analysis with target gene expressions and gut ultrastructure will help to determine more robust benefits of novel feed formulations on intestinal epithelial integrity and gut homeostasis, but it certainly encourages the application of probiotics as alternative treatment strategies against bacterial diseases.

### **How internet of things can enhance aquaculture farms productivity and ensure sustainability?**

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Internet of things is considered as a network of physical objects or « things » embedded with electronics, software, sensors, and network connectivity, which enables these objects to collect and exchange data. In the context of aquaculture, different objects that can be used are overall: (1) physiological sensors able to measure fish acceleration (proxy of metabolic rate), heartbeat rate logger (indicative of fish stress state), electromyogram sensors, measuring muscles activity which is indicative of energy expenditure or camera videos recording fish behaviour (position, locomotion, feeding); (2) environmental sensors able to measure dissolved oxygen, salinity, chlorophyll, blue-green algae, turbidity, or coloured Dissolved Organic Matter or water currents, all together providing valuable insights about the environmental conditions in which fish are living; (3) biomass estimation systems (through acoustic transmission, infrared technologies, or video recording); and (4) feeding optimization system that can detect fish satiation via video and algorithms.

In the internet of things context, those sensors and systems can be connected to provide valuable data in real-time to farmers. Getting real-time data related to the physiology of animals and the environment help to prevent any health or fish welfare issue while avoiding any economic loss for farmers due to pandemic for instance. In addition, a biomass system could allow to continuously monitor fish growth, without stressing fish during biometrics. The feeding optimization system could make feeding operations more efficient, reduce feed costs, and also avoid wasting feed.

In the context of H2020 FutureEUAqua (GA #817737), we tested both physiological and environmental sensors, coupled with a biomass estimation system in different aquaculture farms, allowing real-time monitoring of fish wellbeing and growth as a function of environmental parameters. In conclusion, the internet of things can enhance aquaculture farms productivity and ensure sustainability and will allow the future growth in sustainable, resilient and climate-friendly organic and conventional European aquaculture in a framework of precision livestock farming.

### **PARAFISHCONTROL: From practical guides to combat parasitic infections of gilthead sea bream and sea bass to recommendations for policy makers**

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ParaFishControl project (GA #634429) run from 2015 to 2020. In this workshop, we focused on the main results obtained for the Mediterranean fish species, gilthead sea bream (GSB) and European sea bass (ESB). The studied parasites were the myxozoan *Enteromyxum leei*, the microsporidian *Enterosporea nucleophila*, the monogeneans *Sparicotyle chrysophrii*, the dinoflagellate *Amyloodinium ocellatum* and the isopod *Ceratothoa oestroides*. We have obtained basic and crucial information concerning the genomics and transcriptomics of several parasites, developed models of experimental transmission, and obtained information on the host immune response and host pathology using immunology, histology, transcriptomics, and metabolomics. We have studied the interaction between wild and farmed species, tested some alternative treatments, tried early-stage vaccines or identify vaccine candidates, and developed new diagnostic tools (PCR and ISH). We have run expert consultation forums and epidemiological surveys to identify risk factors in the farms. We have run an extensive survey that has shown that the overall risk of zoonotic helminths in the selected farmed fish species is negligible. This information has been delivered to EFSA to change the current Commission regulation (EU) No. 1276/2011, to add more exemptions from the freezing treatment. The results are gathered in integrated pest management strategies for *E. leei* and *Sparicotyle* ([www.parafishcontrol.eu/parafishcontrol-media/media-centre](http://www.parafishcontrol.eu/parafishcontrol-media/media-centre)), and in a free online tool for farmers to plan parasite

control in Mediterranean aquaculture ([www.openscience.cefas.co.uk/parafish\\_economic](http://www.openscience.cefas.co.uk/parafish_economic)). All these pieces of knowledge have been transmitted through peer review articles, several training courses and four OA guides for parasite management, one of them devoted to ESB and GSB ([www.eafp.org/parafishcontrol-guides](http://www.eafp.org/parafishcontrol-guides)). We have edited the book “Fish Parasites: A Handbook of Protocols for their Isolation, Culture and Transmission” ([www.5mbooks.com/fish-parasites-2218.html](http://www.5mbooks.com/fish-parasites-2218.html)).

## Discussions

The workshop aroused interest among conference participants and 86 delegates took part in this session. Several important questions were raised in the discussion such as: the need for the development of an efficient VNN vaccine for immersion application; implementation and refinement of the biosecurity quantification prototype in sea bream and sea bass aquaculture; and a problem with the shortage of licensed antibacterial and antiparasitic medicines to be used in aquaculture. Some of the presentations highlighted the beneficial effect of functional feeds for the health of the digestive tract and avoidance of deleterious impact of Myxozoan infection to fish i.e., infection with *Enteromyxum leei*. Moreover, some specific diagnostic tools were elaborated as in the case of detection of microsporidians, where real-time PCR is confirming symptoms of the retarded growth when other diagnostic methods are failing or giving false-negative results. The essential goal of this workshop was to explore the perception of the project outputs by the industry; could they use the results as the solutions in day-to-day practice? Similarly, to the findings emphasized by project partners, industrial parties are missing solutions where exact substances, dosages and protocols for disease combating will be offered. In conclusion, it was agreed that the EU projects tackling Mediterranean aquaculture health management should be more focused on cooperative actions between industry and academia, and the challenges faced in real-life production. It is of utmost importance to organize more specific workshops to discuss the exploitation of research results both among sectorial researchers, industrial partners and other interested stakeholders.

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### ***Project sources***

[MedAID](http://www.medaid-h2020.eu) (Mediterranean Integrated Aquaculture Development), available online at: [www.medaid-h2020.eu](http://www.medaid-h2020.eu)

[PerformFISH](http://www.performfish.eu) (Integrating Innovative Approaches for Competitive and Sustainable Performance across the Mediterranean Aquaculture Value Chain), available online at: [www.performfish.eu](http://www.performfish.eu)

[FutureEUAqua](http://www.futureeuaqua.eu) (Future growth in sustainable, resilient and climate-friendly organic and conventional European aquaculture), available online at: [www.futureeuaqua.eu](http://www.futureeuaqua.eu)

[ParaFishControl](#) (Advanced Tools and Research Strategies for Parasite Control in European farmed fish), available online at: [www.parafishcontrol.eu](http://www.parafishcontrol.eu)

[AdriAquaNet](#) Enhancing Innovation and Sustainability in Adriatic Aquaculture), available online at: [www.italy-croatia.eu/web/adriaquanet](http://www.italy-croatia.eu/web/adriaquanet)

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