

RESEARCH ARTICLE

New Challenges and Achievements in Mediterranean Fish Health Management

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The European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) are key species in marine aquaculture, cultivated in both EU and North African/Levant Mediterranean regions. Production disparities between these areas emphasize a significant reliance on trade for juveniles, feed, and other resources, posing notable biosecurity risks. Addressing shared health concerns across the Mediterranean requires a regional approach to ensure successful management. A recent workshop delved into pivotal topics, including insights from concluded Horizon 2020 projects, effectiveness of nutraceuticals in combating parasitic infections, legal considerations surrounding autogenous vaccines, updated knowledge in betanodavirus infections, and biosecurity measures in Mediterranean aquaculture. Discussions also explored upcoming challenges such as sustainability in cage-based production, impact of climate change, enhancing diagnostic capabilities, fortifying biosecurity measures, advocating for education, and fostering networking opportunities.

Dedicated to Panos Varvarigos

Introduction

The European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) are vital species in global marine aquaculture, accounting for 526,000 tonnes of production (FAO 2022). In the Mediterranean, including EU countries and Turkey they contribute 422,837 tonnes (FEAP 2023), while North African, and Levant Mediterranean regions are also significant producers with almost 100.000 tonnes per year (FAO 2022). The industry comprises on-growing facilities mainly cages, hatcheries, feed manufacturers, and other providers including health service (Muniesa et al. 2020). However, the uneven distribution of production between EU and non-EU countries which included mostly cages, highlights a substantial need for trade in juveniles, feed, and other commodities around the region (Aguilera et al. 2019).

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Health challenges, including parasitic infections, threaten Mediterranean aquaculture. The 80s saw threats to production due to bacterial diseases like Vibriosis and Photobacteriosis, which were later controlled by commercial vaccines. However, new challenges emerged like betanodavirus infections, parasitic infestations, and various bacterial threats, some of which exacerbated by rising water temperatures due to climate change (Cascarano et al. 2021). Autogenous vaccines helped manage bacterial pathogens, contributing to increased production in the 2000s. To enhance profitability, industry stakeholders should focus on timely prevention measures, precise disease detection, and effective treatments.

The most important health problems with slight differences are the same in the whole Mediterranean (Vendramin et al. 2016) and a key element of successful and efficient health management is fast, reliable and validated diagnostic techniques capable of detecting the presence of pathogens timely. Diagnostic capabilities vary significantly between European and non-European Mediterranean countries (Zrnčić, Fioravanti, et al. 2021). The latter, despite high production, lack adequate diagnostic capacities, posing a regional risk due to the movement of juveniles.

The workshop aimed to foster a holistic approach to regional health management covering the most relevant problems and possible solutions as a basis for discussion between researchers and stakeholders encompassing: i) the legacy of two recently completed Horizon 2020 research projects on health management; ii) the lack of available licensed therapeutic substances, alternative use of functional feeds and/or natural substances for disease prevention/mitigation; iii) legislative framework for autogenous vaccines production and use, as well as the problems posed by the limited production of licensed commercial vaccines; iv) the updates on the current situation and diagnostic capacity with different genotypes of betanodavirus and v) achievements and challenges in biosecurity.

The discussion covered diverse topics such as possibility of diseases surveillance, sustainability of the cage based production, climate changes impact, education and networking needs at regional level.

Most relevant topics on regional health management

Main outputs from PerformFISH and MedAID Projects by F. Padrós and S. Zrnčić

Between 2017 and 2023, the MedAID (<http://www.medaid-h2020.eu/>) and PerformFish (<http://performfish.eu/>) project significantly impacted Mediterranean aquaculture. Their joint focus on enhancing industry sustainability and competitiveness, particularly in health and welfare aspects, showcased strong collaboration among leaders and participants. Extensive

resources including videos, fact sheets, deliverables and numerous open-access documents available through websites and Zenodo repository reflect the robust work carried out on fish health and welfare.

PerformFish specifically delivered various outputs on diagnostic methods, epidemiological status, best therapeutic practices, vaccines, welfare assessment, and challenge methods. Notably, improved diagnostic techniques and pre-commercial vaccine formulations against several diseases were achieved. Operational Welfare Indicators (OWIs) were developed, validated, and adopted by certification schemes. The project generated scientific papers, training activities, and cooperation with other initiatives like ParaFishControl and MedAID projects.

MedAID summarized its outputs, including risk assessment templates, biosecurity scoring system for farmers, diagnostic methodologies written and published in Diagnostic manual for the main pathogens in European seabass and gilthead seabream, harmonisation of betanodavirus diagnostic techniques through proficiency testing for betanodavirus genotypes. MedAID consortia tested Viral like particles (VLP)-based vaccines against VNN and characterized antibody-mediated immunity, and also measurable welfare indicators for sea bream were defined. MedAID and PerformFish projects partners established the networks for information exchange and for setting epidemiological cut-off values of susceptibility testing for *V. anguillarum* and *V. harveyi*, the most common pathogenic bacteria. Partners from both projects published 40 scientific articles out of which six in cooperation, organised five workshops, and five training courses with one in cooperation, demonstrating a comprehensive approach.

Both projects, with unique strategies, significantly boosted Mediterranean fish farming. The collaborative efforts involving diverse Mediterranean and non-Mediterranean participants stand as a valuable legacy for the future, reinforcing the industry's position within global aquaculture.

The nutraceutical approach against parasites: an overview by A. Gustinelli and ML. Fioravanti

Efforts to sustainable control of parasitic diseases in European seabass and gilthead sea bream farming are escalating due to their threat to fish health and productivity (Fioravanti et al. 2020). Traditional antiparasitic methods pose environmental and health risks, prompting a search for alternatives. With many banned compounds (Lieke et al. 2020), the aquaculture sector is turning to nutraceutical approaches like functional feeds, aiming to enhance growth, immunity, and overall well-being beyond conventional feeds (Encarnação 2016). Recent attention focuses on using these feeds to prevent and manage severe parasitic diseases in Mediterranean fish farming (Firmino et al. 2020). Recent and ongoing European H2020 projects like ParaFishControl, PerformFISH, MedAID, and NewTechAqua have explored

or integrated trials investigating the effects of functional feeds against major parasites affecting gilthead seabream, such as *Sparicotyle chrysophrii* and *Enteromyxum leei*.

Trials using diets enriched with a mixture of caprylic acid, iron and mannan oligosaccharide (MOS) showed promising results against *S. chrysophrii* (Rigos et al. 2016), emphasizing the need for early use and proper management practices for efficacy. Similarly, trials integrating various additives demonstrated significant mitigation of sparicotylosis when administered to juvenile fish at the outset and throughout the high-risk period (generally spring to fall). For *E. leei*, controlled laboratory challenges revealed potential in limiting Enteromyxosis using feed additives like Sanacore® GM (Palenzuela et al. 2020), warranting further field testing.

Recent research advancements underscore the potential of sustainable tools against prevalent parasites in Mediterranean aquaculture. In general, the promising results achieved by the research carried out in the last decade on sustainable control tools against the most impacting parasites in Mediterranean aquaculture, namely *Sparicotyle chrysophrii* and *Ceratomyxus oestroides* (Mladineo 2020) have given the opportunity to underline the need of a bigger effort and further investments on the implementation of field studies on the effects of functional feeds against fish pathogens.

The future of autogenous vaccines in the context of new EU regulations by P. Katharios

Autogenous vaccines, tailored to combat specific farm outbreaks, are vital in preventing diseases among production animals. They are only allowed within the affected farm when no licensed vaccine exists for the disease or if the available vaccine is not effective due to strain differences. This personalized approach fills gaps left by conventional vaccines, ensuring a targeted response to each farm's unique disease profile.

In Mediterranean aquaculture, the diverse range of cultured species makes vaccine development challenging due to market fragmentation and substantial time and financial investments required. Hence, autogenous products play a significant role, especially in combating new pathogens from introduced fish species and climate change impacts or for species with different genotypes that have been known for a long time but for which vaccines are not effective any more. These vaccines, customized to regional aquaculture's evolving pathogens, provide a crucial defense against new microbial species and changing environmental conditions. European seabass aquaculture demonstrates the success of autogenous vaccines against diverse pathogens like *Aeromonas veronii* and *Vibrio harveyi* (Smyrli et al. 2022), where developing a universal vaccine is challenging due to both pathogen

complexity. This success underscores the practicality and effectiveness of tailored vaccine approaches, particularly in aquaculture where a one-size-fits-all solution is not feasible.

Previously, autogenous vaccine production lacked a centralized regulation in Europe. However, the new veterinary medicine Regulation (EU) 2019/6 (Grein, Jungbäck, and Kubiak 2022) mandates Good Manufacturing Practice (GMP) for their production, ensuring consistent and controlled vaccine manufacturing. This update aligns autogenous vaccines with EU standards for other veterinary medicinal products. Presently, GMP guidelines specifically tailored to these vaccines are being prepared for full implementation by 2025.

While ensuring higher quality and safety, the shift to GMP standards might impact the price and availability of autogenous vaccines. The cost of complying with GMP could strain smaller manufacturers. Balancing enhanced quality assurance with economic constraints will be crucial during this regulatory transition.

Viral Nervous Necrosis: what's new? by A. Toffan

Betanodaviruses are endemic in the Mediterranean Sea and cause Viral Nervous Necrosis (VNN), a significant and economically impactful disease in warm water marine species (Bandín and Souto 2020). They infect various fish species and mortality rates vary based on fish age, seawater temperature, viral species and strain, fish density, and other stressors (Panzarin et al. 2012; Bitchava et al. 2019).

Official epidemiological data on the disease's spread are unavailable, but data from the WOA reference Laboratory at IZSVe in Italy indicate a 2 to 10% prevalence of real-time RT-PCR positive samples from 2021-2022, with RGNNV being the most detected viral species. However, factors like sampling clinically healthy fish for routine checks, lack of complete anamnestic data, and samples originating from select Mediterranean countries may bias these findings, rendering them unreliable, albeit being the only systematically collected data available.

The impact of VNN is projected to rise due to increasing seawater temperatures caused by climate change, especially in the Mediterranean, classified as a climate change hotspot. This region has experienced a consistent sea surface temperature rise of 0.035°C/year over 30 years (Pastor, Valiente, and Khodayar 2020). Some pathogens, like RGNNV, show increased virulence in warmer waters (>25 °C), potentially leading to higher mortality rates (Toffan et al. 2016; Vázquez-Salgado, Oliveira, and Bandín 2022). Studies suggest that a 1°C rise in seawater temperature could increase mortality in NNV-infected fish by 2.18–5.37% (Combe et al. 2023), posing a risk to aquaculture and global food security.

40 Years of Health Challenges and Solutions in Mediterranean Fish Farming

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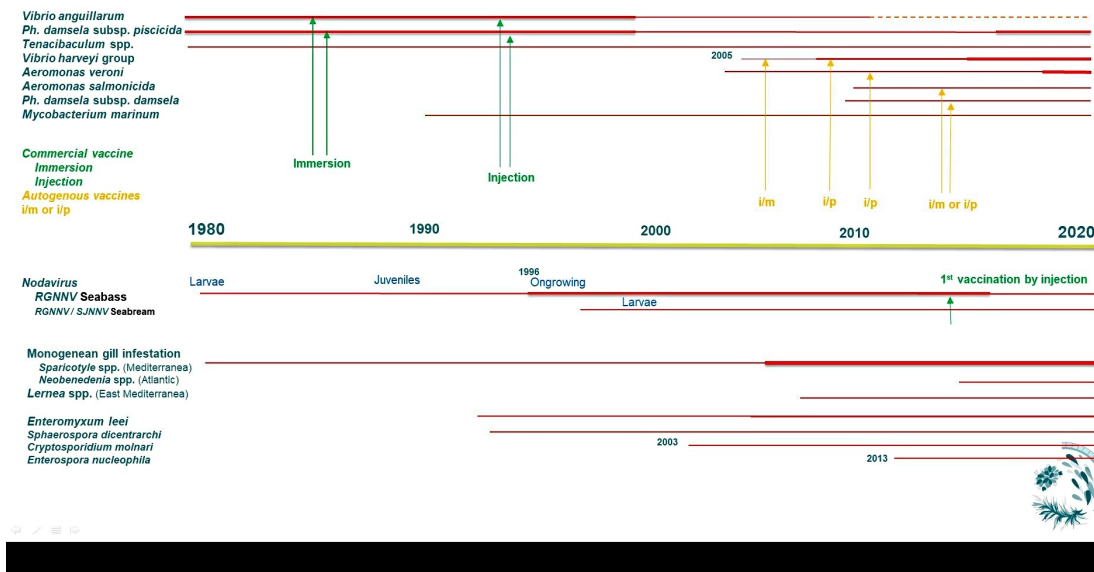


Figure 1. 40 Years of Health Challenges and Solutions in Mediterranean Fish Farming

Legend: blue line – timeline from 1980 to nowadays

Red line – diseases occurrence and intensity (the thicker is line, the impact of the disease is stronger)

green arrow – commercial vaccine

yellow arrow – autogenous vaccine

Plastic pollution, specifically polystyrene nanoplastic particles, has been linked to increased viral replication and clinical signs of NNV infection in sea bass. Exposure to nanoplastics affects the fish's immune response, facilitating disease progression but not altering final susceptibility (González-Fernández and Cuesta 2022).

While the impact of the RGNNV/SJNNV reassortant strain has lessened due to increased biosecurity measures in sea bream hatcheries, attention to this virus remains crucial. This strain, while causing mild clinical signs in sea bass, can lead to high viral loads in asymptomatic survivors, suggesting a potential reservoir role (Toffan et al. 2017, 2021; Vázquez-Salgado et al. 2023; Biasini et al. 2022).

Biosecurity in the Mediterranean aquaculture by A. Le Breton

Mediterranean aquaculture faces unique challenges due to its structure, different from other intensive industries like salmon farming. Unlike the all-in-all-out approach in salmon production, Mediterranean farms rear different fish generations together, posing a risk of disease spread (Figure 1). Traditional practices involve early transfer of juveniles to the sea, which hampers the implementation of complete vaccination strategies and increase the risk of diseases. Over the last 40 years health challenges occurred with different intensity and management measures were developed accordingly.

A significant change came with well boats a decade ago, allowing land-based pregrowing, vaccination, and the transfer of larger juveniles (15-20 grams) to sea cages, reducing cross-contamination risks. This led to decreased initial-phase mortalities, lower pathogen pressure, and cost savings (Fernández Sánchez et al. 2022). Other benefits were generated such as reducing the length of production cycles, the storage of small granulometry feeds and small mesh net for early juvenile stages.

Regulations like EU's 2019/6 improved autogenous vaccine use, but medicine availability remains limited. Biosecurity measures primarily focus on individual farms, but a broader approach considering the entire Mediterranean as an epidemiological unit is crucial. Actions like transport disinfection, monitoring transferred juvenile health, and conducting epidemiological surveys are vital across Mediterranean nations. However, uniformity in frameworks, labs, and methods is lacking in some of the Mediterranean countries.

A coordinated effort involving stakeholders and adopting the FAO's Progressive Management Pathway for Aquaculture Biosecurity program is essential (FAO 2020; Cottier-Cook, Cabarubias, Brakel, et al. 2022). This approach can identify health risks, establish biosecurity systems at national and Mediterranean levels, and ensure the sustainability of Mediterranean aquaculture.

Discussion

The moderated discussion aimed to gather delegates' opinions (n=80) on the future needs of Mediterranean aquaculture. Several key questions were addressed:

1. **Challenges to the Cage-Based Production Model:** The discussion highlighted that the economic returns from Mediterranean farmed species might not cover the costs of recirculation aquaculture systems (RAS). It was suggested that fish farmed in such installations should possess added value. Although the Norwegian RAS industry is expanding, it's not a comprehensive solution. Investigating rotation culture was proposed to combat health challenges posed by monogeneans, a significant constraint in Mediterranean species farming.
2. **Addressing Health Problems Due to Climate Change:** It is well known that there is a shortage of veterinary drugs approved for parasitic and bacterial diseases in Mediterranean area. The conversation stressed the need for genetic selection, aiming not only to genetic resistance to diseases but also for fish more resilient to warm water. Increasing the resilience of farmed fish to parasite infection was highlighted, suggesting strategies like controlled parasite exposure during specific stages of fish development.

Combining classical diagnostic techniques with newer ones like transcriptomics was suggested to enhance disease understanding and management.

3. **Improving Disease Surveillance and Diagnostic Capacities:** Horizon projects identified a lack of diagnostic laboratories in Northern African countries. Emphasis was placed on the need to enhance local diagnostic capacities. Moreover, well-boats were identified as potential disease transmission risks, urging strict disinfection protocols. The discussion debated on who should oversee these protocols, proposing either well-boat managers or third-party companies to provide necessary services. Fish farmers, fish health managers, or veterinarians were recommended to seek certification before using well-boat services.
4. **Feasibility of Fish Disease Surveillance:** The importance of field veterinarians capable of aiding companies in enhancing biosecurity and defining health procedures was highlighted. The role of fish vets as sentinels in aquaculture practices was described by Varvarigos (2020), emphasizing the need for their expertise in identifying emerging pathogens such as *Piscirickettsia salmonis* (Zrnčić, Vendramin, et al. 2021) or *Aeromonas veronii* (Smyrli et al. 2019) and pollution sources. The shortage of field vets sparked a discussion on the necessity for educational workshops tailored to the industry.
5. **Educational Needs in Aquatic Veterinary Services:** There's a call for specialized education focusing on aquatic animals, essential for veterinarians in this sector. Collaboration between aquatic animal experts and fish vets was proposed for the continuous support to fish farmers. Efforts to engage international organizations like FAO, WOAHA, and General Fisheries Commission for Mediterranean and Black Sea (GFCM) to support aquatic vet education, particularly in less developed Mediterranean countries, were suggested.
6. **Enhancing Networking and Collaborations:** Communication among Mediterranean farms regarding health issues was seen as reserved compared to regions like Ireland and Scotland. Suggestions included initiating stronger collaboration among Mediterranean enterprises with potential support from EU and non-EU governments. Drawing parallels with the Great Lakes region in the USA, where collaboration among states improved through a unified commission, it was proposed to establish a Mediterranean Health Commission composed of representatives from each country facing the Mediterranean Sea. GFCM was identified as a potential organization to lead this initiative due to its focus on aquatic animals in the Mediterranean.

Additionally, a strong will was expressed to organize Mediterranean EAFP meetings in non-conference years, starting with a meeting alongside the Italian Society for Fish Pathology (SIFI) meeting in Italy in 2024.

Conclusions

The future of the aquaculture industry will undeniably be influenced by diseases and climate change. This is especially evident in the Mediterranean basin, where a substantial interconnection already exists among farms in various countries. Consequently, there is an urgent requirement to bridge gaps, such as disparities in diagnostic capabilities, availability of drugs and vaccines, and the implementation of biosecurity protocols. Coordinating collective actions is imperative to effectively confront the impending challenges ahead.

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