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WORKSHOP

Fish health in Mediterranean Aquaculture, past mistakes and future challenges

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Introduction

A significant production of finfish in Europe consists of European Sea Bass (Dicentrarchus labrax) and Gilthead Sea Bream (Sparus aurata). According to the Federation of European Aquaculture Producers (FEAP, 2015), European Mediterranean countries including Turkey, produced 148,367 tons of sea bass and 146,467 tons of sea bream in 2014. Nevertheless, the production in the area is considered to be underestimated based on satellite picture analysis of the facilities present in the Mediterranean basin and an estimate of their use and production (Trujillo et al., 2012).

Regarding the legislation on surveillance and control of fish diseases, despite the relevance of the production and economic value of these two marine species, no specific provisions are given in the present EU legislation (2006/88/EC); they are not included in the list of susceptible species to listed diseases mentioned in Annex IV of Council Directive 2006/88/EC, but they are included as vector species for Viral haemorragic septicaemia under specific conditions according to EU regulation (1251/2008/EC) and list of vector species according to EFSA (2007). Furthermore, despite CD 2006/88/EC art.43 include the opportunity that single member states can imply provisions to limit the impact of diseases not listed in Part II of Annex IV, no national surveillance programs have been applied to these fish species.

Due to different levels of implementation of the aquatic animal health law in different European countries, many different qualified players including research and diagnostic laboratories, private testing laboratories, consultants and practitioners have essential information and insight on the actual health situation; however information available to each single player is partial and fragmented and a focal point having an overview on the current spread and prevalence of infectious fish diseases in the Mediterranean basin is missing. This gap is regarded as an obstacle for further development of the industry and to design and coordinate harmonized strategies in the different regions of the basin.

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Since 2012, the European Union Reference Laboratory for Fish Diseases (EURL) have performed a survey involving a number of private and institutional experts depicting the most important diseases in the Mediterranean and their evolution (Olesen & Vendramin, 2012; 2013; 2014; 2015). Data have been compiled and presented at the Annual Workshops organized by the EURL. From the information and experience obtained during these surveys, an initiative was taken to organize a specific workshop (WS) on the subject at the 17th EAFP Conference.

The aim of the WS was to collate information through a live forum discussion, actively involving all present stakeholders, with the conference being a unique opportunity to gather several relevant players for participation. Discussions were mainly focused on the socio-economic impact of viral, bacterial and parasitic diseases of sea bass and sea bream. The final output of the discussions aimed to rank the most important infectious diseases in Mediterranean mariculture prioritizing them according to their economic impact. The expected output of the WS is an agreed working document as a baseline for future initiatives focusing on relevant diseases in the area.

**WS organization**

The total time allocated to the WS was 1 h and 45 min with 3 sessions on the agenda:

i. Introduction describing the aim and topic of the WS providing instruction to participants. Impact was constituted by impact on production, economy and legislative consequences.

ii. Working group activity. Participants were divided into 4 groups according to geographical affiliation (Figure 1). Each group was coordinated by a facilitator, who was a member of the organizing committee.

a. Western Mediterranean (Spain, Canary islands, France and Italian west coast)

b. Adriatic Sea (Italian east coast, Slovenia, Croatia) and Greek Ionian sea

c. Aegean Sea and Turkey

d. Northern Africa and Levant

iii. Plenary session. Representative of each working group presented outputs of the discussions.

The definition of “impact” was clarified as a value that compiles:

- “Impact on production” means the severity of losses in terms of mortalities, reduction of growth, etc.
- “Impact on the economy” indicates the cost of prevention (i.e. vaccination, biosecurity measures, treatment and reduced final product value).
- “Legislative consequences” due to trade restrictions, national plans for control/eradication, sale ban due to antibiotic withdrawal time etc.
- “Prevention” – Are there preventive measures or practices for prevention of specific diseases?
- “Control” – Are there control measures available (i.e. available treatment)?

The scoring system was from 0 to 10 for each point; 0 was to be understood as no impact on expected output and 10 as complete failure of a production cycle. For prevention and treatment the higher the score, the higher are chances to prevent or control the single pathogen.

It was estimated that 60 persons participated in the event, representing fish health managers of
some of the most important corporate groups, private practitioners, experts from feed companies providing technical support to farmers, as well as scientists from research and diagnostic laboratories at universities and governmental institutes, representing most of the key players involved in all levels of the management of infectious fish diseases in the area.

**Output of group activities**

In order to guide the discussions, a list of known pathogens was provided to all groups. The list was open and further problems could be included. Among viral diseases Viral Encephalopathy and Retinopathy/Viral Nervous Necrosis (Munday et al., 2002; Bovo et al., 1999) and Lymphocystis disease (Cano et al., 2007) were included.

Concerning bacterial diseases Vibriosis, Photobacteriosis/Pasteurellosis (due to *Photobacterium damselae* subsp. *piscicida*), Tenacibaculosis (Toranzo et al., 2005) Mycobacteriosis (Colorni et al., 1998), Aeromonasis (Doukaset al., 1998), Streptococcosis and Nocardiosis were considered.

As important parasitic diseases gill flukes including *Sparycotyle chrysophrii* (Sitja-Bobadilla et al., 2010), enteric diseases caused by the myxosporea *Enteromyxum leei* (Diamant et al., 1994) and the microsporida *Enterospora nucleophila* (Palenzuela et al., 2014), infections with *Amylochinum ocellatum* (Paperna, 1980), *Cryptocaryon irritans* (Rigos et al., 2001) and infestations with copepods and isopods (Vagianou et al., 2006) were discussed.

The 5 most important infectious problems are listed per group. The ranking provided apply to
what is considered to be the current mariculture production in the Mediterranean consisting of hatcheries/nurseries for production of juveniles to seed in cages for on-growing phase. Even though not included in the lists some on-growing farms with earthen ponds still have problems with other known pathogens like *Amylodinium ocellatum* or *Cryptocaryon*.

**Western Mediterranean Area**

*Speaker – Daniel Gijon, DVM, Skretting, Spain – 25 participants*

i. VER-VNN is considered the most important disease linked to the economic impact due to mortality rate and the growth reduction in the affected batch. The output of a disease outbreak is considered to be rather unpredictable. The only prevention measure is checking each batch of juveniles before seeding and no control methods are available.

ii. Gill flukes (mainly *Sparycotyle chrysophrii*) in sea bream seem to be widespread in the area and pose a serious threat to the production. The infection often results in high mortality rates and treatment is rather complicated.

iii. Despite the specific differences, commonly known bacterial infections with *V.anguillarum*, *V.harveyi*, *Ph. damselae* subsp. *piscicida* and different species of *Aeromonas* still play an important role. Prevention against vibriosis requires a cycle of vaccination and consists of dip, and injection at a later stage, is usually efficacious in reducing mortality and until now, resistance has not been observed. Concerning the *P.damselae* subsp. *piscicida*, most batches of sea bream are dip vaccinated, but protection does not last for a long period. Some autologous vaccines are available against different *Aeromonas* species. Often antimicrobial resistance is quickly established after infection and therefore sensitivity testing is important to perform the correct treatment.

iv. Tenacibaculosis caused by *Tenacibaculum spp.* represents an important issue particularly for sea bass in hatcheries and nurseries. Various species of the bacteria are involved and it is difficult to identify and distinguish them.

v. Enteromyxosis was included in the list, being a major problem in the past, but seems to have reduced impact over the last two years.

**Central Mediterranean Area**

*Speaker- Amedeo Manfrin, IZSVe, DVM, Italian NRL for Fish Diseases - 20 participants*

i. VER/VNN is also considered the most important disease and the cost of disposal of dead fish is stressed as contributing to the disease costs. Prevention is not possible due to the lack of vaccines. It is difficult to select brood stocks which do not carry the pathogen asymptotically.

ii. Vibriosis is considered the second most important disease with prevention schemes that appears to be quite different in different countries. While in some areas only dip vaccination is performed, many Greek farms are vaccinating 2 or 3 times (the last one by IP injection) to prevent clinical appearance of disease. In some cases antibiotic resistance has appeared. In Italy some failures in the vaccination have been observed. Recently *Vibrio harveyi* has been recognized as an emerging problem...
in sea bass.

iii. Parasitic infestations clustered under the name “gill flukes” are considered the third most important problem. As for the Western Mediterranean it can result in high mortalities in the affected batches and gill anemia in market size fish is a commercial problem. Differences in authorized treatments between countries and persistence of parasite eggs on cage nets were raised.

iv. Photobacteriosis caused by Ph. damsela subsp. piscicida is a significant problem due to the lack of authorized vaccines (i.e. Italy) and the relatively short protection provided to vaccinated batches.

v. Tenacibaculosis scored a rather high impact due to the difficulties of treatments.

**Eastern Mediterranean Area**

*Speaker - Kantham K. Papanna – Fish health manager Nireus, 20 participants*

i. VER/VNN is also the most important disease including the production in the Aegean side of Greece and Turkey. The annual prevalence is dependent on the sea water temperature; at the peak, 26°C to 28°C, it remains for prolonged periods and the impact is serious in the on growing farms. There is good biosecurity in the land based hatcheries and the outbreaks are kept under control.

ii. The impact of Vibriosis is still high considering the cost for prevention which includes implementation of vaccination of all produced batches both by immersion for juveniles and a booster administered by IP injection in cages. Properly planned and executed vaccination programs can effectively prevent outbreaks in sea bass farms.

iii. Photobacteriosis also remains a serious infection, because treatments are unsuccessful if they have not started before the onset of anorexia in affected fish. Vaccines are not 100% effective as regarded for classical Vibriosis due to V. anguillarum.

iv. Isopod infestation is a major problem ranking maximum impact; however the control of this pathogen with therapeutic compounds used in the salmon industry against sea lice demonstrates effectiveness, whereas no prevention methods are available. Medications used for treatment of salmon lice must be licensed locally before being used in disease control.

v. Gill Flukes pose a major problem too. The treatment is quite difficult and still relies on the use of formalin based bath treatment. The licensed veterinary medical product Aquacen-F is the suitable alternative under veterinary prescription.

**Southern Mediterranean Area**

*Speaker – Nadav Davidovich, Fish health veterinary officer Israel, 10 participants*

i. VER/VNN is the disease considered to have the highest impact on production and economy in the southern Mediterranean, including Tunisia and Israel. Therefore the production shifted towards production of sea bream, which is less susceptible to VNN than sea bass.

ii. Gill flukes represent a major sanitary issue in the whole northern African production region including Tunisia, Morocco and Algeria.

iii. Enteromyxosis in bream is considered to be the third most important problem due to the reduction of growth in affected fish in northern African countries.
iv. Photobacteriosis still remains a major problem in the whole area, both bass and bream are affected, and as mentioned in other groups, the efficacy of the vaccine does not last for a long period.

v. Mycobacteria infection is quite important due to its impact in closed recirculating facilities, based on the zoonotic features of this pathogen and on the difficulty in treating the infection properly.

Discussion and Conclusion
This workshop was regarded as a successful event both in terms of number of participants and outputs of discussions. Approximately 60 experts actively participated in the working groups providing significant inputs and drawing a baseline for the sanitary issues in the Mediterranean mariculture of sea bass and sea bream. Interestingly, it should be emphasized that, with slight differences, the most important problems are the same in the whole area.

All groups agreed that VER/VNN is the most important problem in Mediterranean mariculture. Further characterization of the disease is needed aiming to discriminate existing types of Nodavirus infections in relation to the virus genotype and species.

According to the area, the second and the third place were shared between bacterial and parasitical infectious diseases with some spatial differences. The efficient management of the bacterial pathogens *V. anguillarum* and *Ph. damsela* subps. *piscicida* remains priority despite availability of vaccines and antibiotic treatments.

Amongst the parasitic diseases, the most important parasitic infestations are caused by gill flukes, mainly due to difficulty in antiparasitic treatments.

Future research should be focused to precisely characterize the impact of the single disease with consideration of all zootechnical issues related to infection both as a cause and a consequence.

Risk factors need to be analyzed and assessed to understand the different impact of particular diseases in different Mediterranean areas.

The preliminary geographical clusters established at this WS should be characterized better based on the following features:
- Type of cage or land systems
- Proximity of farms and companies
- Biosecurity measures in force like coordinated inputs and treatments, following “all in all out”, single year class, synchronized production
- Farming of multiple species in the same site
- Legislative control
- Vaccination programs applied

Consequently, it is foreseen to extend the forum addressed in such a survey to involve associated farmers and pharmaceutical industry. The overall idea is to integrate inputs from all stakeholders, being able to combine inputs and develop a sustainable health management strategy for the further development of the industry in the whole area.

The following key topics need to be specifically addressed:

i. What needs to be modified in the production scheme and organization of the farms?

ii. What preventive and curative medicines
are required in the near future from the pharmaceutical industry?

iii. How to manage epidemiological survey at the regional level without interfering with confidentiality of data for the producers?

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