Survey on laboratories and consultants working in the diagnostics of European seabass and gilthead seabream diseases: preliminary results

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Abstract

European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) are prevailing species in Mediterranean marine finfish aquaculture, and despite an increasing demand of the global markets for high-quality seafood, production and technical performance of these two species in the EU has stagnated over the last few years. Two EU Horizon 2020 projects, MedAID and PerformFISH were launched in 2017 to increase the competitiveness and sustainability of the Mediterranean marine fish aquaculture sector. Since the impact of diseases has been one of the factors attributed to the stagnation, both projects envisaged that the concept of healthy fish was a prerequisite for sustainable and profitable aquaculture. A key element of disease surveillance and health management is the availability of fast, reliable, efficient and validated diagnostic techniques capable of detecting the presence of pathogens and timely diagnosis of diseases in fish stocks. Collaborative activities between both consortia launched specific activities to identify all actors involved in diagnostics and to evaluate their diagnostic capacities. An online "Questionnaire on diagnostic capacities in the Mediterranean basin" was carried out. The results obtained showed disproportionate diagnostic capacity between European and non-European Mediterranean countries. European countries in general showed a high level of diagnostic capacity with many advanced or specialised labs dealing with the main diseases of concern for sea bass and sea bream. There was evidence of lower diagnostic capacities in non-European Mediterranean countries in contrast with their high degree of production, which poses a significant regional risk considering the important movement of juveniles in the region. These findings indicate the necessity to address the health management in the region in a more holistic, cooperative and harmonised way. An important finding was a lack of capacity to diagnose viral diseases although VNN has been identified to be the main health threat. Efforts should be engaged in capacity building in the countries missing particular techniques and improvement and training is a priorty. For this purpose, national focal points should be established to create an international network aimed at improving and harmonising all future activities in the field of diagnostics of Mediterranean fish diseases.

Introduction

In the last decade farming of marine fish species had a growth of 73% globally (Tveteras et al., 2020). Although the majority of these aquaculture activities are situated in China and Indonesia, followed by salmon farming in Norway and

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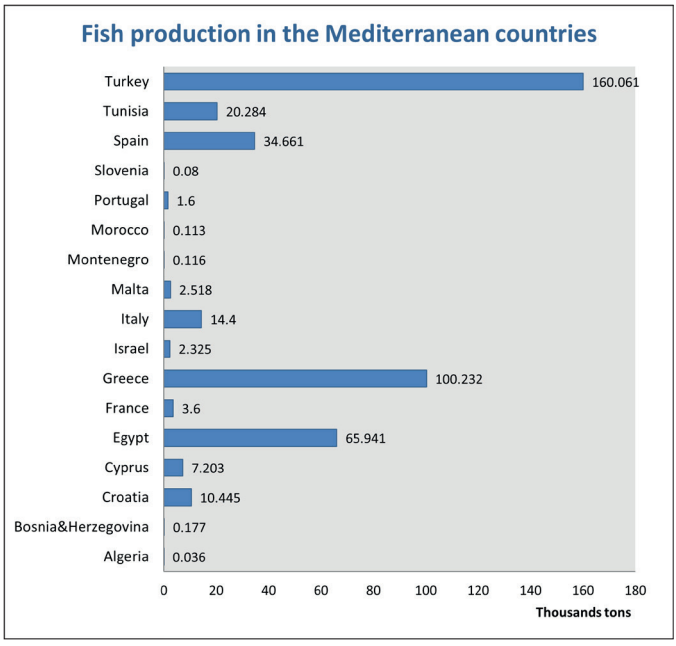

Figure 1. Production of European sea bass and gilthead sea bream in Mediterranean countries

Chile, some of the leading marine fish producers, namely Turkey, Greece and Egypt are part of the Mediterranean basin (FAO, 2020). Mediterranean marine finfish aquaculture is dominated by two main species, the European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) (Massa et al., 2017). The main market for the above-mentioned species is the European Union (EU), the world's largest market for seafood (EUMOFA, 2019). Despite a huge demand of the EU market for seafood, production of seabass and seabream in EU countries has stagnated over the last few years, whereas in non-EU countries production has increased significantly. Production of both species started some forty years ago and nowadays, has increased up to 426,774 tons (FAO, 2018).

The highest volumes are produced in Turkey (160,061 t), followed by Greece (100,232 t), Egypt (65,941 t), Spain (34,661 t), Tunisia (20,289 t) and Italy (14,400 t) (Figure 1). Other significant producers in the Mediterranean are Croatia (10,445 t), Cyprus (7,203 t), France (3,600 t), Malta (2,518) and Israel (2,325 t). Smaller producers with less than 1,000 tons are Morocco, Algeria, Slovenia and Bosnia and Montenegro.

Generally, the impact and burden of diseases has been one of the main factors attributed to the stagnation and are a serious challenge to the future development of this specific marine farming sector despite the efforts engaged into minimising the disease impacts. For this reason, disease control needs to be an overall priority for the development and improvement of the Mediterranean aquaculture sector (Vendramin et al., 2016).

In 2017, two EU Horizon 2020 projects, MedAID (Mediterranean Aquaculture Integrated De-

velopment) and PerformFISH (Integrating Innovative Approaches for Competitive and Sustainable Performance across the Mediterranean Aquaculture Value Chain) have been launched to increase the competitiveness and sustainability of the Mediterranean marine fish aquaculture sector. The main goal of both projects is to improve key performance indicators (KPIs) of Mediterranean mariculture and the concept of healthy fish is envisaged as a prerequisite for sustainable and profitable aquaculture in the Mediterranean area. A key element of disease surveillance and health management is the availability of fast, reliable, efficient and validated diagnostic techniques capable of detecting the presence of pathogens and timely diagnosis of diseases in fish stocks (Zrnčić et al., 2019).

For this reason, it was necessary to gain more accurate knowledge on all relevant actors in diagnostics including national reference laboratories, research laboratories, private laboratories, consultants and farm health experts, including an assessment of their diagnostic capacities. Consequently, a "Survey on laboratories and consultants working in the diagnostics of European seabass and gilthead seabream diseases" was launched as a collaborative activity by the two consortia to determine the strength, weakness and possibilities of improvement in the future overall diagnostic capacities.

The purpose of this paper is to present the results of the survey specifically aimed to 1) set up the database of laboratories and consultants working in diagnostics of European seabass and gilthead seabream and to facilitate collaboration among them, and 2) to evaluate and define diagnostic capacities and methodologies applied for the most relevant pathogens providing the basis for optimisation and standardisation of diagnostic techniques.

Methodology

Identification and recruitment of participants

Stakeholders in each country of the Mediterranean basin were identified by project partners and recruited to participate in an online questionnaire-based survey. Recruitment methods involved an email invitation of the identified stakeholders and the collection of basic data such as country of residence, name of laboratory, ownership (private or public), type of laboratory (research, diagnostic or industrial), and contact information (address, phone, contact person, email) taking into consideration the EU data protection rules (general data protection regulation). Datasets were organised in a specific database. In the next step, contact persons were addressed by email with a request to confirm their involvement in diagnostics of sea bass and sea bream diseases and to express willingness or denial to participate in the diagnostic capacity survey.

Questionnaire

An online questionnaire was created and conceived to provide an insight into the details of the laboratory capacity and methodology applied in each laboratory. After a first question giving consent by the participant on data management within the PerformFISH-MedAID survey, sets of questions were divided into 5 sections: 1) General information on each laboratory; 2) Methods applied to diagnose parasitic diseases; 3) Methods applied to diagnose bacterial diseases; 4) Methods applied to diagnose viral diseases and 5) Other methods applied to diagnose all aforementioned diseases, such as histology, immunohistochemistry or other

immunological methods, *in situ* hybridization and next-generation sequencing (NGS). Some additional questions about the prevalence of the most important parasitic, bacterial, viral or other diseases were included at the end of each section as well as willingness to participate in the validation of particular diagnostic methods and training. The example of questions asked for assessing the diagnostic capacity on parasitic diseases is shown as follows:

1. Do you commonly perform parasitological exams in your Lab?
   Yes
   No
2. If yes, please specify methodology:
   Fresh mount smears
   Stained smears
   Molecular methods
   Electron microscopy (SEM, TEM)
   Other
3. Level of taxonomic identification
   Species
   Genus
   Family
4. Specify the staining methods you use for detection of certain parasites
5. Specify the parasite and molecular methods you use for diagnostics
6.Specify the EM method you use for detection of certain parasites
7. Please specify which parasites are more commonly diagnosed in European sea bass in your Lab
8. Please specify which parasites are more commonly diagnosed in gilthead sea bream in your Lab

The questionnaire "Questionnaire on diagnostic capacities in the Mediterranean basin" was published using Google forms. An invitation letter comprising the link to the online questionnaire was delivered to all participants who agreed to participate in the survey. A reminder was delivered after two months encouraging some missing laboratories to fill it in.

Data analysis

Submitted answers were collected in another dataset, respondents were coded and answers were grouped. Diagnostic methods applied for the diagnosis of particular groups of pathogens were analysed aiming to find the capacity of each respondent. It was obtained by implementing the scoring scheme according to the criteria shown in Table 1. Within a scoring scheme, three categories were set up: basic, advanced and specialised capacity to diagnose each group of disease. A detailed description of each scoring category was agreed consensually between two consortia.

The data obtained for each section of the questionnaire were grouped, scored and basic statistical analysis was used for calculation of shared techniques and comparisons of the laboratories and countries capacities, and ability, to diagnose particular groups of pathogens.

Results

In total 123 stakeholders were identified as being involved in diagnostics of sea bass and sea bream diseases (Table 2). Most of them (76%) correspond to southern European EU countries. The non-European Mediterranean countries represent 22% and northern European EU and non-EU countries, Denmark and UK, (both partners in MedAID and PerformFISH project, respectively, only 2%), which clearly showed higher diagnosis capacities in the EU European countries.

Table 1. Criteria for scoring the capacity to diagnose a particular group of diseases

Group of disease by pathogen	Capacity	Description
Parasitic diseases	Basic	Fresh mount/staining techniques
	Advanced	Basic plus molecular methods of electron microscopy (EM)
	Specialised	Basic methods plus molecular methods plus EM
Bacterial diseases	Basic	Isolation with biochemical identification and sensitivity testing
	Advanced	Basic plus molecular methods and/or serology and/or MALDI-TOF
	Specialised	Basic plus all other techniques
Viral diseases	Basic	Molecular methods
	Advanced	Basic plus the isolation of viruses in cell cultures
	Specialised	Advanced plus additional methods such as TEM, serology
Other techniques	Basic	Histology
	Advanced	Basic plus other methods such as immunohistochemistry (IHC)
	Specialised	Advanced plus in situ hybridization, immunology or NGS

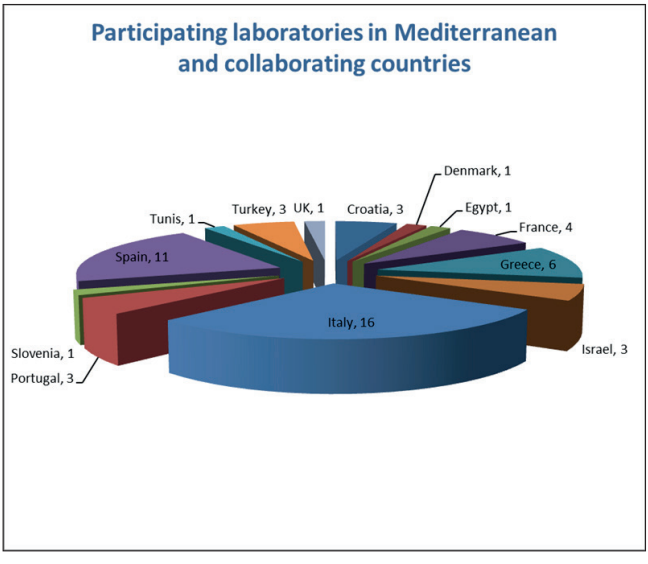

Figure 2. Number of participants in the survey per respective Mediterranean country

Most of the laboratories (76%) were recognised as public laboratories, while 24% were defined as private. Interestingly, many of these stakeholders developed both research and diagnostic (59%) activities while the others were either research or diagnostic. A smaller number was

Table 2. Summarised profile of the stakeholders involved in diagnostics of Mediterranean fish diseases in a particular country

Country	Total no of laboratories	No of public	No of private	Research laboratory	Diagnostic laboratory	Industrial laboratory	Readiness to participate in the survey
Algeria*	1	1	0	1	1	0	1
Egypt*	2	2	0	2	2	0	1
Israel*	6	5	1	4	4	1	2
Morocco*	1	1	0	1	1	0	1
Tunisia*	3	3	0	2	3	0	3
Turkey*	14	10	4	11	14	0	3
Sub Total Non EU Med	27	22	5	21	25	1	11
Croatia	6	5	1	6	3	1	3
France	20	11	9	12	17	3	5
Greece	13	7	6	8	6	6	6
Italy	20	15	5	17	19	5	14
Portugal	6	6	0	4	4	0	3
Slovenia	1	1	0	1	1	0	1
Spain	28	24	4	26	16	1	13
Sub Total EU Med	94	69	25	74	66	16	45
Denmark**	1	1	0	1	1	0	1
UK**	1	0	1	1	1	1	1
IN TOTAL	123	92	30	96	92	17	59

*Mediterranean non-European countries
**European non-Mediterranean countries, partners in PerformFISH and MedAID projects

identified as industrial laboratories (14 %). Surprisingly, readiness to participate in the survey was received from only half of the contacted laboratories (48 %).

In total 55 laboratories filled in and sent the questionnaire. All respondents gave the consent for all information provided in the survey for storing, processing, analysing and publishing by relevant MedAID-PerformFISH partners. The number of respondents per respective countries is shown in Figure 2. Among respondents, 72.5% belong to the public and 27.3% to the private sector. Most laboratories perform both diagnostic and research activities (37% and 67.3%) while 12.7% of the laboratories develop only research activities and 20% only diagnostic activities. A reduced number of laboratories (16.4%) only receive samples or only collect them in the field (14.5%) and the majority (69.1%) receive samples and also visit farms for sampling. Most of responding laboratories (89.1%) are involved in clinical assessment, 94.5% perform necropsies, 80% conduct diagnostics based on parasitological techniques, 80% on bacteriological techniques and only 47.3% on virological techniques; 49.1% of labs perform diagnostic methods other than those included in the sections 2, 3 and 4.

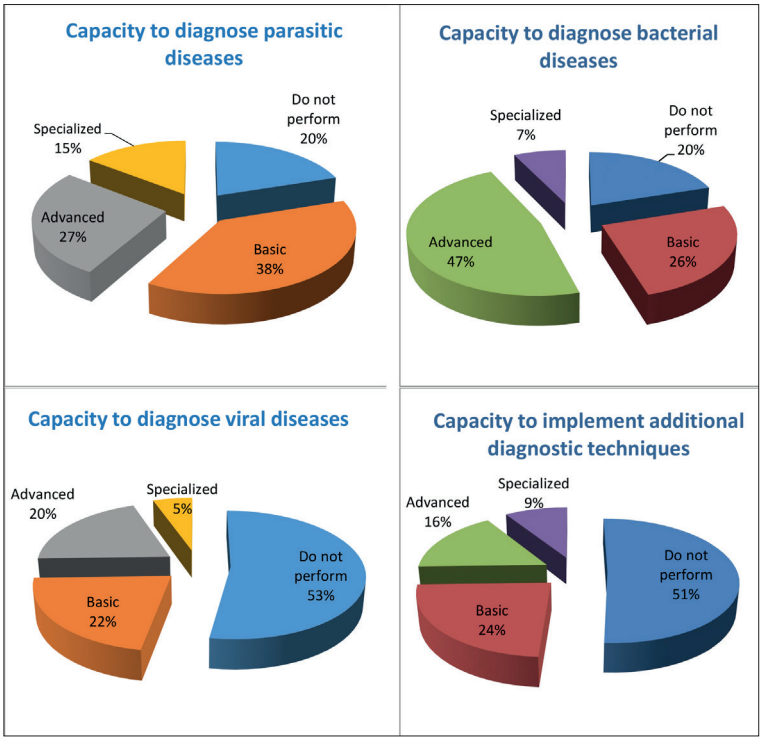

Figure 3. Laboratories' capacity to diagnose a different group of diseases

Concerning the capacity for diagnostics of a particular group of diseases (Figure 3), among laboratories involved in diagnostics of parasites, 38% are categorised as undertaking basic diagnostics, 27% as advanced diagnostics and 15% as specialised diagnostics. In the field of bacteriology, 26% fall into the category of basic diagnostics, 47% in advanced diagnostics and much less (7%) in specialised microbiological techniques. Regarding viral disease diagnostics, 22% were categorised as basic, 20% as advanced and only 5% as specialised. Additional diagnostic techniques such as histology, immunohistochemistry, *in situ* hybridization, other immunological methods and NGS were performed by less than half (49%) of the respondents. Amongst these stakeholders, 24% fell in the basic category, 16% in advanced, and 9% in the specialized category. It was confirmed that 45.5% were performing general histopathology but a reduced number (23.6%) have immunohistochemistry (IHC) capacities and only a 9.1% indicate the capacity to undertake *in situ* hybridization (ISH); 27.3% of the laboratories use immunological methods and 27.3% apply New Generation Sequencing (NGS) techniques. Finally, most of the respondents (90.6%) declared a willingness to participate in diagnostic methods validation and 51 (92.7%) to attend training courses in diagnostics.

Discussion

Diseases continue to hamper the growth of Mediterranean aquaculture (Muniesa et al., 2020) and successful disease surveillance and health management are based on efficient diagnostic capacities. Most of the fish farming companies

and farms carry out disease control by internal health units or subcontracting external experts and laboratories, although there is high variability in expertise and capacities amongst farms. It is also highlighted that although there are no notifiable diseases for sea bass or sea bream included in the EU legislation and for the OIE, countries do have the obligation of monitoring the occurrence of abnormal mortalities events and/or emergent diseases.

Results of the survey showed that there is a significant number of laboratories involved in diagnostic work throughout the Mediterranean basin. However, the data obtained through the "Questionnaire on diagnostic capacities in the Mediterranean aquaculture" indicated considerable differences among the different parts of the basin. Assessment of the geographical distribution of identified laboratories showed a marked difference between non-European and European (mostly EU) Mediterranean countries (Table 2 and Figure 2). European countries showed in general a high level of diagnostic capacity with many advanced or specialised labs dealing with the main diseases of sea bass and sea bream. An important fact is that non-European countries produce more than half (248,760 tons) of the total production of sea bass and sea bream and that Turkey, Egypt and Tunisia are major contributors to the total amounts produced in the Mediterranean basin (Figure1). Correlating the laboratory capacity to the production quantities indicated that laboratory capacities do not correlate to production quantities. The lower diagnosis capacities in non-European Mediterranean countries was in fact in contrast with their high production volumes. When comparing, for instance, Spain and Egypt, that have similar production outputs of sea bass and sea bream, there is a marked difference in laboratory capacity. Egypt has basic capacity in diagnostics of parasites, advanced in diagnostics of bacterial diseases, but has no capacity for diagnostics of viral diseases at all. On the contrary, Spain was represented with 11 laboratories in the survey and generally, they are all categorised as advanced to specialised, with specialised laboratories in diagnostics of all groups of pathogens (parasites, bacteria, viruses and other techniques). Similar capacities, with specialised laboratories in all groups of pathogens, is characteristic of Italy and France. Greece, Croatia, Israel and Turkey have advanced laboratories for all groups and specialised in diagnostics for parasites. Portugal are also grouped among those countries "advanced to specialized" in diagnostic capacity, but they have not reported laboratories for diagnostics of viral diseases in this questionnaire. Tunisia has advanced diagnostics testing capacity for each group of the pathogens. These findings indicate the necessity to address the health management in the region in a more holistic, cooperative and harmonised way. Vast differences highlight the necessity for coordinated efforts in strengthening the diagnostic capacities all across the Mediterranean. It is particularly relevant in the assessment of the health of fry/juveniles, as the trade between different companies and countries in the Mediterranean situation (Cidad et al., 2019) is a relevant epidemiological issue that should be taken into account.

Unfortunately, data was not obtained on the laboratory capacities in Algeria and Morocco, representing smaller producers, similarly to Slovenia which declared basic capacities although they have the capacity for more sophisticated methods if the industry requires it. There were no respondents from Cyprus and

Malta although certain amounts are produced in both countries.

Comparing capability to diagnose a different group of pathogens showed similar skills for detection of parasitic and bacterial diseases with parasitology leading in specialised techniques and bacteriology in advanced. However, the ability to detect viruses was less present with only 14 laboratories applying cell culture techniques for virus detection. Moreover, 18 laboratories did not even have basic techniques for detecting viruses despite viral nervous necrosis (VNN) representing one the most devastating diseases in the hatchery and on-growing stages of sea bass farming. Furthermore, recently recombinant strains of VNN have been detected as emerging pathogens in sea bream hatcheries (Toffan et al., 2017; Volpe et al., 2020). All the obtained data emphasise the fact that many laboratories need the training for implementation of new diagnostics and improvement of existing diagnostic capacities.

Similarly, less than half respondents declared the implementation of "other techniques" and it was amazing that only 13 laboratories declared the usage of histology, a diagnostic tool considered a central technique in diagnostics as it has the advantage of identifying a wide variety of expected and unknown pathogens, as well as lesions (Kent et al., 2013).

Encouraging was the high level of interest of respondents to participate in the validation of diagnostic methods. However, some of them highlighted a need to validate methods for detection of different bacteria, some would like to participate in the validation of histology, some in diagnostics of myxozoan and microsporidian parasites, some in viruses, while some of them expressed willingness to validate detection of all groups of pathogens causing diseases of sea bass and sea bream.

Based on the collected data it is obvious that efforts should be engaged in capacity building in the countries missing particular techniques and that updating and training of the personnel of the existing laboratories is imperative for accurate future disease diagnostics.
For this purpose, national focal points should be established to create an international network aimed at improving and harmonising all future activities in the field of diagnostics of Mediterranean fish diseases.

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