



## Quantification of biosecurity measures in Mediterranean European seabass and gilthead sea bream farms

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### ABSTRACT

In the Mediterranean region, aquaculture drives economic growth and food supply through the production of seabass and sea bream. However, the region faces disease risks attributable to aquaculture intensification. Biosecurity is crucial for maintaining fish health and sustaining the aquaculture industry. This study adopted a novel risk-based scoring tool, originally developed for terrestrial animals, to benchmark biosecurity levels in seabass and sea bream. The tool considers all relevant aspects of aquaculture biosecurity, subdivided into external and internal biosecurity, with an accompanying questionnaire subjected to expert scoring. Land-based hatcheries and pre-growing facilities exhibited higher preventative measures, achieving overall biosecurity scores of 73.0 % and 75.0 %, respectively. These scores stem from stringent external and internal preventative measures, contrasting with lower overall biosecurity scores in land-based on-growing (50.0 %) and sea-based on-growing (59.0 %) facilities. Maintaining biosecurity during on-growing phases seems demanding due to uncontrolled interactions with the seawater environment and potential water quality issues. This benchmarking tool allows farmers to audit their biosecurity programs and pinpoint areas for improvement. These include vaccination, establishing biosecurity programs and keeping records, given the low scores across farm types. Improving vaccination practices, enhancing record keeping for traceability, and implementing robust biosecurity programs are crucial for improvement. Ultimately, the study emphasizes the need for tailored measures that foster synergy between external and internal components and enhance overall biosecurity in Mediterranean aquaculture.

### 1. Introduction

The focus of aquatic animal production has shifted toward intensive aquaculture due to the increasing depletion of wild fish stocks resulting from overfishing (FAO, 2020). Mediterranean aquaculture practices are

following the growing trend of aquaculture intensification, focussing on two main marine species, namely, the European seabass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). Turkey is the major producer of these species in the Mediterranean, with about 148,000 MT of seabass and 109,000 MT of sea bream, followed by Greece, which

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produces 44,000 MT of seabass and 62,000 MT of sea bream (FishStatJ, 2022).

Improper management of intensive animal production systems can negatively impact animal health and welfare through pathogenic agents (Gelaude et al., 2014; Pandey and Upadhyay, 2022). The viral pathogen of highest economic importance in Mediterranean aquaculture is Viral Encephalopathy and Retinopathy/Viral Nervous Necrosis (VER/VNN), belonging to the genus Betanodavirus (Toffan et al., 2017; Toffan and Panzarin, 2020). The bacterial pathogens and parasitic agents of highest economic importance are *Vibrio anguillarum* and *Photobacterium damselae* subsp. *Piscicida* (Phdp), and gill flukes, respectively (Varvarigos, 2020; Mladineo et al., 2023; Vendramin et al., 2016). These agents reduce the profitability of farming seabass and sea bream in the Mediterranean through decreased growth and production rates (Bandin and Souto, 2020; Blanco et al., 2000; Fernández Sánchez et al., 2022). It is therefore imperative to aim at preventing disease incidences in Mediterranean aquaculture rather than cure them. Biosecurity encompasses all preventive measures against pathogens entering (external biosecurity) and spreading (internal biosecurity) within an aquaculture facility (Rohana et al., 2023; Scarfe and Palić, 2020).

The proactive adoption of biosecurity measures yields positive impacts in food production systems, yet, their implementation is limited in the aquaculture industry. In terrestrial animals, health management has advanced with the adoption of a quantitative risk-based assessment tool (Biocheck.UGent) in livestock and poultry (Damiaans et al., 2020; Dewulf et al., 2018; Gelaude et al., 2014; Laanen et al., 2010). Piloting Biocheck.UGent in these food production systems has guided the evaluation of biosecurity measures for better implementation. To the best of our knowledge, only one study has adopted the Biocheck.UGent tool to quantify biosecurity in aquatic animals, focussing on ornamental fish (Sembapperuma and Padminisiri, 2023). One of the assumed reasons for the limited application of risk-based biosecurity scoring in aquaculture is the absence of an objective quantification system. Developing objective biosecurity quantification systems establishes quantifiable, reproducible, standardized, and systematic methodologies for risk-based biosecurity scoring in a transparent way. Without such systems, assessing and comparing biosecurity levels within and among different aquaculture firms can be biased and hinder effective disease prevention.

Despite the economic significance of pathogens affecting Mediterranean seabass and sea bream production, there is currently no biosecurity quantification system in place. As of 2019, the value of Mediterranean seabass and sea bream was estimated at USD 2.24 billion, underscoring the urgent need for improved biosecurity implementation to secure these economic gains. Using Mediterranean seabass and sea bream as a pilot, this study aims to adopt the Biocheck.UGent scoring system for biosecurity evaluation in food fish. This benchmarking tool can optimize external and internal biosecurity measures at the farm level to ensure targeted actions that offer maximum possible protection in Mediterranean aquaculture. Additionally, the scores of specific biosecurity measures implemented for both external and internal biosecurity can be mapped, and high risk areas for fish disease spread identified for targeted surveillance.

## 2. Materials and methods

The data in this study originate from a survey conducted as part of the H2020 project MedAID, which aims to assess the sustainability of the Mediterranean fish farming sector. The MedAID partner countries participating in the survey are Croatia, Tunisia, France, Egypt, Greece, Italy, Spain, and Turkey. Eighty-eight (88) fish farms were selected from these participating countries. Although the farms were not randomly sampled, they were chosen to represent various production systems across the Mediterranean basin, including hatchery, pre-growing, and on-growing farms.

### 2.1. Questionnaire design

A questionnaire was drafted based on diseases of economic importance in farming Mediterranean seabass and sea bream, their risk factors for introduction, and corresponding biosecurity measures (Toffan et al., 2017; Vendramin et al., 2016). Next, a fish health expert group and aquatic epidemiologists reviewed the questionnaire. Subsequently, the revised questionnaire underwent a pilot testing phase involving fish farmers for further revisions. The final version of the questionnaire consisted of 19 sections with a total of 160 self-explanatory questions. The sections include for instance, information on the general characteristics of the farm, production statistics, production management, health management, disease reporting, diagnostic capacity, and biosecurity measures (Supplementary information).

### 2.2. Questionnaire administration

Given the diversity of participating farms, the questionnaire was translated into the official languages of the participating Mediterranean countries. Each of the participating farms signed a confidentiality agreement to ensure their anonymity. The questionnaire was then administered through face-to-face interviews between the periods of August 2018 and July 2019. The study team, comprising of fish health experts and aquatic epidemiologists, conducted the interviews, which lasted about 1–2 h. When face-to-face interviews were not possible, the interviews were conducted through telephone calls. Each interview took place in the presence of a national fish health expert or aquatic epidemiologist to mediate language barriers and ensure proper data collection.

### 2.3. Biosecurity profiling for Mediterranean seabass and sea bream farms

The expert group consulted the initial questionnaire to select questions (supplementary information) relevant to the two main biosecurity categories of external and internal biosecurity. This resulted in a varied number of questions for external and internal biosecurity in land-based hatcheries, pre-growing and on-growing farms on land, including seabased on-growing farms. Additionally, both the external and internal categories of biosecurity were divided into eight subcategories as follows (Table 1). The subcategories of external biosecurity are “location of the farm”, “facility entry and exit”, “introduction of live fish”, “feed and water supply”, “biovectors”, “personnel and visitors”, “use of divers”, and “equipment and vehicles transport into farm.” The subcategories of internal biosecurity are “vaccination”, “disease management”, “rearing management”, “feed management”, “waste management”, “harvesting”, “equipment, cleaning and disinfection on-site”, and “biosecurity program and record keeping.”

#### 2.3.1. Prioritization of the biosecurity measures

Biosecurity measures are not equally relevant, as disease transmission pathways have different efficiencies (Naug, 2010). It is therefore important to adopt risk-based scoring systems for prioritizing biosecurity measures to make disease prevention effective (Backhans et al., 2015; Laanen et al., 2010). We followed the risk-based scoring methodologies of Gelaude et al. (2014) and Laanen et al. (2010) in consultation with nine experts to weigh biosecurity measures applicable to European Seabass and Sea bream. The expert group has a background in aquaculture, epidemiology, and veterinary medicine to ensure a well-rounded approach in prioritizing and weighing biosecurity measures.

According to the adopted methods, 100 weight points are available for distribution by the experts between the external and internal biosecurity subcategories (Table 1), based on their relevance in disease prevention. Subsequently, biosecurity questions selected by the experts under external and internal biosecurity subcategories were scored with weights ranging from 1 to 10. The weight of 1 to 10 refers to the importance of the biosecurity questions, with 1 indicating lower

**Table 1**  
Average weights/points assigned by the experts to each subcategory.

Subcategories	Subcategory weight			
	Hatcheries (On-land)	Pre-growing (On-land)	On-growing (On-land)	On-growing (Open-sea)
Location of the farm	4.8	5.9	6.6	10.0
Facility entry and exit	6.7	6.5	6.0	–
Introduction of live fish	11.2	11.5	10.0	11.0
Feed and water supply	8.8	10.1	8.4	6.0
Biovectors	4.9	4.2	5.5	6.0
Personnel and visitors	7.9	6.2	6.2	6.0
Use of divers	–	–	–	6.0
Equipment and vehicles transport into farm	8.7	7.6	7.3	7.0
<b>External biosecurity total</b>	<b>53.0</b>	<b>52.0</b>	<b>50.0</b>	<b>52.0</b>
Vaccination	7.1	8.5	5.4	6.0
Disease management	8.5	8.7	8.4	11.0
Rearing management	6.1	6.7	7.3	6.0
Feed management	4.4	4.3	5.1	4.0
Waste management	5.4	5.6	6.0	5.0
Harvesting	–	–	4.1	4.0
Equipment, cleaning, and disinfection on-site	8.5	7.9	7.2	7.0
Biosecurity program and record keeping	7.0	6.3	6.5	5.0
<b>Internal biosecurity total</b>	<b>47.0</b>	<b>48.0</b>	<b>50.0</b>	<b>48.0</b>
<b>TOTAL</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

importance and 10 indicating the highest importance in disease prevention (Table 2). For each question, various answers are possible, with scores of 1 and 0 assigned accordingly. According to Gelaude et al.

**Table 2**  
Weight of questions and scores assigned to answers within the “Facility entry and exit” subcategory of hatcheries.

Question	Question weights	Answer possibilities			
		Best	Score	Worst	Score
1. Do you keep the gate locked when not in use?	5	-Yes	1	-No	0
				-Don't know	0
2. Are there fences, gates and or signs on the facility which prevent people and vehicles from entering without permission?	5	-Yes	1	-No	0
				-Don't know	0
3. Do you control and monitor visitors and vehicles entering and exiting the facility?	6	-Yes	1	-No	0
				-Don't know	0
4. Are there footbaths at all entrances to the facility?	9	-Yes	1	-No	0
				-Don't know	0
5. Are vehicles disinfected on entry to the facility?	9	-Yes	1	-No	0
				-Don't know	0

(2014), 0 means total absence of preventive measure/full presence of the risk while, 1 means full presence of preventive measure/total absence of the risk. For example, a score of 1 is assigned for a ‘Yes’ response, indicating the presence of a biosecurity practice, while both ‘No’ and ‘Don’t Know’ responses receive a score of 0 (Table 2). While the general questionnaire included the nominal response, ‘not applicable,’ it was omitted in Table 2. In certain farms, such as sea-based on-growing facilities, specific measures were not feasible (e.g., question 1 – Do you keep the gate locked when not in use?), and were therefore omitted from the analysis. This also applies to scoring some subcategories; for instance, ‘harvesting’ was omitted from hatcheries and land-based pre-growing farms. Nevertheless, the algorithm considered only relevant questions and subcategories during the analysis, ensuring that a 100 % score remained achievable and that the score is only based on the total number of applicable questions. Moreover, farms engaging in multiple activities are treated as separate entities for each activity. For instance, if a farm operates as both a hatchery and pre-growing facility, the scores are calculated independently for each activity.

2.3.2. Quantification of the biosecurity score

The scores of 0 or 1 as responses to the questions are multiplied by the weight of the specific question to obtain the relative result for each question. Subsequently, the results from all the individual questions within a particular subcategory are summed together and divided by the maximum possible score for that subcategory. Next, the proportional result of the subcategory is multiplied by the weight of the subcategory to obtain the subcategory score (Gelaude et al., 2014: Eq. 1). The final score for internal and external biosecurity is calculated by summing the different subcategory scores (Gelaude et al., 2014: Eq. 2). The total of the different subcategory scores is equivalent to the final score of internal and external biosecurity. The overall biosecurity score is calculated using Eq. 3 (Gelaude et al., 2014). Fig. 1 summarizes the quantification steps applied to both external and internal biosecurity to calculate the overall biosecurity.

$$Subcategory\ score = \frac{\sum_{i=1}^n (question\ score_{(i)} * weight_{question(i)})}{maximum\ score\ of\ subcategory} \tag{1}$$

$$Category\ score = \sum_{j=1}^m (subcategory\ score_{(j)} * weight_{subcategory(j)}) \tag{2}$$

$$Overall\ biosecurity\ score = (External\ biosecurity * weight\ for\ external) + (Internal\ biosecurity * weight\ for\ internal) \tag{3}$$

To exemplify this scoring system in hatcheries, of the 53 points allocated to external biosecurity, the experts assigned a relative weight of 6.7 to the subcategory “Facility entry and exit” (Table 1). As highlighted in Table 2, this subcategory has five questions with optional nominal responses: ‘Yes,’ ‘No,’ and ‘Don’t Know’. If a farmer’s response to the questions were as follows: Question 1: Yes; Question 2: Yes; Question 3: No; Question 4: Yes; Question 5: No, then the score would be calculated as (1\*5) + (1\*5) + (0\*6) + (1\*9) + (0\*9) = 19. That is 19 out of a maximum score of 34, assuming ‘Yes’ responses to all five questions; 19/34 = 0.56 or 56 %. This score is multiplied by the subcategory weight: 0.56\*6.7 = 3.8, to determine the contribution of “Facility entry and exit” to external biosecurity score. The overall biosecurity score can then be calculated by summing the weighted scores of both external and internal categories.

2.4. Data processing and analysis

Responses to the questionnaire were carefully reviewed to avoid unintentionally missing any answers. Complete responses were recorded into Microsoft Excel and assigned a unique identification number for confidentiality. A descriptive analysis was performed to outline the farm

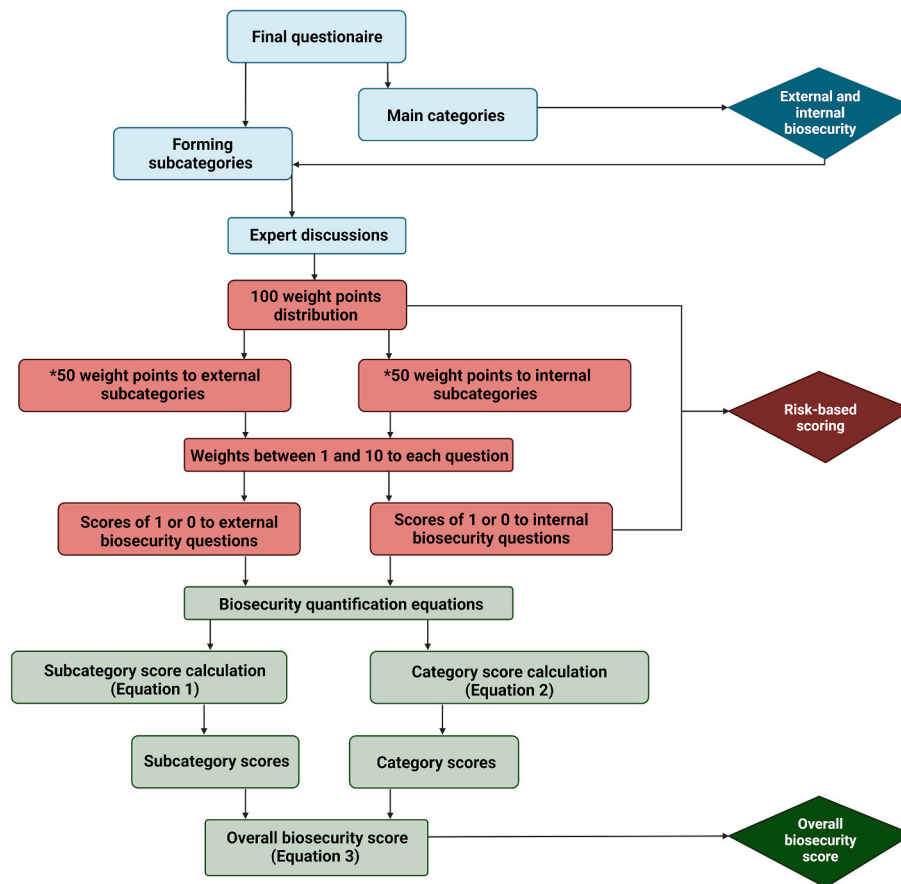


Fig. 1. Flowchart of biosecurity quantification in seabass and sea bream.<sup>1</sup>

<sup>1</sup> \*In theory, the 100-weight points can be distributed 50 each across external and internal subcategories. However, these points may be redistributed unevenly based on prioritization of the various subcategories.

characteristics in terms of production systems/activities and location (Table 3). To assess normality, Shapiro-Wilk tests were conducted on the biosecurity scores across production systems at a significance level of

**Table 3**  
Farm distribution based on production systems/activities and location.

Production system/activity	Land-based	Open seawater	Coastal ponds	Lagoons	Total n (%)
Hatchery	19				19 (21.6 %)
Pre-growing	6				6 (6.8 %)
On-growing	5	44	2	2	53 (60.2 %)
Processing	2				2 (2.3 %)
Multiple activities					
Hatchery, Pre-growing	2				2 (2.3 %)
Hatchery, On-growing				1	1 (1.1 %)
Pre-growing, On-growing	1	2			3 (3.4 %)
On-growing, Packaging	1	1			2 (2.3 %)
<b>Total n (%)</b>	<b>36 (40.9 %)</b>	<b>47 (53.4 %)</b>	<b>2 (2.3 %)</b>	<b>3 (3.4 %)</b>	<b>88 (100 %)</b>

0.05. If the resulting *p*-value exceeded 0.05, indicating no significant deviation from normality, the data were considered approximately normally distributed. Pearson and Spearman correlations were used to check for association between subcategories scores and overall biosecurity scores, as well as between external and internal biosecurity scores. These associations can provide insight into the interrelatedness of biosecurity subcategories and suggest how optimizing certain biosecurity practices could enhance overall external and internal biosecurity. Pearson correlation was used when Shapiro-Wilk test *p*-values exceeded 0.05, while Spearman correlation was applied when the *p*-values were 0.05 or lower. Following Chan (2003) guidelines on the strength of linear relationships, correlation coefficient values of at least 0.8 signify a very strong relationship, 0.6 to 0.8 represent a moderately strong relationship, while 0.3 to 0.5 and < 0.3, indicate fair and poor relationships, respectively.

### 3. Results

#### 3.1. Response characteristics

The eighty-eight (88) farms were distributed in countries as follows; Spain (*n* = 27), Greece (*n* = 13), Turkey (*n* = 13), France (*n* = 11), Italy (*n* = 8), Egypt (*n* = 7), Tunisia (*n* = 6) and Croatia (*n* = 3). Of the 88 farms, 97.0 % (85 farms) were engaged through physical visits while 3.0 % (3 farms) were engaged through telephone calls. The seawater phase encompassed 52 farms located in open seawater (47), coastal ponds (2), and lagoons (3) (Table 3). Collectively, the sea-based farms were

**Table 4**  
Overall biosecurity score of land-based hatcheries.

	Mean (%)	SD	Median (%)	Min (%)	Max (%)
<b>External biosecurity</b>					
Location of the farm	57.0	24.2	58.3	29.1	100
Facility entry and exit	82.5	15.3	73.5	47.0	100
Introduction of live fish	63.7	25.9	77.2	17.7	100
Feed and water supply	67.5	10.9	69.1	40.2	82.3
Biovectors	68.1	18.6	69.8	38.7	91.3
Personnel and visitors	82.6	10.7	83.7	66.2	100
Equipment and vehicles transport into farm	76.8	13.8	75.9	56.1	100
<b>Subtotal of external biosecurity</b>	<b>71.5</b>	<b>8.2</b>	<b>73.2</b>	<b>54.2</b>	<b>88.5</b>
<b>Internal biosecurity</b>					
Vaccination	71.4	44.9	100	0	100
Disease management	69.3	11.7	68.8	39.4	87.2
Rearing management	76.0	11.7	69.7	55.8	100
Feed management	90.4	7.9	91.0	82.1	100
Waste management	69.3	17.2	78.3	32.4	91.8
Equipment, cleaning, and disinfection on-site	79.9	10.6	79.2	60.4	100
Biosecurity program and record keeping	85.0	30.8	100	0	100
<b>Subtotal of internal biosecurity</b>	<b>74.7</b>	<b>9.7</b>	<b>78.4</b>	<b>59.0</b>	<b>88.7</b>
<b>Overall biosecurity</b>	<b>73.0</b>	<b>7.0</b>	<b>73.0</b>	<b>58.0</b>	<b>83.0</b>

dominant (59.1 %) followed by 36 land-based farms at 40.9 %. Some farms carry out multiple production activities, with the main activities being on-growing (53 farms, 60.2 %) and hatchery (19 farms, 21.6 %). In terms of spatial arrangement, 73 % (64 farms) have at least one facility located within a 10 km radius, which typically includes pre-growing, on-growing, or processing facilities. In contrast, 27 % (24 farms) do not have any facilities within the 10 km radius. Concerning environmental exposure, 56 % (49 farms) operate outdoors without any protective cover. Most of these farms were on-growing facilities located in open seawater, coastal ponds, and lagoons.

### 3.2. Biosecurity score evaluation based on farm types and location

The 19 land-based hatcheries accrued an overall biosecurity score of 73.0 % (median 73.0 %), as shown in Table 4. The external biosecurity subtotal score was 71.5 % (median 73.2 %), while the internal biosecurity subtotal score was 74.7 % (median 78.4 %). Considerable variations were observed in the average scores within and between subcategories. For external biosecurity, the subcategory “Location of the farm” had the lowest score of 57.0, while “Personnel and visitors” had the highest score at 82.6. In internal biosecurity, the subcategory “Feed management” recorded the highest score at 90.4, whereas both “Disease management” and “Waste management” recorded the lowest score at 69.3 (Table 4).

The 6 pre-growing land-based farms had an overall biosecurity score of 75.0 % (median 74.0 %) (Table 5). The subtotal score for external category was 69.2 % (median 68.5 %), while the subtotal score for internal biosecurity was 80.4 % (median 80.6 %). Some of the average subcategory scores differ substantially. In the external biosecurity, the subcategory “Introduction of live fish” had the lowest score at 43.2, and the subcategory with the highest score was “Feed and water supply” at 89.4. For internal biosecurity, the subcategory “Vaccination” recorded the highest score of 100, whereas the subcategory “Waste management” had the lowest score at 68.2 (Table 5).

The overall biosecurity score for the 5 land-based on-growing farms was 50.0 % (median 49.0 %) (Table 6). The farms had a subtotal score of 51.5 % (median 48.7 %) for external biosecurity and 48.9 % (median 49.3 %) for internal biosecurity. When evaluating the average subcategory scores, the lowest score in external biosecurity was “Location of the farm” at 24.2, while “Equipment and vehicles transport into farm” had

**Table 5**  
Overall biosecurity score of land-based pre-growing farms.

	Mean (%)	SD	Median (%)	Min (%)	Max (%)
<b>External biosecurity</b>					
Location of the farm	43.5	19.8	46.1	23.0	76.9
Facility entry and exit	84.3	19.3	90.6	53.1	100
Introduction of live fish	43.2	7.6	41.8	31.0	52.7
Feed and water supply	89.4	7.1	92.6	80.4	95.1
Biovectors	63.2	11.1	62.5	46.8	81.2
Personnel and visitors	86.7	8.1	86.8	76.3	100
Equipment and vehicles transport into farm	77.4	6.2	76.7	67.1	86.3
<b>Subtotal of external biosecurity</b>	<b>69.2</b>	<b>5.0</b>	<b>68.5</b>	<b>62.5</b>	<b>75.9</b>
<b>Internal biosecurity</b>					
Vaccination	100	0	100	100	100
Disease management	72.4	9.0	71.4	59.2	87.1
Rearing management	68.8	10.8	69.2	51.2	84.6
Feed management	85.0	14.3	85.8	66.6	100
Waste management	68.2	8.6	65.1	60.5	78.9
Equipment, cleaning, and disinfection on-site	75.3	16.2	71.3	59.1	100
Biosecurity program and record keeping	94.1	9.1	100	81.9	100
<b>Subtotal of internal biosecurity</b>	<b>80.4</b>	<b>7.0</b>	<b>80.6</b>	<b>71.9</b>	<b>90.8</b>
<b>Overall biosecurity</b>	<b>75.0</b>	<b>4.0</b>	<b>74.0</b>	<b>70.0</b>	<b>80.0</b>

**Table 6**  
Overall biosecurity score of land-based on-growing farms.

	Mean (%)	SD	Median (%)	Min (%)	Max (%)
<b>External biosecurity</b>					
Location of the farm	24.2	43.3	0	0	100
Facility entry and exit	48.3	30.7	45.1	19.3	100
Introduction of live fish	36.8	11.4	32.8	22.8	52.8
Feed and water supply	71.3	17.0	83.7	51.3	83.7
Biovectors	38.0	18.9	30.8	25.5	68.3
Personnel and visitors	55.2	18.9	57.7	30.9	78.8
Equipment and vehicles transport into farm	82.3	8.3	80.8	70.5	91.1
<b>Subtotal of external biosecurity</b>	<b>51.5</b>	<b>13.2</b>	<b>48.7</b>	<b>35.2</b>	<b>70.6</b>
<b>Internal biosecurity</b>					
Vaccination	20.0	44.7	0	0	100
Disease management	61.4	12.7	55.2	49.8	81.9
Rearing management	68.3	18.1	69.4	47.2	86.1
Feed management	76.4	18.9	79.3	44.8	95.8
Waste management	45.2	15.5	36.8	28.9	63.1
Harvesting	79.5	14.9	81.4	58.1	100
Equipment, cleaning, and disinfection on-site	48.1	14.1	47.4	34.0	70.7
Biosecurity program and record keeping	26.9	41.2	14.2	0	100
<b>Subtotal of internal biosecurity</b>	<b>48.9</b>	<b>5.9</b>	<b>49.3</b>	<b>41.2</b>	<b>57.5</b>
<b>Overall biosecurity</b>	<b>50.0</b>	<b>8.0</b>	<b>49.0</b>	<b>38.0</b>	<b>59.0</b>

the highest score at 82.3. For internal biosecurity, the subcategory “Vaccination” recorded the lowest score at 20.0, while “Harvesting” achieved the highest score at 79.5 (Table 6).

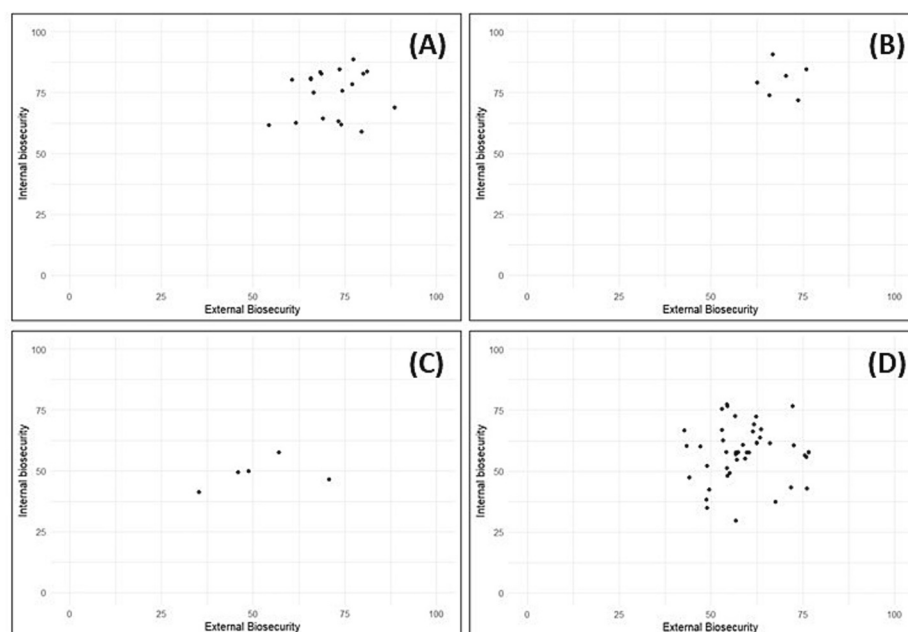
In total, the overall biosecurity score for all 52 farms operating during seawater phase was 59.0 % (median 59.0 %). The subtotal scores were 59.0 % (median 57.0 %) for external biosecurity and 58.4 % (median 57.7 %) for internal biosecurity (Table 7). Assessing the average subcategory scores in external biosecurity revealed the lowest score of 21.5 for “Location of the farm,” while “Feed supply” had the highest score of 94.1. For internal biosecurity, the subcategory “Vaccination” recorded the lowest score of 43.1, in comparison to “Feed management,” which had the highest score of 87.0 (Table 7).

**Table 7**  
Overall biosecurity score of sea-based on-growing farms.

	Mean (%)	SD	Median (%)	Min (%)	Max (%)
<b>External biosecurity</b>					
Location of the farm	21.5	41.5	0	0	100
Introduction of live fish	58.9	11.6	58.9	26.7	83.8
Feed supply	94.1	16.2	100	30.0	100
Biovectors	57.0	16.9	55.0	20.0	95.0
Personnel and visitors	72.0	17.7	70.5	20.5	100
Use of divers	67.8	19.4	72.8	10.8	100
Equipment and vehicles transport into farm	71.0	11.9	71.6	45.2	95.2
<b>Subtotal of external biosecurity</b>	<b>59.0</b>	<b>9.2</b>	<b>57.0</b>	<b>42.7</b>	<b>76.5</b>
<b>Internal biosecurity</b>					
Vaccination	43.1	50.0	0	0	100
Disease management	65.1	15.9	67.7	25.1	89.8
Rearing management	69.3	17.4	67.3	34.7	100
Feed management	87.0	14.7	95.7	35.7	100
Waste management	53.2	14.2	54.4	13.2	85.2
Harvesting	69.3	12.3	62.9	44.4	94.8
Equipment, cleaning, and disinfection on-site	69.3	18.2	78.3	30.0	91.2
Biosecurity program and record keeping	72.6	38.6	100	0	100
<b>Subtotal of internal biosecurity</b>	<b>58.4</b>	<b>11.1</b>	<b>57.7</b>	<b>29.7</b>	<b>77.4</b>
<b>Overall biosecurity</b>	<b>59.0</b>	<b>7.0</b>	<b>59.0</b>	<b>42.0</b>	<b>74.0</b>

### 3.3. Correlation between external and internal biosecurity

Fig. 2 illustrates the distribution of external and internal biosecurity scores across all farm types based on location. In land-based hatcheries, Pearson's correlation showed a poor positive correlation (0.126) between external and internal biosecurity (Fig. 2A). Conversely, there was a poor negative correlation between external and internal biosecurity in both land-based pre-growing (Fig. 2B:  $-0.018$ ) and sea-based on-growing (Fig. 2D:  $-0.017$ ) facilities. In land-based on-growing facilities, a fair positive correlation (0.38) was observed between external and internal biosecurity (Fig. 2C).



**Fig. 2.** Scatterplot of internal and external biosecurity scores. Hatcheries located on land (Fig. 2A). Pre-growing farms located on land (Fig. 2B). On-growing farms located on land (Fig. 2C). On-growing farms located on open seawater (Fig. 2D).

## 4. Discussions

In contrast to questionnaire-based surveys, adopting a risk-based scoring system for terrestrial animals has enabled biosecurity quantification in Mediterranean seabass and sea bream. This tool allows for benchmarking and distinguishing between high and low biosecurity levels in these fish species, segregated by location and production activities.

Risk-based scoring offers several advantages, such as providing reproducible, quantifiable, consistent, and systematic methods for evaluating and benchmarking biosecurity measures. Consequently, the scoring outcomes can inform specific areas for improvement and facilitate targeted interventions in the overall biosecurity plan. However, there are limitations, including personal biases from self-assessment of biosecurity practices and subjectivity arising from differences in respondents' knowledge of adequate biosecurity measures. This highlights the importance of expert consultations, as adopted in the study to support individual opinions with scientifically informed risk estimates. Moreover, while the fundamental principles of many biosecurity plans are generally the same, their implementation varies depending on the fish cultured, their life cycle, relevant pathogens, and the type of farming system (Ozby et al., 2014). Therefore, producers must tailor their biosecurity measures to reduce the economic burden of diseases. Both producers and regulatory bodies can benefit from this benchmarking tool to identify and address weaknesses in biosecurity practices. Regulatory bodies in particular can use the scoring outcomes to set industry standards and monitor biosecurity compliance. To execute this effectively, regulatory authorities must combine visual inspections with the recording of producers' responses. In this study, confirmatory physical assessments conducted for 97.0 % of the farmers reduce the likelihood of inaccurately reporting biosecurity practices. However, the remaining 3.0 % lacking physical presence may lead to overestimation and inaccurate reporting of biosecurity practices. Therefore, regulatory authorities should not rely solely on subjective responses, as this can give the semblance of biosecurity compliance that may not be entirely accurate.

From the results, land-based hatcheries and pre-growing farms had the highest scores for both external and internal biosecurity. The higher internal biosecurity in these facilities can be attributed to indispensable

biosecurity measures and husbandry practices implemented in preparation for the upcoming seawater phase. In hatcheries, production must take place in a controlled environment with optimal nutrition to ensure good health and welfare in juvenile fish. This is reflected in certain subcategory scores that contribute to the overall higher internal biosecurity scores in hatcheries. These subcategories include feed and rearing management, having a biosecurity program and record keeping, and maintaining equipment and site cleanliness through cleaning and disinfection (Table 4). In seabass and sea bream, VER/VNN and Phdp are vertically transmissible through infected eggs and gonadal tissues, and horizontally through contaminated water, fish or feed (Borrego et al., 2017; Breuil et al., 2002; Castri et al., 2001). Vaccination as a preventative measure is essential in brooders before spawning and in juvenile fish before the on-growing seawater phase (Buonocore et al., 2019; Hanif et al., 2005). This is reflected in the higher scores obtained for vaccination as an internal biosecurity measure in both land-based hatcheries (Table 4) and land-based pre-growing farms (Table 5). It may be that vaccination is prioritized during the freshwater phase, considering the low scores recorded in both land-based on-growing (Table 6) and sea-based on-growing (Table 7) facilities. Booster vaccines are sometimes administered during the on-growing stages, but this is not common in open-sea production, unless a very specific pathogen, e.g., *Aeromonas*, affects large seabass. Nevertheless, pre-growing and on-growing facilities that rely on juveniles for fattening must endeavour to purchase vaccinated fish. Notably, there are considerable disparities in vaccination scores. The minimum and maximum scores for vaccination were 0 and 100, respectively, across land-based hatcheries and all on-growing facilities on land and sea. These findings imply that there is scope for improvement in vaccination practices, as some farms are not vaccinating their fish. Additionally, land-based pre-growing farms have placed emphasis on feed management, incorporating it into their internal measures alongside having a strong biosecurity program and keeping records (Table 5). Unlike the freshwater phase in hatcheries and pre-growing facilities, the on-growing facilities on land and sea recorded the lowest scores for external and internal biosecurity. For sea-based on-growing, the lower internal and external biosecurity scores (Table 7) can be attributed to the open seawater environment, which presents challenges to effective biosecurity control. Similar to vaccination, there was a high disparity in the scores for biosecurity program and record keeping, highlighting areas for improvement. Apart from land-based pre-growing facilities, minimum and maximum scores of 0 and 100, respectively, were assigned to the other facilities for biosecurity program and record keeping. This means some farms are not following any biosecurity program nor are they keeping records of management practices. These observations can be attributed to differences in the traditional and legal basis of disease control, diagnostic capacities, and financial resources among the participating countries.

Implementing external biosecurity in sea-based farms may be difficult due to uncontrolled interactions with biovectors such as wild fish and birds, as well as the inability to manage water quality in open-sea cages (Holmer, 2009; Melotti et al., 1995; Melotti et al., 1994). However, feed management and supply received the highest score among both internal and external biosecurity in sea-based on-growing farms (Table 7). Automated feeding systems are commonly used during the on-growing seawater phase of seabass and sea bream. These systems limit human intervention, reducing the chances of horizontal transmission via contaminated feed, as the feeders are programmed to deliver precise amounts of feed at scheduled intervals. Apart from feed handling, sea-based on-growing farms exert strong control over certain external biosecurity measures, such as personnel and visitor oversight, as well as managing equipment and vehicle transport (Table 7). Effective management of wellboats is also crucial for controlling vehicular traffic, as their shared use across different sea-based sites can pose significant disease transmission risks. Similarly, the use of divers across multiple farms presents comparable biosecurity challenges, potentially facilitating the spread of pathogens if stringent precautions are not enforced.

Thus, the sea-based on-growing farms in this study appear to prioritize the management of divers, as reflected in the relatively high score among external measures (Table 7). Managing external biosecurity in land-based facilities appears to be much easier compared to sea-based farms. This is because maintaining hygiene and monitoring fish health status are within the direct control of land-based facilities. Among the subcategories of external biosecurity in land-based hatcheries, the highest priority was given to controlling personnel and visitors, followed by regulating entry and exit into the facility (Table 4). Consequently, the same subcategories were prioritized in land-based pre-growing farms but did not surpass feed management and water supply (Table 5). In land-based on-growing farms, equipment and vehicle transport into the farm ranked highest among external biosecurity measures. This can be attributed to heightened precautionary measures, as these facilities receive juvenile seabass and sea bream from external companies such as hatcheries. Land-based pre-growing facilities are typically the receiving end for seabass and sea bream eggs (EFSA, 2008), emphasising the seemingly high scores obtained for maintaining equipment and vehicle cleanliness (Table 5). However, biosecurity measures applied during the introduction of live fish into land-based pre-growing facilities received the lowest scores among external subcategories. It is recommended that the health status of newly introduced fish is monitored during quarantine until they are deemed fit for transfer to pre-growing units (EFSA, 2008). For eggs, it is recommended to clean them in sterilized water followed by disinfection in iodine (VESO, 2005).

Most participating farms obtained the lowest score for the location of the farm within the subcategory of external biosecurity. This is particularly true for land-based facilities and sea-based on-growing farms. Similarly, 73.0 % (64) of the farms have at least one facility located within a 10 km radius, while 56.0 % (49) are exposed to the environment. According to Salama and Murray (2011), there is a higher risk of disease occurrence arising from inappropriate spatial arrangement and site selection in aquaculture. Furthermore, waste management obtained the lowest overall score among the subcategories of internal biosecurity, especially in land-based hatcheries and pre-growing farms. This indicates that these farms are at a higher risk if measures for wastewater management, including the removal and containment of dead fish and other waste are not consistently followed. Therefore, the timely removal of fish cadavers and other organic wastes is imperative to prevent the spread of diseases (Zornu et al., 2023). The results of the correlation analysis indicate a poor correlation across all types of production systems. It is important to note that a high correlation between external and internal biosecurity measures is neither an objective nor a criterion for evaluating effective biosecurity. In cases where both external and internal biosecurity scores are consistently low, a high correlation may still exist, indicating inadequate practices in both areas. For example, a fish farming operation might implement strict external biosecurity measures, such as controlling facility access, installing sanitation footbaths, and conducting regular inspections of incoming shipments. Conversely, if internal biosecurity measures such as rigorous fish handling, feed management, and hygiene protocols are not equally robust, the overall biosecurity may still be compromised. Therefore, the primary goal is strong adherence to both external and internal biosecurity measures for effective biosecurity. Correlation analysis is a useful tool for understanding the relationship between internal and external measures. By examining these relationships, we can develop strategies that promote comprehensive biosecurity practices. We may find that farms with high compliance in external biosecurity also tend to have better internal practices, such as regular staff training on biosecurity protocols. However, if we discover that farms with strong external measures do not prioritize internal protocols, we can develop targeted strategies to improve internal practices. Establishing clear guidelines that link external measures with internal protocols will help ensure cohesive biosecurity efforts.

A limitation of this study is the small sample sizes for land-based pre-growing ( $n = 6$ ) and on-growing ( $n = 5$ ) facilities, which may lead to

high variability and increased susceptibility to outliers, potentially affecting the strength of observed correlations. Future studies could address this limitation by ensuring proportional sample sizes across all farm types, enabling more reliable statistical interpretations. Additionally, enhancing biosecurity effectiveness may involve investigating the relationship between biosecurity scores and both disease incidence and prevalence. Correlating biosecurity measures with disease data would provide insights into the effectiveness of preventative measures, especially when higher biosecurity scores align with lower disease incidence. Such data could inform targeted interventions to improve biosecurity where it matters most, ultimately helping to reduce the disease burden in aquaculture.

## 5. Conclusions

For the first time in food fish, this study benchmarks biosecurity levels in Mediterranean seabass and sea bream using risk-based scoring. Land-based hatcheries and pre-growing farms exhibit higher overall biosecurity due to stringent external and internal biosecurity measures. However, the on-growing phases present challenges in maintaining biosecurity due to the limited control over seawater environments. Ensuring external biosecurity is particularly challenging in sea-based farms due to uncontrolled interactions with the external environment and potential water quality issues. This benchmarking tool allows farmers to audit their biosecurity programs and pinpoint areas for improvement. These include vaccination, establishing biosecurity programs and keeping records, given the low scores and high disparities across farm types. Improving vaccination practices, enhancing record keeping for traceability, and implementing robust biosecurity programs are crucial steps for improvement. Ultimately, the study emphasizes the need for tailored measures that foster synergy between external and internal components and enhance overall biosecurity in Mediterranean aquaculture.

## CRedit authorship contribution statement

**Margarida Gomes Leandro:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Jacob Zornu:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Alain Le Breton:** Writing – review & editing, Validation, Methodology, Investigation. **Nadia Chérif:** Writing – review & editing, Validation, Methodology, Investigation. **Bernardo Basurco:** Writing – review & editing, Validation, Methodology, Investigation. **Dolores Furones:** Writing – review & editing, Validation, Methodology, Investigation. **Ana Muniesa:** Writing – review & editing, Validation, Methodology, Investigation. **Anna Toffan:** Writing – review & editing, Validation, Methodology, Investigation. **Manuela Dalla Pozza:** Writing – review & editing, Validation, Methodology, Investigation. **Eleonora Franzago:** Writing – review & editing, Validation, Methodology, Investigation. **Snježana Zrncić:** Writing – review & editing, Validation, Methodology, Investigation. **Panos Varvarigos:** Writing – review & editing, Validation, Methodology, Investigation. **Hosam Saleh:** Writing – review & editing, Validation, Methodology, Investigation. **Hasmat Cagirgan:** Writing – review & editing, Validation, Methodology, Investigation. **Mona Dverdal Jansen:** Writing – review & editing, Methodology. **Edgar Brun:** Writing – review & editing, Methodology. **Saraya Tavornpanich:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization.

## Declaration of competing interest

The authors declare no conflicts of interests, and the sponsors did not influence the perspectives shared in this article.

## Data availability

The authors do not have permission to share data.

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## Appendix A. Supplementary data

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