



# Expert knowledge-based system for risk assessment of the occurrence of *Amyloodinium ocellatum* in semi-intensive fish farms

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## Abstract

The implementation of a system to assess the risk of *Amyloodinium ocellatum* occurrence in rearing ponds in fish farms located in southern Spain is a fundamental aspect to ensure the economic viability of these facilities. For this purpose, a computer program (called Amy) for Windows PCs and an application for mobile devices (AmyAPP), based on the Android operating system, were developed integrating transformation functions and weightings associated with environmental parameters and fish behavioural factors from which it is possible to estimate the level of risk of occurrence of *A. ocellatum*. The weights for each of the environmental parameters and behavioural factors were estimated from the responses of a panel of experts (the fish farmers) using a Delphi methodology. The results indicate that, under operational validation, Amy/AmyAPP responses were statistically sensitive to the occurrence of *A. ocellatum* outbreaks in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) rearing ponds.

**Keywords** Sea velvet · Ectoparasite outbreak · Delphi methodology · Computer program · Mobile device

## Introduction

One of the main aspects that conditions the economic viability of any aquaculture facility is the impact caused by the occurrence and prevalence of different pathogens (Johnson et al. 2004; Bondad-Reantaso et al. 2005; Hernández-Llamas et al. 2013, 2014; Masson et al. 2013; Føre et al. 2018; Lieke et al. 2020; Wang et al. 2021; Rai et al. 2023). In the case of

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the land-based semi-intensive fish farms located in the southern of the Iberian Peninsula, the main economic losses have been caused by the incidence of sea velvet (*Amyloodinium ocellatum*) which is a euryhaline-thermophilic dinoflagellated ectoparasite that causes a rapid infection, initially asymptomatic, that usually can lead to mortality rates of 50–80% and 100% in its most severe epidemiological manifestation (Kuperman and Matey 1999; Saraiva et al. 2011; Moreira et al. 2018, 2019; Massimo et al. 2022).

In the Iberian Peninsula, the first cases were reported in 1994 on sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) reared in the Óbidos coastal lagoon and estuary of river Sado (Portugal) (Menezes 2000; Soares et al. 2011) and, since then, the parasite has spread to southern Portugal and Spain, appearing periodically in farmed ponds causing significant mortalities. In both countries, the effects of sea velvet have been very severe, to the extent that in recent years some fish farms lost all their production and were forced to close down. Therefore, it is essential for the managers of these farms to have effective procedures in place to estimate the occurrence of the parasite in advance, in order to apply control and prophylaxis measures to minimise the mortality rate.

In this sense, the fish farmers apply two basic procedures for early detection of one of the three stages (parasitic trophont, reproductive tomtont and infective dinospores) that comprise the life cycle of *A. ocellatum*. First, they can carry out a microscopic examination of water pond samples in order to detect free dinospores in the water column. This method has the advantage that it is cheap but it is operationally impractical as the concentration of dinospores in water is normally very low even during epidemic events. Second, they complement the possible detection of free dinospores with the observation of the infective trophont stage in some fishes. However, the presence of this stage is an indication that the infection could be very advanced and therefore it is usually too late to apply a treatment.

On the other hand, fish farmers are aware of the importance that environmental conditions and some water quality parameters in previous time periods have on the explosive emergence of the parasite. Although they have an intuition of how these parameters could favour the appearance of an outbreak, they do not know the cause-effect relationships that allow the parasite to proliferate in a culture tank. Knowledge of the role of environmental patterns and the weight of water quality parameters could facilitate the development of monitoring programs to establish thresholds for parasite prevention, control and prophylaxis (Snieszko 1974; Eissa et al. 2022). The main problem with the data from expert knowledge is that it is hardly quantifiable and extractable.

A way to elicit information from experts in a specific field is to use an iterative process called the Delphi method (Dalkey and Helmer 1963). This is a method that structures a communication process of a group of experts to solve a complex problem. Its predictive capacity is based on the systematic use of an intuitive judgement made by this group of experts (fish farmers) who share information and perceptions (Linstone and Turoff 1975). The Delphi method has been successfully applied in some recent studies (Hai et al. 2015; Marvin et al. 2020; Van den Burg et al. 2022).

The main goal of this work was the design and development of a friendly-low-cost procedure based on expert knowledge to assess the risk of *Amyloodinium ocellatum* occurrence in rearing ponds in fish farms. For that, two specific objectives were established: (i) to determine the weight of the main environmental parameters and of the fish behaviour in the ponds by means the design of a questionnaire, specifically addressed to those responsible for the pathological control of fish farms, which collected and managed their responses by applying the Delphi method; and (ii) the implementation of this information in a computer application and a mobile APP that provides a rapid response on the occurrence risk of an *A. ocellatum* outbreak in the culture ponds.

## Materials and methods

### Study area

This study was aimed to the main semi-intensive aquaculture fish farms scattered in Huelva, Sevilla and Cádiz provinces (western of Andalusian region, bordering with Portugal) (Fig. 1) which are focused on rearing of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). In these locations, the aquaculture in traditional ponds, namely 'improved extensive farming' supposed a significant production which has made an important contribution to the regional economy in last decades (Gutiérrez-Estrada et al. 2012, 2022). These fish farms are characterised by a fish rearing in land trapezoidal ponds with a very variable size (between 250 and 25,000 m<sup>2</sup>) and depths not exceeding 3 m.

### Questionnaire and Delphi method

We designed and developed a questionnaire whereby to elicit the expert opinions of fish farmers in relation to the weight that several environmental and fish behavioural factors have on the occurrence risk of *A. ocellatum* in their aquaculture facilities. This way, the objective of this questionnaire was to characterise how fish farmers qualitatively relate different environmental and behavioural events to the presence of the parasite. These fuzzy associations are constructed on the basis of the fish farmer's experience in such a way that a series of associative rules are established to provide a belief level (very low, low, normal, high, very high) about the occurrence of an outbreak.

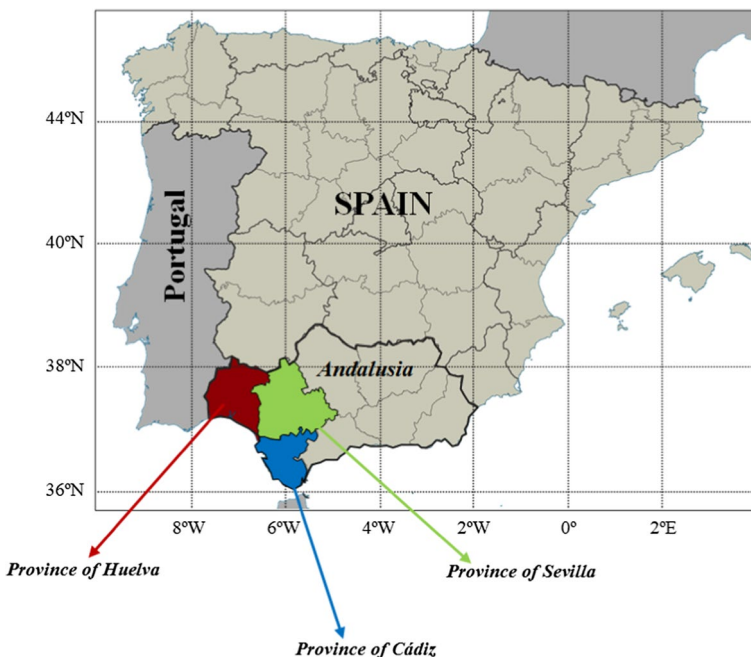


Fig. 1 Study area

A battery of questions were initially selected based on an exhaustive review of *Amyloodinium ocellatum* literature in the main national (Spanish) and international databases (ISI Web of Science; CAB Abstract; CABI Compendium; Indices CSIC; SCIELO; Scopus) which were subsequently screened and revised by an independent aquaculture auditor. The screening process provided a total of sixteen questions grouped in three types: (a) type A=questions related with operational aspects of the fish farms (Table 1); (b) type B=questions associated with a qualitative value (very low, low, normal, high, very high) and related with the weight (importance) that several environmental parameters have on the occurrence of *A. ocellatum* in the ponds (Table 2); and (c) type C=questions associated with a qualitative value (very low, low, normal, high, very high) and related with the weight (importance) that several fish behaviour factors that could confirm the presence of *A. ocellatum* in the ponds (Table 3).

The questionnaire was processed following the classical Delphi method described by Linstone and Turoff (1975) but making use of electronic communication (<https://www.onlineencuesta.com>). This approach requires that an administrator send the questionnaire to the expert panel together with a document containing precise instructions to facilitate the fish farmer's response (sending phase). Anonymity and non-communication between the members of the expert panel must be guaranteed at all times. In this way, more experienced experts are prevented from influencing the response of less experienced ones. A total of 10 fish farms participated in this study.

Once the administrator receives the responses from the fish farmers (response phase), the administrator prepares a report that presents the results on questions type B and C in an aggregated form (report phase). For that, median and percentiles (25th and 75th) for the responses to each question were calculated. The first report is sent back to the expert panel asking them to answer the questions again (re-sending phase). The fish farmer can change the initial answer to each question (depending on the aggregated data provided by the administrator) or keep the initial answer. When the administrator receives the new set of responses, the administrator compares the results with those obtained in the initial submission. If the administrator finds that overall there are significant statistical differences between the responses from the first and second mailing, the administrator proceeds to send a third round of surveys to the panel. This process (sending → response → report → re-sending) is repeated until that the administrator finds no significant statistical differences between two consecutive response sets. This will mean that the expert panel will have reached a state of consensus and therefore the process will be concluded.

To determine if panel reach consensus on a determined factor, the mean score for each question between two consecutive response sets was compared using a Kruskal–Wallis test (Siegel and Castellan 1988). Likewise, Kendall's correlation (*Tau*) was calculated for each question type B and C which allowed to test if a question was answered in the same way between two consecutive rounds (Siegel and Castellan 1988; Schmidt 1997).

## Weighting factors and transformation functions

Once the group of experts had reached a state of consensus, each of the environmental parameters (EP) and fish behavioural factors (FB) was weighted (Tables 4 and 5). For that, a total of 100 risk points were distributed between EP and FB in function of averaged values obtained from the expert responses. For a specific factor *i*, the risk weighted value (WRisk<sub>*i*</sub>) can be calculated as:

**Table 1** Questions type A related with operational aspects of the fish farms and possible associated responses

Questions type A	Responses	Sea bream ( <i>Sparus aurata</i> )	Sea bass ( <i>Dicentrarchus labrax</i> )	Other
1. What species are reared in your fishfarm?	Sea bream ( <i>Sparus aurata</i> )			
2. What is the rearing size/age?	Free text			
3. What is your level of experience regarding the occurrence of <i>A. ocellatum</i> outbreaks and their treatment in your facility?	VL (1)	L (2)	N (3)	H (4) VH (5)
4. Has your fish farm had several outbreaks (in previous years) of <i>A. ocellatum</i> ?	No (0)	Yes (1)		
5. What is the rearing density when the <i>A. ocellatum</i> outbreak is detected?	VL (1) < 0.2 kg/m <sup>2</sup>	L (2)	N (3)	H (4) VH (5) > 5 kg/m <sup>2</sup>
6. What is the rearing density of neighbouring fish farms when an outbreak of <i>A. ocellatum</i> is detected in your fishfarm?	VL (1) < 0.2 kg/m <sup>2</sup>	L (2)	N (3)	H (4) VH (5) > 5 kg/m <sup>2</sup>  There is NOT I don't know
7. How often is the water in the pond completely renewed?	VL (1) Once every two weeks	L (2)	N (3)	H (4) VH (5) More than once a day
8. When mortality is detected in the ponds, are dead specimens removed?	No, never (0)	Yes, some times (1)	Yes, always (2)	
9. Do you routinely carry out pond bottom cleaning of your fish farm?	No (0)	Yes (1)		

\* As a guideline, upper and lower limits were provided

Some answers have associated a categorical value (in brackets). VL, very low; L, low; N, normal; H, high; VH, very high

**Table 2** Questions type B related with environmental parameters and possible associated responses

Questions type B	Responses
10. What do you think is the weight (importance) of each of the following variables in the occurrence of <i>A. ocellatum</i> ?	
10.1 Water temperature	VL (1) L (2) N (3) H (4) VH (5)
10.2 Dissolved oxygen	VL (1) L (2) N (3) H (4) VH (5)
10.3 Salinity	VL (1) L (2) N (3) H (4) VH (5)
10.4 Turbidity	VL (1) L (2) N (3) H (4) VH (5)
10.5 Tidal coefficient	VL (1) L (2) N (3) H (4) VH (5)
11. In relation to water temperature, what temperature level do you think triggers an outbreak of <i>A. ocellatum</i> ?	VL (1) < 15 °C L (2) N (3) H (4) VH (5) > 30 °C Free text**
12. In relation to dissolved oxygen, what dissolve oxygen level do you think triggers an outbreak of <i>A. ocellatum</i> ?	VL (1) < 2 mg/l L (2) N (3) H (4) VH (5) > 6 mg/l Free text**
13. In relation to turbidity, what turbidity level do you think triggers an outbreak of <i>A. ocellatum</i> ?	VL (1) L (2) N (3) H (4) VH (5) Free text** Secchi or others
14. In relation to salinity, what salinity level do you think triggers an outbreak of <i>A. ocellatum</i> ?	VL (1) L (2) N (3) H (4) VH (5) Free text** PSU, g/l, or others
15. In relation to tidal, what tidal level do you think triggers an outbreak of <i>A. ocellatum</i> ?	VL (1) < 0.4 L (2) N (3) H (4) VH (5) > 1 Free text**

\* As a guideline, upper and lower limits were provided

\*\*\*For these questions, fish farmers were able to provide additional information on quantitative measurements of the associated parameter. The answers have associated a categorical value (in brackets). VL, very low; L, low; N, normal; H, high; VH, very high

**Table 3** Questions type C related with fish behaviour factors and possible associated responses

Questions type C	Responses				
<i>16. In relation to symptoms, what weight (importance) would you give to the presence of specimens near the water intake, the decrease in feeding level, the presence of dark coloured specimens with slow swimming and the presence of parasites on the gills?</i>					
<i>16.1 Specimens near the water intake</i>	VL (1)	L (2)	N (3)	H (4)	VH (5)
<i>16.2 Decrease in feeding</i>	VL (1)	L (2)	N (3)	H (4)	VH (5)
<i>16.3 Presence of dark coloured specimens</i>	VL (1)	L (2)	N (3)	H (4)	VH (5)
<i>16.4. Specimens with slow swimming</i>	VL (1)	L (2)	N (3)	H (4)	VH (5)
<i>16.5 Presence of parasites on the gills</i>	VL (1)	L (2)	N (3)	H (4)	VH (5)

The answers have associated a categorical value (in brackets). VL, very low; L, low; N, normal; H, high; VH, very high

**Table 4** Weighting and transformation functions of environmental parameters (EPs)

Environmental parameters (EP)	Weighting (WRisk <sub>i</sub> )	Units	Transformation function
Water temperature	10.56	°C	$f(x) = a + b_1x + b_2x^2 + b_3x^3$ $a = 2.0230; b_1 = -0.3863;$ $b_2 = 0.022233; b_3 = -0.000350$ $\min_x = 14^\circ\text{C}; \max_x = 39^\circ\text{C}$
Dissolved oxygen	9.48	mg/l	$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$ $\mu = 2.92\text{mg l}^{-1}; \sigma = 0.29\text{mg l}^{-1}$ $\min_x = 0.0\text{mg l}^{-1}; \max_x = 16.00\text{mg l}^{-1}$
Salinity	8.12	Qualitative	$f(x) = \frac{x-1}{4}$ $\text{Salinity}(x) = \begin{cases} \text{if } x = \text{fresh water} \rightarrow 1 \\ \text{if } x = \text{very low - salt water} \rightarrow 2 \\ \text{if } x = \text{low - salt water} \rightarrow 3 \\ \text{if } x = \text{salt water} \rightarrow 4 \\ \text{if } x = \text{very high - salt water} \rightarrow 5 \end{cases}$
Turbidity	8.66	Qualitative	$f(x) = \frac{x-1}{4}$ $\text{Turbidity}(x) = \begin{cases} \text{if } x = \text{very low} \rightarrow 1 \\ \text{if } x = \text{low} \rightarrow 2 \\ \text{if } x = \text{normal} \rightarrow 3 \\ \text{if } x = \text{high} \rightarrow 4 \\ \text{if } x = \text{very high} \rightarrow 5 \end{cases}$
Tidal	8.12	Tidal coefficient	$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$ $\mu = 0.64 \text{ Coeff.}; \sigma = 0.24 \text{ Coeff.}$ $\min_x = 0.05\text{Coeff.}; \max_x = 1.05\text{Coeff.}$

$$\text{WRisk}_i = \frac{\sum_{\forall N} \text{Factor}_i \cdot N^{-1}}{\sum_{i=1}^j \sum_{\forall N} \text{Factor}_i \cdot N^{-1}} \cdot 100 \tag{1}$$

where *N* is the number of experts who effectively responded to the questionnaire and *j* is the number of parameters and factors analysed.

**Table 5** Weighting and transformation functions of fish behavioural factors (BFs)

Behavioural factors (BFs)	Weighting (WRisk <sub>i</sub> )	Units	Transformation function
Individuals close the water intake	10.53	Qualitative	$f(x) = \frac{x}{5}$ Fishes (x) = $\begin{cases} \text{if } x = \text{none} \rightarrow 0 \\ \text{if } x = \text{very few} \rightarrow 1 \\ \text{if } x = \text{few} \rightarrow 2 \\ \text{if } x = \text{normal} \rightarrow 3 \\ \text{if } x = \text{many} \rightarrow 4 \\ \text{if } x = \text{lots of} \rightarrow 5 \end{cases}$
Decrease in feeding	9.93	Qualitative	$f(x) = \frac{x}{5}$ Feeding (x) = $\begin{cases} \text{if } x = \text{anything} \rightarrow 0 \\ \text{if } x = \text{very low} \rightarrow 1 \\ \text{if } x = \text{low} \rightarrow 2 \\ \text{if } x = \text{normal} \rightarrow 3 \\ \text{if } x = \text{high} \rightarrow 4 \\ \text{if } x = \text{very high} \rightarrow 5 \end{cases}$
Presence of dark-coloured specimens	10.83	Qualitative	$f(x) = \frac{x}{5}$ Dark specimens (x) = $\begin{cases} \text{if } x = \text{none} \rightarrow 0 \\ \text{if } x = \text{very few} \rightarrow 1 \\ \text{if } x = \text{few} \rightarrow 2 \\ \text{if } x = \text{normal} \rightarrow 3 \\ \text{if } x = \text{many} \rightarrow 4 \\ \text{if } x = \text{lots of} \rightarrow 5 \end{cases}$
Specimens with slow swimming	11.13	Qualitative	$f(x) = \frac{x}{5}$ Slow swimming (x) = $\begin{cases} \text{if } x = \text{none} \rightarrow 0 \\ \text{if } x = \text{very few} \rightarrow 1 \\ \text{if } x = \text{few} \rightarrow 2 \\ \text{if } x = \text{normal} \rightarrow 3 \\ \text{if } x = \text{many} \rightarrow 4 \\ \text{if } x = \text{lots of} \rightarrow 5 \end{cases}$
Presence of parasites on the gills	12.64	Qualitative	$f(x) = \frac{x}{5}$ Parasites on the gills (x) = $\begin{cases} \text{if } x = \text{none} \rightarrow 0 \\ \text{if } x = \text{very few} \rightarrow 1 \\ \text{if } x = \text{few} \rightarrow 2 \\ \text{if } x = \text{normal} \rightarrow 3 \\ \text{if } x = \text{many} \rightarrow 4 \\ \text{if } x = \text{lots of} \rightarrow 5 \end{cases}$

Each EP or FB is measured in its own units. This makes impossible to know which factor is of greater importance and also does not allow the comparison of factors or to obtain an overall risk value. Therefore, each EP or FB is transformed onto a standardised scale easily comparable and interpretable that ranges from 0 to 1, whereby higher scores correspond to higher risk occurrence of *A. ocellatum*. This way, the risk level of the *A. ocellatum* occurrence associated to a specific EP<sub>i</sub> or FB<sub>i</sub> can be calculated as:

$$\text{Risk}_i = \text{WRisk}_i \cdot f(\text{EP}_i \text{ or } \text{FB}_i) \tag{2}$$

In function of how EP or FB is measured in the fish farms, two classes of functions were applied. In the cases of water temperature, dissolved oxygen and tidal coefficient different continuous and bell functions were used. For the other factors (salinity, turbidity, individuals near the water intake, decrease in feeding, presence of dark coloured specimens with slow swimming and presence of parasites on the gills), functions increasing discontinuous were applied (Tables 4 and 5). Finally, the overall risk is a multi-metric indicator which is calculated as:

$$\text{Overall Risk} = \sum_{\forall i} \text{Risk EP}_i + \sum_{\forall i} \text{Risk FB}_i \tag{3}$$

To facilitate the interpretation of the indicator, a total of four risk categories were used: (i) low risk (green status; overall risk between 0 and 25); (ii) average risk (yellow status; overall risk between > 25 and 50); (iii) high risk (orange status; overall risk between > 50 and 75); and (iv) very high risk (red status; overall risk between > 75 and 100).

### Software implementation

The procedure described in previous sections was implemented in a computer program (Amy 1.01) developed and compiled in Microsoft® VisualBasic 6.0 (compatible with Windows PCs) (Fig. 2) and also in a mobile APP (AmyAPP 1.0) programed and compiled in Android Studio Dolphin 2021.3.1 (Fig. 3) (compatible with Android devices). Both programs display in Spanish language with friendly windows where the fish farmer can select the level of each parameter or factor by means different sliders. The programs provide the weighted values associated to each EP or FB, the overall risk and a set of recommendations in function of the estimated risk.

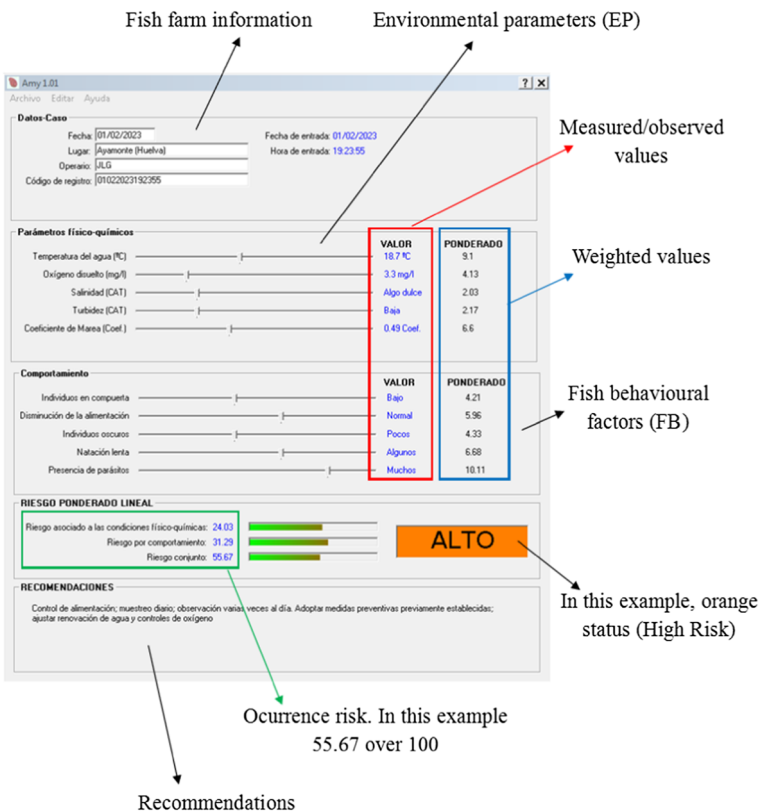
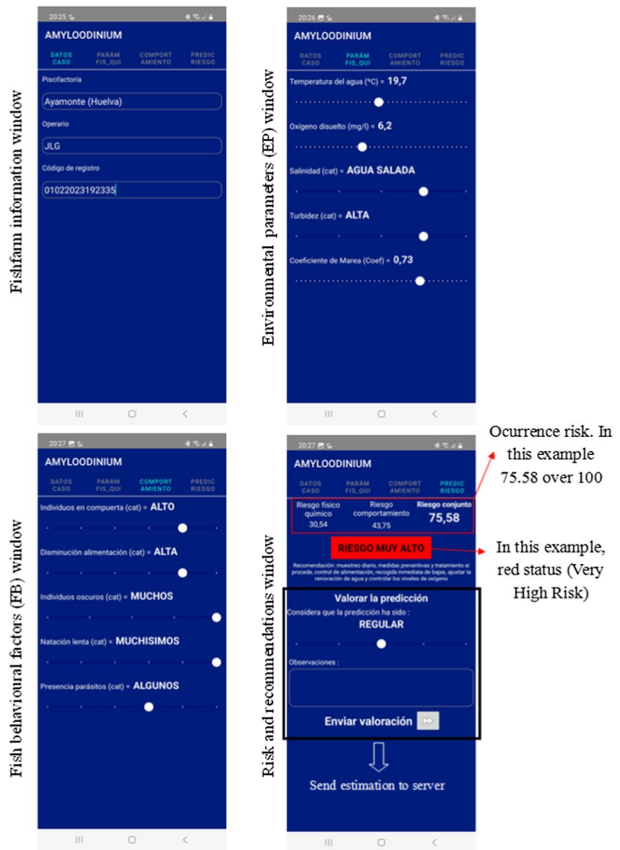


Fig. 2 Main window of Amy 1.01 computer program

**Fig. 3** Windows of AmyAPP for Android mobile devices



**System test**

To test the system, a total of 9 fish farms located in the Huelva and Cádiz provinces were randomly selected. In each fish farm, the manager was informed about the installation and setup of Amy 1.01 and how it works. Also, the fish farm operators were informed of how to use Amy 1.01 and were instructed to provide a system validation. The validation protocol consisted of a comparison between the traditional assessment carried out at each facility with that provided by Amy 1.01. To this end, managers and operators were asked to use Amy at the same time when carrying out the traditional risk assessment. Five days after the initial estimation, managers reported on the risk adjustment capacity predicted by Amy 1.01. A chi-square test was applied to assess the fit between Amy’s estimate and the fish farmers’s assessment (Martín-Andrés and Luna del Castillo 1994).

**Results**

**Questionnaire and weighting factors**

A total of 10 fish farms from Huelva, Sevilla and Cádiz provinces participated in this study and returned the first-round questionnaire. Since the questionnaire was reviewed in

the design phase by an external auditor, none of the questions was left unanswered. This way, all participants answered the 16 questions concerning operational aspects of the fish farm, the weight (importance) that several environmental parameters have on the occurrence of *A. ocellatum* in the ponds and the weight (importance) of several fish behaviour factors that could confirm the presence of *A. ocellatum* in the ponds. The panel included only those responsible for the management and pathological control of the fish farms.

Approximately 2 months elapsed between the first and second round. In the second round, all participants returned the questionnaire. No statistical differences were found between the scores' associated questions after the second round (Kruskal–Wallis test  $H(14,278)=72.59$ ;  $p<0.001$ ). A similar result was found when the overall results between rounds for the B and C set of questions were compared (Kruskal–Wallis test  $H(1278)=39.30$ ;  $p<0.001$ ). Also, the comparison of the answers for each question after the second round showed significant Kendall's correlations except in the case of the question number 12 ( $Tau_{Q12}=0.32$ ;  $p>0.05$ ). On the other hand, all responses showed a high stability with percentages of changes in all cases lower than 6% (Table 6). Therefore, after the second round, the administrator determined that the experts reached a situation of consensus.

The mean, 25th percentile, 50th percentile (median) and 75th percentile of the scores assigned by the contestants to each factor after the second round are shown in Table 6. The results reflected an occurrence rate of *A. ocellatum* very high in the fish farms located in the southwest of Andalusian region. At least 70% of the fish farms had an outbreak in the last years (question number 4; mean=0.70). On the other hand, the experience level of the fish farmers panel regarding the occurrence of *A. ocellatum* outbreaks was not statistically different from 3 (question number 3; mean=2.60±0.84) with the majority of the experts' answers being above the 50th percentile.

The mean scores for most behavioural factors (mean score questions type C=4.06) were slightly higher than for environmental parameters (mean score questions type B=3.30). In the case of questions type B, the most weighted parameter was the water temperature (mean=3.90) while the least important one was the dissolved oxygen (mean=2.92). Regarding questions type C, the most important factor was the presence of parasites on the gills with a score of 4.67 while the factor with a lower weight was the presence of individuals near the water intake.

## System validation

Nine fish farms randomly selected were used. Routine checks were carried out at these facilities throughout the summer of 2022. Control visits and phone calls were made and no outbreaks were detected until September. A total of 2 cases (C1 and C2 code) were reported with signs of infection classified by the operators as average risk. One of these cases (C1) was assessed by Amy 1.01 as high risk. In both cases, the risk associated to EP was higher than FB. Globally, comparison of the assessments of the fish farmers and Amy 1.01 provided no significant differences ( $\chi^2=2.00$ ;  $p=0.57$ ).

## Discussion

A process based on the Delphi method was successfully applied to use the experience and knowledge of fish farmers into a model that provides the outbreak occurrence risk of *Amyloodinium ocellatum* in the rearing ponds of sea bream (*Sparus aurata*) and sea bass

**Table 6** Score (mean, standard deviation, 25th, 50th and 75th percentiles) associated to each question type A, B and C after the second round. Also, Kendall's *Tau* values and percentage of change for questions types B and C after the second round are shown

	Mean	Standard deviation	Percentile			Kendall <i>Tau</i>	% change**
			25th	50th	75th		
Questions type A (see Table 1)							
Question 3	2.60	0.84	2.00	3.00	3.00	-	-
Question 4	0.70	0.48	0.25	1.00	1.00	-	-
Question 5	3.75	1.50	2.00	3.00	5.00	-	-
Question 6	2.00	0.81	1.75	2.00	2.25	-	-
Question 7	3.63	1.06	3.00	3.50	4.25	-	-
Question 8	2.80	0.42	3.00	3.00	3.00	-	-
Question 9	0.77	0.44	1.00	1.00	1.00	-	-
Questions type B (see Table 2)							
Question 10.1	3.90	1.10	4.00	4.00	4.75	0.91*	2.50
Question 10.2	3.50	0.71	3.00	3.00	4.00	0.79*	-5.00
Question 10.3	3.00	0.47	3.00	3.00	3.00	0.47*	0.00
Question 10.4	3.20	1.03	2.25	3.00	4.00	0.97*	-2.50
Question 10.5	3.00	0.82	3.00	3.00	3.00	0.84*	-5.00
Question 11	3.31	1.18	3.00	3.00	4.00	0.96*	3.13
Question 12	2.92	0.29	3.00	3.00	3.00	0.31	-5.56
Question 13	3.44	0.75	3.00	3.00	4.00	0.76*	-2.78
Question 14	3.50	1.17	3.00	4.00	4.00	0.69*	-5.56
Question 15	3.22	1.20	3.00	3.00	4.00	0.84*	0.00
Mean	3.30						
Questions type C (see Table 3)							
Question 16.1	3.89	1.27	4.00	4.00	5.00	0.92*	-2.78
Question 16.2	3.67	1.12	3.00	4.00	4.00	0.86*	5.56
Question 16.3	4.00	1.12	3.00	4.00	5.00	0.87*	0.00
Question 16.4	4.67	0.71	5	5	5	0.81*	-5.56
Mean	4.06						

\*  $p < 0.05$ ; \*\*a negative sign indicates a shift to a higher value in the second round

(*Dicentrarchus labrax*) located in the southwest of Spain. By means this methodology, the weighting of key environmental parameters and fish behaviour factors that favour the occurrence of this parasite was carried out, which allowed the development of a computer program and a mobile APP to estimate the risk level of *A. ocellatum* occurrence.

## Delphi method

It is clear that the success or failure of the implementation of the Delphi methodology depends on the selection of the expert panel. In this sense, although several authors highlight the number of experts on the panel as the main factor which conditions the results obtained (Homenuck et al. 1974), the bibliography on Delphi methodology does not

provide an exact recommendation on the number of persons who should compose the expert panel. For example, Buxton et al. (1991) and Hai et al. (2015) used a panel with a limited number of experts (between 6 and 10). In contrast, other authors such as Marvin et al. (2020) needed over one hundred experts. Therefore, it seems that the capacity to elicit an expert knowledge is more dependent on the specificity of the problem than on the number of panellists. In our case, the relatively deep knowledge of the expert about the system analysed justifies the use of a reduced expert panel.

Also, the knowledge level of fish farmers on the *A. ocellatum* occurrence has been reflected on the number of rounds needed to reach an overall consensus status. It was evident that the fish farmers understood that the environmental parameters and fish behaviour factors analysed play a crucial role in the risk of *A. ocellatum* occurrence. This way, only two rounds were necessary to reach a consensus level close to 100%. Although the iterative process (sending → response → report → re-sending) should continue until to reach a complete consensus, some classical studies have reported that essentially no significant changes are detected after the second round of evaluation (Dalkey 1969). Likewise, our results indicated that the change percentages for each question between the first and second round did not exceed in any case 6% which is significantly lower than the level (15%) that some authors have suggested as threshold for adding a new Delphi round (Linstone and Turoff 1975).

### Integrating expert knowledge

Each transformation function (continuous or discontinuous) has adequately captured the potential influence of the parameter on the occurrence of the parasite and the type of operational measurement carried out in the fish farms. In this sense, for the parameters salinity and turbidity and FB factors, the operators carry out a non-quantifiable estimation (fuzzy approach) that usually is not recorded and therefore, in these cases, discontinuous transformation functions were used. This type of approach, from which heuristic decisions on management and control are made, is common in these fish farms (Gutiérrez-Estrada et al. 2012; Pulido-Calvo et al. 2014). Instead, water temperature and dissolved oxygen were associated to continuous transformation functions because the operators take and record their measurement using the corresponding device (thermometer and oximeter). Likewise, a continuous transformation function was used for the tidal coefficient because its value is known at all times since it is necessary to properly plan the inflows and outflows of water from the ponds.

The water temperature affects a wide range of physiological aspects of fish pathogen and parasites (Snieszko 1974) and therefore, these condition the outbreaks of *A. ocellatum* in the ponds. It is not surprising that fish farmers have identified water temperature as the most important environmental factor among those analysed (weighting = 10.56). This agrees with Paperna (1984) who indicated that the optimal temperature range for *A. ocellatum* reproduction was 18–30 °C and the minimal division time was at 23–27 °C. Thus, a polynomial continuous transformation function slightly right-skewed with a maximum value at 30 °C, like the one used in this work, reflexes the preferential parasite development temperature in the rearing ponds.

The second best weighed parameter was the dissolved oxygen (weighting = 9.48). For this parameter, a more conservative transformation function was applied (a symmetric bell function centred on 2.92 mg l<sup>-1</sup>). This is because the direct effects of high or low oxygen levels on the life cycle of *A. ocellatum* are currently unknown. Some inconclusive works indicate that low oxygen concentration could reinforce an indirect impact of *A. ocellatum*. This way, a low oxygen concentration would cause a

depression of the respiratory function of fish as a consequence of alteration of the respiratory organs by the parasitic trophonts (Kuperman and Matey 1999). Pereira et al. (2011) reported that it is necessary a better understanding of the role played by dissolved oxygen on the occurrence of *A. ocellatum* mainly in an interaction context with other environmental parameters like phytoplankton concentration. Therefore, a precise understanding of the direct and indirect relationships between dissolved oxygen concentration and parasite emergence is a key aspect to be studied in future works in order to assign a more accurate transformation function to the level of risk.

Salinity, turbidity and tidal coefficient were similarly weighted (salinity = 8.12; turbidity 8.66; tidal coefficient: 8.12). Salinity and turbidity were associated to discontinuous increasing transformation function, while tidal coefficient was associated with a more conservative symmetric bell function. Salinity is a parameter that the fish farmers associate to *A. ocellatum* occurrence despite relatively small variations under normal conditions which could be related with to the fact that freshwater is an effective treatment in eliminating the parasite from gills (Cruz-Lacierda et al. 2004). In this way, a discontinuous increasing transformation function adequately captures the perception of the fish farmers as well as the behaviour of the parasite in relation to salinity.

Turbidity and tidal coefficient are strongly related with salinity. Tidal coefficient is an important parameter for these fish farms because pumping of clean water only can be carried out when the tidal coefficient is higher than + 0.5 (Gutiérrez-Estrada et al. 2022). This supposes that water pumped to ponds is basically sea water. The circulation of clean salt water promotes oxygenation and the removal of nitrogenous nutrients. If pumping is not carried out, the concentration of these nutrients increases, leading to an increase in primary production and a consequent increase in turbidity. This way, the fish farmers associate a lack of water renewal with an increase in turbidity and with an increase of occurrence's risk of *A. ocellatum*. The relationship between turbidity and the increase of fish pathogen also has been observed in eutrophic waters (Collins 1970). Therefore, the transformation functions associated to turbidity and tidal coefficient seem adequate to describe the risk level assigned by the fish farmers.

Normally, in an *A. ocellatum* outbreak control context, information extracted from symptoms is of a categorical nature. That is to say, the fish farmer observations about individuals close the water intake, the decreasing in feeding, the presence of dark-coloured specimens or specimens with slow swimming are used to define the control measures and risk of occurrence. Thus, for all these symptoms, an increasing discontinuous transformation function seems appropriate.

### **Amy response assessment**

A requirement for the admissibility of a risk assessment system like Amy is that it presents to the expert the assumptions set compatible with the available data. This strategy was applied in this study, based on the assumption that the condition for the reliability of Amy was the comparison of the estimated risk level with the operational assessments (Molino et al. 1996). According to such strategy, 89% of evaluated cases (8/9) were classified by fish farmers as 'totally correct', 11% (1/9) as 'approximate' and 0% as 'incorrect'. This level of accuracy is similar to that achieved by other systems focusing on fish pathology. For example, Gutiérrez-Estrada et al. (2005) developed an expert system (SEDPA) for disease diagnosis in eel rearing systems which provided 'incorrect' response rates close to 3.4%. Deng et al. (2013) proposed a neural network-based

model for fish disease diagnosis that simplified knowledge acquisition and showed high adaptability and fault tolerance for uncertain and incomplete knowledge. These authors reported that the diagnostic accuracy of their system was 90%. More recently, Molares-Ulloa et al. (2022) have developed a machine learning model to forecast the risk of bio-toxin accumulation in mussel farming with accuracy close to 92%.

Finally, one advantage of the system developed highlighted by the fish farmers is that there is no interrelation between the risks associated with PD and FB. This additive approach makes possible to obtain a level of risk based on only one of the components of the system without the need for an assessment of each of the items. This is an important factor to take into account because the risk associated with BF depends exclusively on the visual evaluation of the farmer.

## Conclusions

In view of the results presented in this work, it can be convincingly stated that the computer program and mobile application (Amy and AmyAPP) developed for the assessment of the risk of occurrence of *A. ocellatum* outbreaks in sea bream and sea bass farms in southern Spain have shown effective behaviour and therefore it is possible to prevent the occurrence of this parasite in rearing ponds.

Both applications have been developed on the basis of information from a panel of experts (fish farmers) who are the best informed about the pathological problems in their farms. Therefore, the usefulness of the application of the Delphi methodology for the extraction of knowledge accumulated by fish farmers in a context of lack of data, quantitative information and causal relationships associated with the occurrence of this parasite in fish farms has also been demonstrated.

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**Data availability** Data will be made available on reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

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