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Automated prediction of spawning nights using machine learning analysis of flatfish behaviour

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ABSTRACT

Senegalese sole (*Solea senegalensis*) broodstock exhibit distinct behaviours (Rest the Head, Guardian, Follow, and Locomotor activities) that are important for breeding success. Understanding and monitoring these behaviours are essential to understand successful breeding of Senegalese sole. However, manually analysing these behaviours represents a significant challenge for human observers and is a labour-intensive process. Moreover, due to reproductive dysfunctions in Senegalese sole, aquaculture operations currently depend on wild origin breeders for successful spawning a reliance that is unsustainable in the long term. Therefore, to address these limitations, this study introduces a custom-designed framework based on computer vision and machine learning techniques. The model integrates object detection and tracking mechanisms to recognize and monitor reproductive behaviours of Senegalese sole within aquaculture environments. By combining advanced tracking algorithms, our model effectively extracts and analyses behavioural patterns from video datasets. The automated model behavioural analyses compared with manual analyses demonstrated strong performance, with accuracy, precision, and specificity exceeding 87 %, and a Pearson correlation of $R = 0.99$ between manual observation data and automated data. The model analysed videos to accurately identify behaviours with minimal human intervention, thereby saving a substantial number of hours and opened up the possibility to analyse behaviours over longer periods, generating more data. This is the first study to automatically analyse reproductive behaviours across full-night video recordings in Senegalese sole, providing new insights into how behavioural patterns relate to spawning. These behavioural changes in relation to spawning enable the model to effectively predict spawning and non-spawning nights with accuracies ranging from 70 % to 100 %. Such predictive capability can reduce dependence on wild origin breeders, support timely gamete collection, improve reproductive planning, and serve as a potential tool for hatchery automation.

1. Introduction

Senegalese sole (*Solea senegalensis*) is an important aquaculture species with high commercial potential [2,3,19]. The species traits for aquaculture include robustness, resistance to disease, and rapid growth [12,19]. These qualities have positioned the Senegalese sole as one of the most economically promising marine fish species in Spain [3,4]. However, large-scale industrial production of Senegalese sole is hindered by significant reproductive challenges, mainly the failure of cultured individuals to perform courtship and spawn spontaneously despite possessing viable gametes [6,17]. This behavioural reproductive dysfunction [1,9,10] has forced aquaculture activities to rely on wild-origin breeders for egg production, a practice that is unsustainable in the long term [2,7,18]. This kind of behavioural problems in aquaculture has led to a new tendency on automatic monitoring of fish activity. Its aim is understanding fish behaviour for improving health, welfare, and reproduction in aquaculture.

Recent advancements in computer vision, machine learning, and artificial intelligence (AI) have enabled precise and scalable monitoring of animal behaviour, reducing the labour and time required for traditional manual observations [22,27,28]. Automated behavioural analysis has the potential to adapt aquaculture practices by enabling precise

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monitoring of fish behaviour, including feeding dynamics such as appetite intensity and feed consumption rates [28]. The ability to recognize behavioural activities from video recordings underscores the need for tools that automatically quantify behaviour and clarify the relationships between different behaviour patterns [27]. Recent advancements in automated behaviour analysis systems have significantly enhanced our understanding of fish behaviour. For instance, Barreiros et al. [5] developed a method to analyse zebrafish behaviour by conditioning them to respond to sensory stimuli, such as red LED lights and vibracall sounds, linked to food delivery through micro-controlled components. Similarly, Du et al. [8] introduced an automated deep learning model to study the breeding behaviours of spotted knifejaw (*Oplegnathus punctatus*). This model analysed video data to identify critical behaviours such as chasing, spawning, and aggregation, providing valuable insights into the reproductive cycle and informing breeding strategies. These developments highlight the potential of automated behavioural analysis to transform aquaculture management and research.

The reproductive success of Senegalese sole has been closely linked to their courtship behaviours [1,9,10]. Accurately analysing these behaviours is essential for understanding the species' reproductive strategy and for developing effective broodstock management. Carazo et al. [6] described the courtship behaviours that are closely associated with successful mating and spawning. These behaviours preceded successful mating events and, therefore, behavioural detection offers a valuable opportunity to predict spawning nights. However, previous studies based on comparisons have shown that these behaviours are less frequent or even absent in cultured sole showing a reproductive behavioural dysfunction [9,10,17]. Understanding the dynamics of spawning nights is equally important, as these nights play a central role in the reproductive cycle and overall success of Senegalese sole spawning. In an effort to better understand the reproductive behaviours of Senegalese sole, Fatsini et al. [10] analysed courtship behaviours manually in both wild and cultured breeders. However, the dynamic nature of courtship behaviours in Senegalese sole presented a challenge for human observers. Manual analysis with repeated observations helped reduce bias, but the process was highly time-consuming and limited the extent of behavioural analysis possible.

The aim of this study is twofold, first, it introduces an automated model for detecting reproductive behaviours in Senegalese Sole, which have been demonstrated to be important for breeding success. The aim is to avoid manual detection, which is time consuming and not viable for long term or continual behavioural analysis. An automated model would provide the information in due time to proceed with the required tasks for increasing the successful breeding. Second, the model enables a larger volume of behavioural analysis to provide more information, which can be used automatically to predict spawning nights. Thus, the present study provides an automated model for detecting behaviours, such as rest the head, guardian, follow and locomotor activity, new data on sole spawning behaviour and accurate prediction of spawning nights.

2. Materials and methods

2.1. The initial database of knowledge and videos

The present study reanalysed videos of Senegalese sole from Fatsini et al. [10], focusing on the mixed-origin groups Mix1 and Mix2. These videos were recorded during the spawning season and analysed by human observers to generate courtship behaviour data, providing a baseline for the current study. The Control group was not included in the automated analysis as no spawning was registered.

2.1.1. Experimental setting

A total of 30 cultured Senegalese sole breeders (weight: 1192.8 ± 158.2 g) and 17 wild breeders (weight: 907.5 ± 192.4 g) were utilized. The cultured breeders (first generation from wild breeders) were from a homogenous stock that had never successfully reproduced. The wild animals used had previously spawned viable eggs in captivity. Three experimental groups were established in 10 m^3 tanks with a controlled recirculation system (IRTAmar®) at IRTA La Ràpita to simulate natural spawning conditions: a control group of cultured breeders and two mixed-origin groups, Mix1 and Mix2, containing both cultured and wild individuals.

2.1.2. Data collection protocol for behaviour analysis

An underwater camera was positioned in each of the three tanks and videos of fish behaviour in the tanks were recorded for the spawning season (March to June) of each of four years. The recording was focused on the part of the night when spawning occurred and periods recorded ranged from 14:00 to 07:00 during the first two years of the study, from 14:00 to 00:00 in the third year, and from 17:00 to 01:00 in the last year of the study.

Five spawning and five non-spawning nights were randomly selected from each year for analysis, all within the known spawning season of Senegalese sole (March-June). Across the four spawning seasons analysed, recordings ranged from 480 to 1020 min per night, with longer recording periods in the first two years and progressively shorter ones in later years as the focus narrowed to the behavioural window of interest (Supplementary Table S1). Human observers analysed the peak hour of behavioural activity (19:00 to 20:00). Within this hour, behaviours were identified and counted during one minute at five-minute intervals, yielding 12 observation points per hour. Therefore, over five nights analysed across three tanks, the total observation time examined by human observers was 180 min (15 h × 12 min per hour). The behaviours counted during this period were Rest the Head (RTH), Guardian, Follow, and Coupled swimming, which were pre-defined and previously described by Carazo et al. [6].

RTH: This behaviour entails a sole resting its head on some part of another sole's body.

Guardian: Involves one sole, often a male, resting its head on another sole while actively guarding the sole from a third sole (usually another male).

Follow: The sole swim in a procession, with one or more fish following the lead fish. The following sole mimics the movements of the lead fish, and these behaviours can last several minutes.

Coupled swimming: This behaviour features a pair, a male and a female, swimming together with the dorsal side of the male pressed against the ventral side of the female. This synchronized swim leads them to the water's surface for gamete release, often visible as an opaque cloud.

For the Locomotor Activity (LA), Fatsini et al. [10] analysed 5 spawning and 5 non-spawning nights from the time period 17:00 to 00:00, LA were identified continuously during the entire recording period for the three different tanks and ranged from 480 to 1080 h per year. LA was assessed by putting a line across the middle of the screen dividing the field of vision of the camera in two, and the number of times breeders crossed the line during an hour was counted for every hour recorded. Note LA and the behaviours can appear more than once at the same time. In this case, all of the behaviours were considered.

2.2. Automatic fish behaviour detection

The aim of our model was to automatize the detection of fish behaviour in Senegalese sole and investigate correlations that could predict whether a spawning night would occur.

2.2.1. Data collection and preprocessing

The videos used to develop and test the model were the videos identified above from spawning season 4 and tanks Mix1 and Mix2. To train the model, cropped images were taken from the videos produced in the study Fatsini et al. [10]. The behavioural dataset consisting of cropped images, was stored in Roboflow database for this study [20].

The dataset included 6000 cropped images. Initially, 3000 images were manually extracted to represent specific sole behaviours: RTH, Guardian, and Fish Alone (FA). These cropped images were obtained by selecting and labelling bounding boxes around the fish and their behaviours, with sizes ranging from 50 × 50 pixels to 250 × 250 pixels, depending on the bounding box area for FA, RTH, and Guardian behaviours. This labelled dataset was then used to train a CNN (Convolutional Neural Network), which learned to detect and classify the three behaviours that were to be detected by the computer vision system.

To improve the model's ability to generalize across varying conditions, the dataset was expanded from the original 3000 images using augmentation techniques including rotation (±15°), 90° rotation (clockwise and counter-clockwise), horizontal flipping, shear (±15° horizontally and vertically), and adjustments to saturation, brightness, and exposure (±25 %). Each original image was augmented to generate additional training instances. The final dataset was divided into training (70 %), validation (18 %), and test (12 %) subsets. Preprocessing steps, such as Auto-Orient, Resize, Grayscale conversion, Auto-Adjust Contrast, and Object Isolation, were performed to prepare the images for model training and evaluation. The cropped image dataset used for training was balanced across static and dynamic behaviours. However, testing data from full-night recordings was naturally imbalanced, as most frames did not contain the target behaviours, leading to lower recall values for behaviours such as Guardian.

2.2.2. Model implementation and algorithm

The model was based on the You Only Look Once (YOLO) object detection algorithm, integrated with the DeepSORT algorithm. YOLO is a state-of-the-art object detection system that recognizes and classifies objects in real-time with high accuracy and speed [23]. YOLOv8 was selected for this study due to its optimal balance between accuracy, speed, and model size, making it particularly suitable for real-time processing and computational efficiency in aquaculture environments. Compared to other widely used detection frameworks such as Faster R-CNN and Mask R-CNN, which are known for their high detection accuracy but are often computationally intensive and less suitable for real-time applications, YOLOv8 provides a more efficient alternative [21]. DeepSORT was integrated to provide object tracking capabilities, enabling the tracking and linking of individual fish across frames through a multiple-object detection approach [26].

In this study, we used YOLOv8x, the largest and most robust variant of the YOLOv8 family, introduced by Ultralytics. YOLOv8x uses a CSPDarknet53-based backbone with 68 layers and utilizes an anchor-free detection mechanism for enhanced object localization and classification. This architecture significantly improves the detection of small, overlapping, and partially occluded objects, which is particularly advantageous when analysing fish behaviour in dense aquaculture environments. The model was trained for up to 30 epochs, with early stopping implementation to prevent overfitting using an input image size of 640 × 640 pixels, a batch size of 16, and a learning rate of 0.001. Training was conducted on a workstation running Microsoft Windows 10 Pro (version 10.0.19045), equipped with a 12th Gen Intel® Core™ i5–12,600 processor (6 cores, 12 threads, 3.3 GHz base frequency), 16 GB of RAM, and an HP Z2 Mini G9 Workstation Desktop PC.

2.2.3. Automated model workflow and analysis of behaviours

Our model was composed of the specific parts described in Fig. 1. The input of the system was the video (in the present study the video was previously recorded, but this could be performed in real-time) from a camera located in a fish tank and the output was the counts of behaviours, predicted fish behaviour and the chance of being a spawning night.

The system operated through the following eight steps (Fig. 1):

1. **Camera**: A 2D camera was positioned just below the water surface in the corner of each tank to record fish behaviour. Ideally, the camera's field of view would cover the entire tank. However, this is often not possible, and individuals may temporarily disappear from the image when they move outside the frame. The camera setup was designed to minimize image distortion due to the camera's angle of view. The cameras used in the present study were square black and white CCD cameras (model F60B/ N80–50 G, KT&C, Korea) with a 150° wide-angle field of view. The selected cameras featured resolutions ranging from 2 MP to 8 MP, equipped with SONY CMOS sensors and a horizontal field of view between 85° and 100° To ensure clear image capture under varying lighting conditions, they were also fitted with a combination of three white light and four IR light.
2. **Frame Extraction**: The images (or frames) were generated at a rate of 24 frames per second (fps).

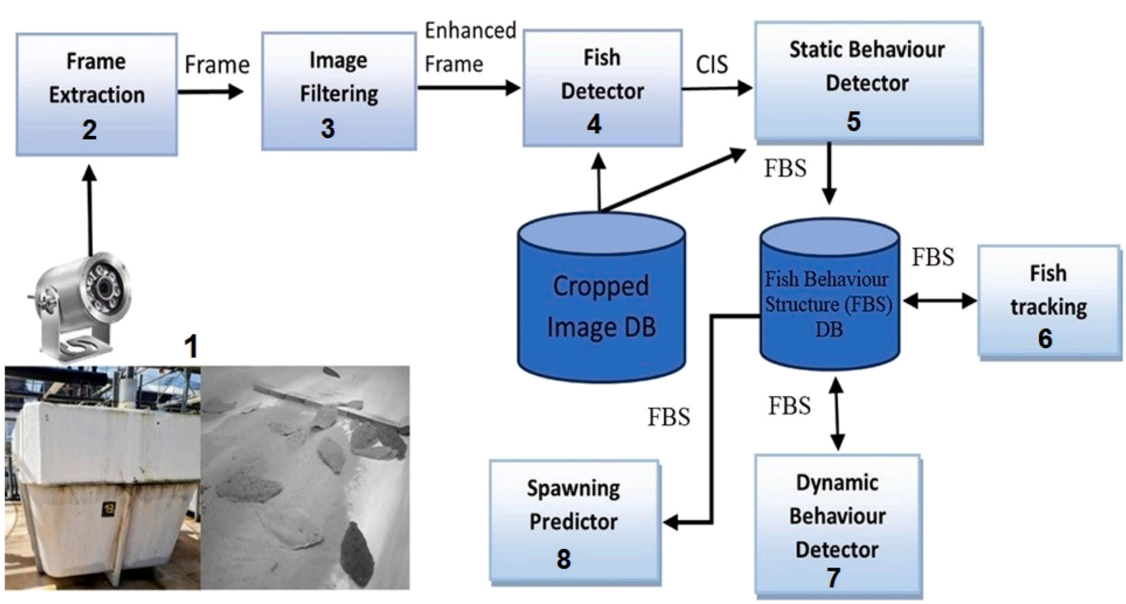

Fig. 1. Principal scheme of our model composed of eight logical blocks: Camera, Frame Extraction, Image Filtering, Fish Detector, Static Behaviour Detector, Fish Tracking, Dynamic Behaviour Detector, and Spawning Predictor, along with two databases: Cropped Image Structure (CIS) and Fish Behaviour Structure (FBS).

3. **Image Filtering**: Edge extraction was applied to filter the images, which means enhancing the borders of the objects (Enhanced image).
4. **Fish Detector**. This module identifies and extracts fish from frames as Cropped Image Structures (CIS) using a YOLOv8-based CNN. It was trained using a set of manually cropped images containing fish, stored in a Cropped Image Database (Cropped Image DB), which was divided into training, validation, and test subsets. During the model training, the algorithm searches the current frame and extracts cropped images that resemble images contained in the Cropped Image DB. These cropped images were related to the behaviours of interest. The output of this module was these Cropped Image Structures (CIS). Given that several fish can be detected in several parts of a frame, more than one CIS can be generated per Frame.

   A CIS is composed of the following fields:

- Frame number: Unique number for the frame from which the CIS was obtained.
- CIS number: Unique number that identifies each CIS.
- Position and size: Coordinates of the CIS center within the frame and its dimensions (length $\times$ width in pixels).

5. Static Behaviour Detector:

The Static Behaviour Detector identifies static behaviours and records them in the Fish Behaviour Structure (FBS), which is stored in a central database (FBS DB). This database, shared with the Fish Tracking, Dynamic Behaviour Detector, and Spawning Predictor modules, enables all four modules to exchange and update information (Fig. 1).

The FBS was composed of the following fields:

- Identifiers: FBS Unique number, Frame number, and CIS number
- Position: The position of the CIS in the frame.
- Fish ID number: Unique identifier for each fish.
- Time: Hour when behaviours were recorded.
- Behaviours: Counts of static behaviours (RTH, Guardian, FA) from the Static Behaviour Detector (Module 5) and dynamic behaviours (LA, Follow) from the Dynamic Behaviour Detector (Module 7).

The Static Behaviour Detector applies a YOLO-based CNN to cropped images (CIS), classifying them into RTH, Guardian, or FA. These classifications are stored directly in the corresponding FBS fields.

6. Fish Tracking: The fish tracking module was implemented using DeepSORT algorithm, which assigns unique ID to each detected fish and links it across frames based on appearance and motion [26]. Each fish was assigned a unique ID upon detection, which allows for continuous tracking. If a fish leaves the camera's field of view and re-enters, a new ID is assigned. Fish movement was computed only when a single fish is present in the CIS (i.e., when the static behaviour is classified as FA). For this purpose, the module maintains a database of FBS from previous frames, ensuring accurate tracking of fish identities and movement over time.

7. Dynamic Behaviour Detector: This module analysed the dynamic behaviours (LA and Follow) when the position of static behaviour FBS changed across frames, using Fish ID number and position of all FBS within the same Frame. LA was defined as continuous movement lasting at least 3 s to capture only meaningful activity and exclude minor displacements. This threshold is appropriate for the Senegalese sole, a sedentary species with generally low locomotor activity that also exhibits specific movements such as the buried movement, where fish move but remain in the same place [6, 11]. Continued movement over various frames was counted as one movement. For the Follow once movement was detected, the module assessed the distance between moving fish within the same frame. If two or more fish were moving and the distance between the fish was maintained within a specific threshold, the module classified the behaviours as Follow. This threshold was set at 80 pixels, defining the maximum distance for close proximity, which is a key characteristic of Follow, consistent with the definition provided in the behavioural ethogram for Senegalese sole [6]. The outputs of the module were:

Appearances of LA: The count of how many times LA appeared.

Appearances of Follow: The count of how many times Follow appeared.

8. Spawning prediction: This module predicts whether fish are likely to spawn during the night. This prediction was made using behavioural data collected earlier on the same day, from 17:00 to 23:00. This component used a linear regression that was trained using the Ordinary Least Squares (OLS) method to estimate the coefficients that best fit the behavioural indicators to predict spawning events. The input of linear regression consisted of five behavioural indications from FBS such as RTH, Guardian, Follow, LA, Time, along with a bias term. The Spawning predictor was modelled as a linear regression equation as follows,

$$Prediction_{spawn_{night}} = A \times A_{RTH} + B \times A_{Guardian} +$$

$$C \times A_{Follow} + D \times \text{ Locomotor Activity} + E \times Time + BIAS$$

Equation 1. Chance of spawning nights based on behavioural inputs (RTH, Guardian, Follow,LA) and Time, along with the learned weights (A, B, C, D, E) and bias term. In this equation, A, B, C, D, and E are the coefficients representing the influence of each behaviour, while RTH, Guardian, Follow, LA, and Time are the input variables affecting the chance of a spawning night, with a value between -1 and 1. A value of 1 represents the maximum chance of a spawning night, while -1 represents the maximum chance of a non-spawning night. The BIAS term serves as the intercept in the linear regression model, reflecting the baseline likelihood of a spawning event.

The linear regression returns a negative number when the chance of spawning is low and a positive one when the chance of spawning is high. Note the Spawning Predictor was run hourly since during each hour the five values are modified in the new FBS.

2.2.4. Model development

Five different models (Model 1 to 5) were developed and compared for the two static behaviours RTH and Guardian. Five spawning nights from group Mix1 were used to develop the models. The five models differed as different Cropped Image Structures (CIS) were used to develop each model using the Leave-One-Out-Cross-Validation (LOOCV) technique. Each model used CIS from 4 nights for training the model that was then used to analysis the fifth night, for example Model 1 was trained with CIS from nights 2, 3, 4 and 5 and used to analysis night 1, Model 2 was trained with CIS from nights 1, 3, 4 and 5 and used to analysis night 2, etc. This strategy ensured that the models for RTH and Guardian were generalized across different spawning nights, could provide accurate detection and demonstrated the effect on the model of the CNN training process. Detection of RTH and Guardian behaviours was treated as a binary classification task, where each frame was classified as either exhibiting the behaviour or not. LOOCV validation was not applied to the dynamic behaviours Follow and LA, only a single model was trained for these. This is because CIS selection and training had limited impact on fish tracking across frames for dynamic behaviours. The model for the Follow was tested on the same five spawning nights from group Mix1 that were used to develop and test Models 1 to 5 for the static behaviours.

Once the tested model had been developed, it was used to describe the behaviour and LA on five spawning and five non-spawning nights from each of the two groups Mix1 and Mix2. The LA described by the automated model was compared with observer counts that were available for all the night analysed. The description of the behaviour and LA was then used by the spawning prediction module to provide the likelihood that specific hours and behaviours had for predicting the spawning or non-spawning of the two groups.

2.2.5. Model testing and statistical analysis

The counts from the automated models for all the behaviours (RTH, Guardian, Follow and LA) were compared to the human observer counts (ground truth) reported by Fatsini et al. [10]. Note the behaviours RTH, Guardian, and Follow were only counted by observers during the peak hour of activity from 19:00 to 20:00, while LA was counted for all hours recorded 17:00 to 01:00. Therefore, comparisons were only made for these hours. The counts were described with the statistical classifications, True Positive (TP) (model correctly identified a behaviour), True Negative (TN) (model correctly identified the absence of a behaviour), False Positive (FP) (model identified a behaviour that was not present) and False Negative (FN) (model did not identify a behaviour). Performance metrics for behaviour classification were calculated to evaluate model effectiveness. Accuracy represented the proportion of correctly classified frames (TP + TN) / (TP + TN + FP + FN), while precision measured the reliability of positive detections as TP / (TP + FP). Recall (sensitivity) quantified the model's ability to detect behaviours when present, calculated as TP / (TP + FN), and specificity assessed the model's ability to avoid false alarms, calculated as TN / (TN + FP).

The linear correlation between the counts obtained by the observers [10] and the models was examined with the Pearson correlation. Paired Student's *t*-tests were applied to compare the means of RTH, Guardian, and Follow counts, as the same timepoints were analysed by both methods. For LA, a GLM was performed with hour of measurement and counting method (observer or automated model) as independent variables. The means of counts of behaviours on spawning and non-spawning nights over time were compared with a GLM with the two independent variables spawning or non-spawning night and hour of measurement. These statistical comparisons were made with SigmaStat (Systat Software Inc., Germany). Means were expressed with one standard deviation and $P < 0.05$ was considered to indicate a statistical difference.

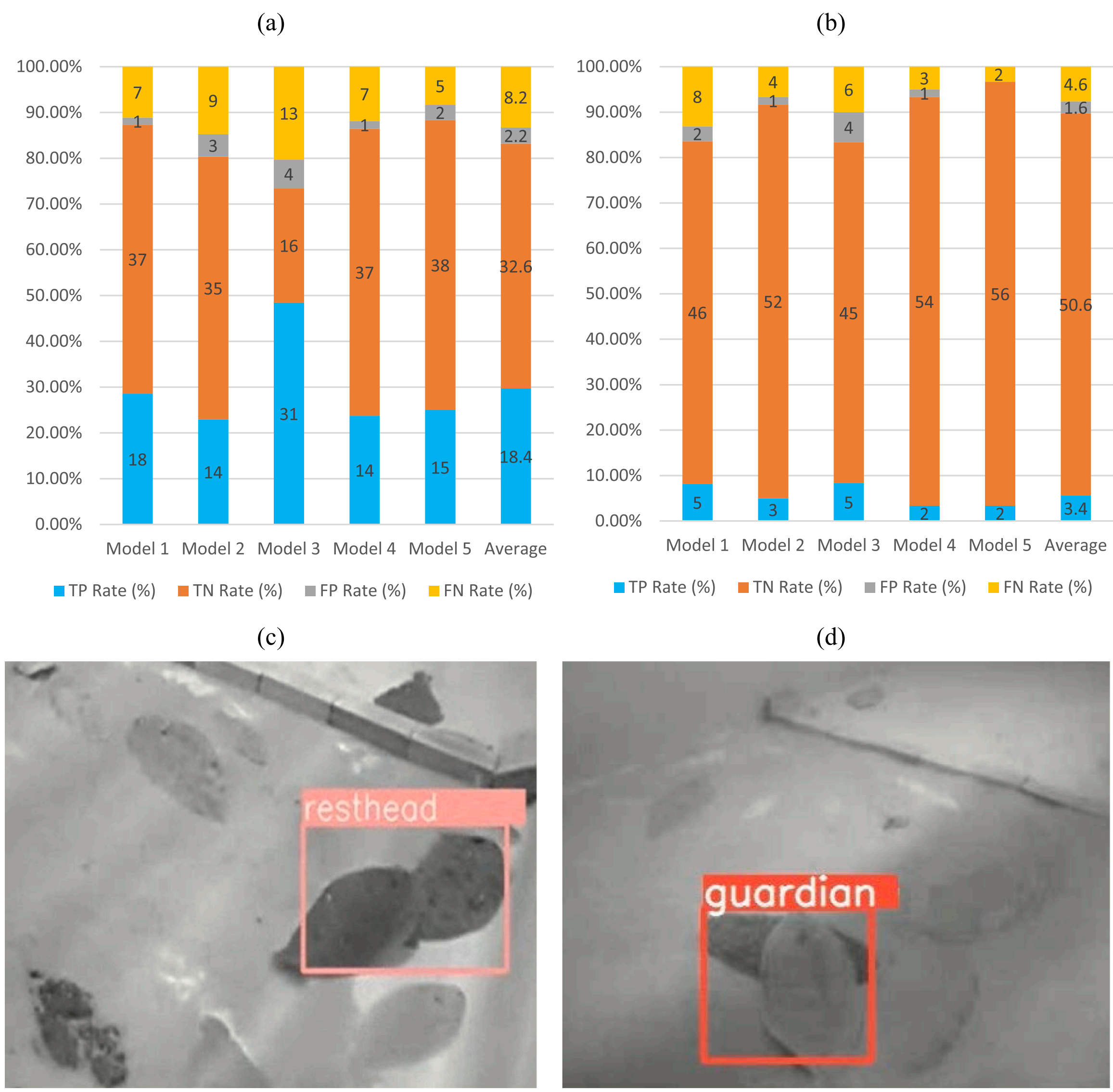

Fig. 2. Automated detection of RTH and Guardian behaviours during the peak hour (19:00–20:00) on spawning nights of group Mix1. (a) Model performance for RTH, (b) model performance for Guardian, (c) instance of RTH detected by the model, and (d) instance of Guardian detected by the model. Performance metrics are reported as TP, TN, FP, and FN (see Section 2.2.3 for definitions) and the numbers on each bar are the actual counts of behaviours.

3. Results

3.1. Fish detector and static behaviour detector: the detection of the RTH and Guardian behaviours with five models

The performance of each of the five models is shown in Fig. 2a for RTH behaviour, which shows consistent high detection rates (TP + TN) that ranged from 73.44 % (Model 3) to 88.33 % (Model 5). Notably, Model 3 achieved a higher TP rate of 48.44 % compared to the other models, which had TP rates ranging from 22.95 % to 29.74 %. Model 3 maintained relatively low FP and FN rates. These combined characteristics made Model 3 the optimal choice for RTH behaviour detection, indicating more reliable performance.

Fig. 2b presents the performance for Guardian behaviour detection. The five models had consistent high detection rates (TP + TN) that ranged from 83.33 % (Model 3) to 96.66 % (Model 5). Model 1 achieved a TP rate of 8.20 %, providing higher detection of Guardian behaviour than Model 5 (3.33 %), while maintaining a low FP rate of 3.28 % and a high TN rate of 75.41 %. These performance metrics made Model 1 the most suitable choice for Guardian behaviour detection. Each model was tested on a night excluded from its training set, confirming that the observed performance reflects generalization rather than overfitting. Detection of RTH and Guardian behaviours was treated as a binary classification task, where each frame was classified as either exhibiting the behaviour or not. Fig. 2c and d show instances of RTH and Guardian behaviours detected by the respective selected models.

3.2. Fish tracking and dynamic behaviour detector: The detection of follow behaviour and LA on five nights

For Follow, the analysis shown in Fig. 3a indicates that FN were more frequent than FP. FN often occurred due to tracking difficulties, such as when the model misinterpreted sand as a fish or classified static fish as dynamic. A key source of these errors was the limited camera view, which did not cover the entire tank. Identifying such error sources is important for refining the model and addressing challenges associated with dynamic behaviour analysis. Despite these challenges, the model achieved an average accuracy of 89.75 % and strong specificity 98.37 %, meaning that it reliably identified frames without Follow as negatives, and a precision of 86.57 %, with recall at 52.29 %, reflecting that some

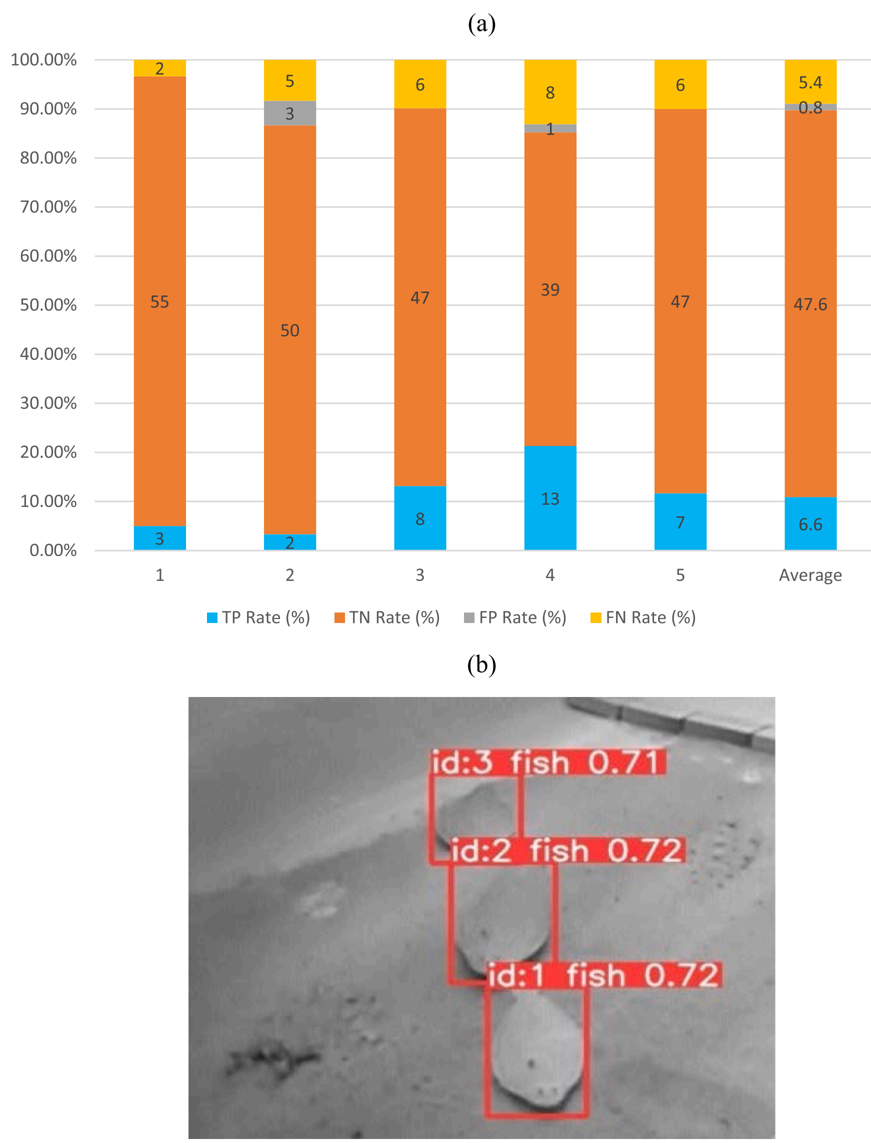

Fig. 3. Automated model accuracy on five spawning nights (19:00–20:00) of group Mix1 for the Follow. (a) Model accuracy (%) based on TP, TN, FP, and FN (see Section 2.2.3 for definitions). The numbers on each bar are the actual counts of behaviours. (b) Example of Follow detected by the automated method. Each detection is represented by a bounding box and with an associated confidence score on top indicating the reliability of behaviour identification.

true instances of Follow were not detected. Similarly to Fig. 2b, the appearance of TN was larger than TP since there were many more video minutes without Follow than with it. Note also that the percentage of false detections (FP) was very low. The model ability to identify the Follow behaviour, highlighted fish individuals with unique fish IDs (Fig. 3b). Each bounding box indicates not only the tracked fish but also the confidence score assigned by the model for the detected behaviour. This tracking feature helps differentiate individual fish and support consistent identification across frames, particularly useful for distinguishing behavioural interactions, although accuracy may be reduced under challenging conditions such as occlusion or overlapping fish.

3.3. General performance of the behaviour's classifier

Generally, the performance of the final model used in the behaviour classifier was strong during the peak hour of activity on the ten spawning nights for groups Mix1 and Mix2 when compared to the observer manual true data set. The classifier's performance metrics accuracy, recall, precision, and specificity along with their standard errors, provide a detailed view of its effectiveness in detecting RTH, Guardian and Follow behaviours (Fig. 4). For RTH, the model achieved 83.17 % accuracy and 69.00 % recall, while Guardian showed high accuracy 89.72 %, with lower recall 43.35 %. Follow achieved similarly high accuracy 89.75 % with moderate recall 52.29 %. These results indicate that while recall varied depending on the behaviour type, the model consistently achieved high performance in other metrics, particularly in rejecting false positives, with specificity exceeding 92 % across all behaviours and reaching as high as 98.37 % for Follow. Precision was also strong, with values above 73 % for all behaviours and over 86 % for RTH and Follow, showing that the classifier was highly reliable when it did detect a behaviour.

To provide an overall perspective, averages across all three behaviours were calculated. Accuracy, precision, and specificity averaged 87.55 % $\pm$ 2.22 %, 83.25 % $\pm$ 6.34 %, and 95.84 % $\pm$ 1.72 %, respectively. Recall averaged 54.88 % $\pm$ 3.15 %, reflecting the relatively lower number of true positive detections compared to false negatives.

The Pearson correlation between the automated behaviour classifier counts and manual observer counts was $R = 0.985$ ($P < 0.0001$) across all behaviours combined, indicating a strong agreement between the two methods. When examining individual behaviours, the relationships were similarly strong: RTH showed a coefficient of $R = 0.993$ ($P < 0.0001$), Guardian had $R = 0.892$ ($P = 0.0005$), and Follow demonstrated a measure of agreement of $R = 0.986$ ($P < 0.0001$). These results indicate a strong agreement between the automated behaviour classification and manual observer counts.

3.4. LA and fish behaviour concerning spawning nights

The automated model demonstrated high correlation with manual observer counts for LA, although some variations existed in activity magnitude between methods (Fig. 5a and b). Pearson correlation coefficients were $R = 0.85$ ($P = 0.00014$, $n = 14$) for group Mix1 and $R = 0.88$ ($P < 0.0001$) for group Mix2. However, there were significant differences ($P < 0.05$) between the mean counts obtained with the two methods, with manual observers generally counting more locomotor activities. Although the difference in magnitude between methods was significant, the patterns over the night were broadly similar for Group Mix2, while Group Mix1 showed more noticeable differences between methods. The overall trend observed in both types of analysis (automated and manual) showed differences between the two groups. Group Mix1 was less consistent, particularly for non-spawning nights where manual counts remained high for longer into the night. In contrast, group Mix2 showed higher LA during the hours 18:00 to 19:00 for spawning nights, followed by lower LA later in the night (22:00 to 23:00 h) (Fig. 5a and b). Both methods identified significant differences ($P < 0.05$) in activity between spawning and non-spawning nights. There were also significant differences within specific hours in group Mix2, whereas no such differences were observed in group Mix1. In group Mix2, significant differences ($P < 0.05$) in LA were found between spawning and non-spawning nights at 18:00 in the observer data analysis and at 17:00 and 18:00 in the automated analysis. The automated analysis also found a significant decline ($P < 0.05$) in LA during the night that was not found by the observer analysis. This suggests that the automated approach detected changes in LA that manual counts may have overlooked due to smaller fluctuations. Importantly, the overall nightly patterns remained consistent between approaches, indicating

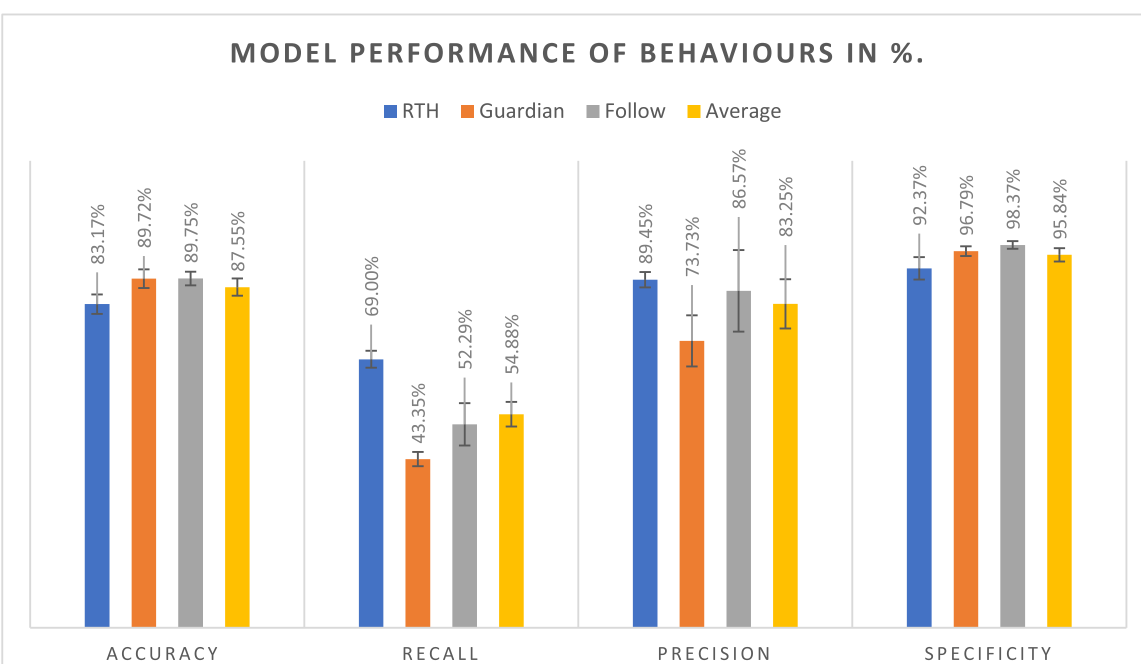

Fig. 4. Performance metrics for the automated behaviour classifier. The figure compares the average accuracy, precision, recall, and specificity (in %) of the models used for RTH, Guardian, and Follow behaviours. The error bars represent the standard errors of each metric.

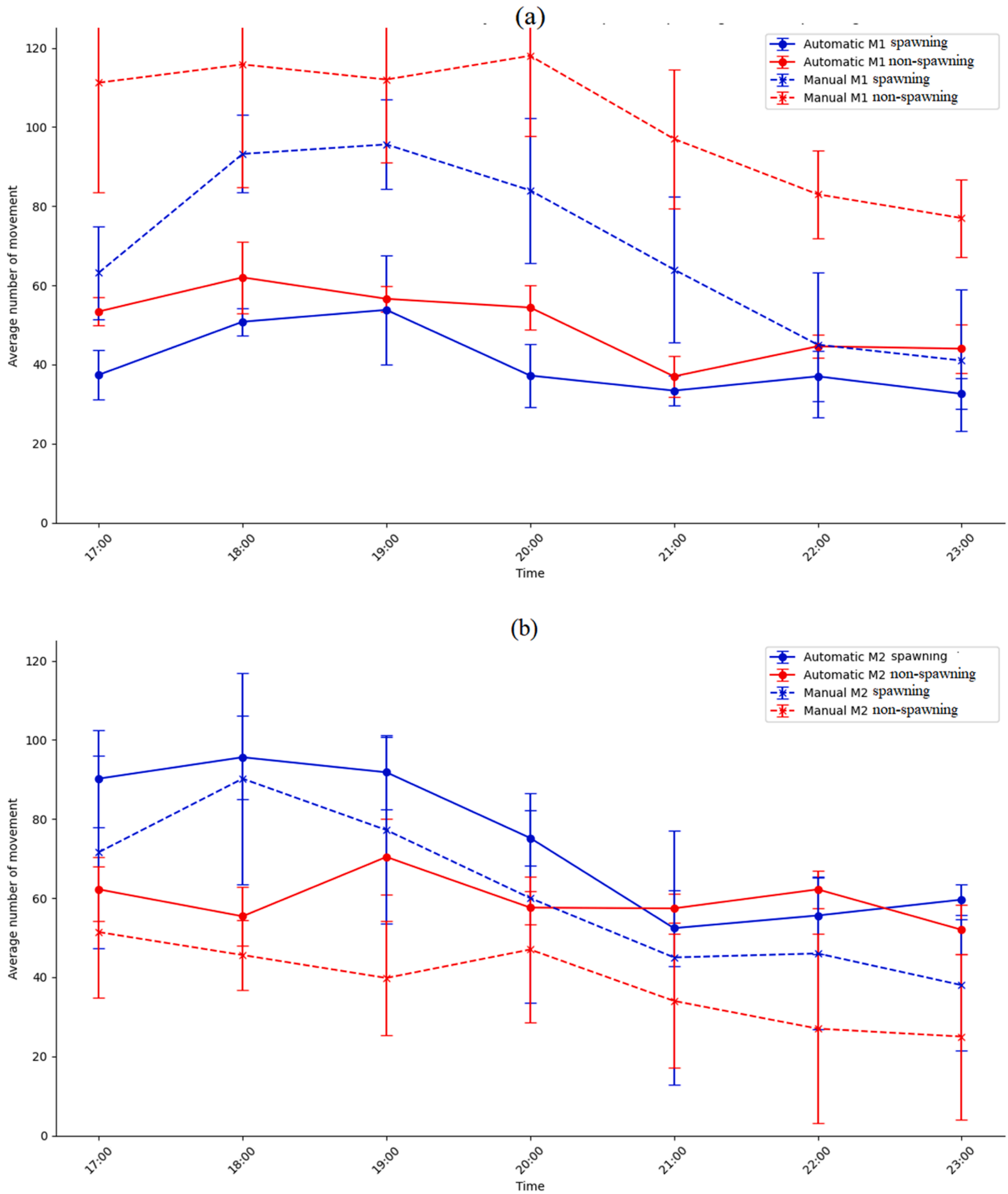

Fig. 5. LA across five spawning and five non-spawning nights, shown as mean ± standard error. Behavioural activity was quantified using manual counts from Fatsini et al. [10] and the automated model. (a) Group Mix1 and (b) Group Mix2.

that both methods captured main behavioural trends, while the automated system provided additional detail for subtle differences.

The automated model detected RTH, Guardian and Follow over the entire night from 17:00 to 23:00 on both spawning and non-spawning nights for group Mix1 and Mix2 (Fig. 6a and b). This represents the first time these behaviours have been analysed continuously throughout the night. The general pattern was similar to the LA with higher behaviour counts in the period 18:00 to 19:00 with behaviour counts declining between 22:00 to 23:00. However, the pattern of each behaviour varied between groups Mix1 and Mix2. In group Mix1, RTH and Guardian behaviours showed significant decreases over time ($P < 0.01$). Both behaviours also occurred more frequently on spawning nights than on non-spawning nights. Follow also declined significantly over time ($P < 0.01$), but did not differ between spawning and non-

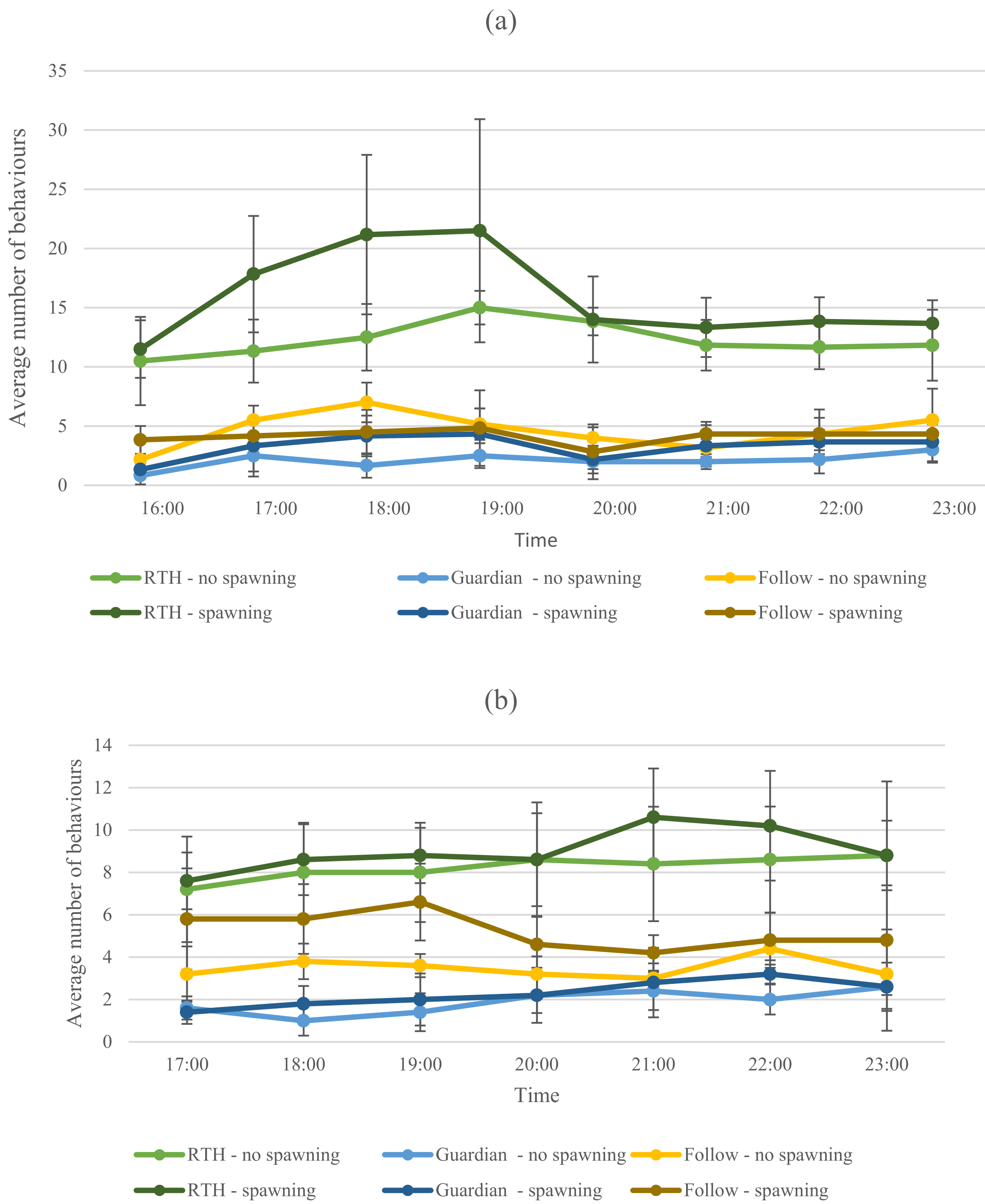

Fig. 6. Behaviour detections by automated model (mean and standard deviation) for RTH, Guardian and Follow in spawning ($n = 6$) nights versus non-spawning ($n = 6$) nights per hour over an extended period in Mix1 (a) and Mix2 (b).

spawning nights. In group Mix2, RTH did not exhibit differences in relation to time, but was significantly higher ($P < 0.05$) on spawning nights. Guardian counts increased significantly ($P < 0.05$), but had no differences in relation to spawning night. Follow exhibited significantly ($P < 0.01$) higher counts on nights with spawning compared to nights without spawning and no differences over time.

3.5. Prediction for spawning nights

The spawning prediction module successfully distinguished spawning from non-spawning events, achieving accuracies between 90 % and 100 %. The model correctly predicted 9 out of 10 nights in Mix1 (90 %) and all 10 nights in Mix2 (100 %). Only one night was incorrectly predicted in Mix1 (Fig. 7a). This misclassification occurred during early evening hours (17:00–19:00) when behaviour activity was low, making it difficult to distinguish between spawning and non-spawning patterns. From 20:00 onwards, when behaviour activity increased, all predictions were correct. All nights in Mix2 were correctly predicted from 18:00 onwards (Fig. 7b). The prediction of spawning nights was based on the four behaviours RTH, Guardian, Follow, and LA over time.

For the detailed comparison, each group was analysed using different combinations of behavioural factors to evaluate prediction accuracy. When tested individually, LA was the strongest single predictor, reaching accuracies of 80 to 84 % in both groups, while RTH, Guardian, and

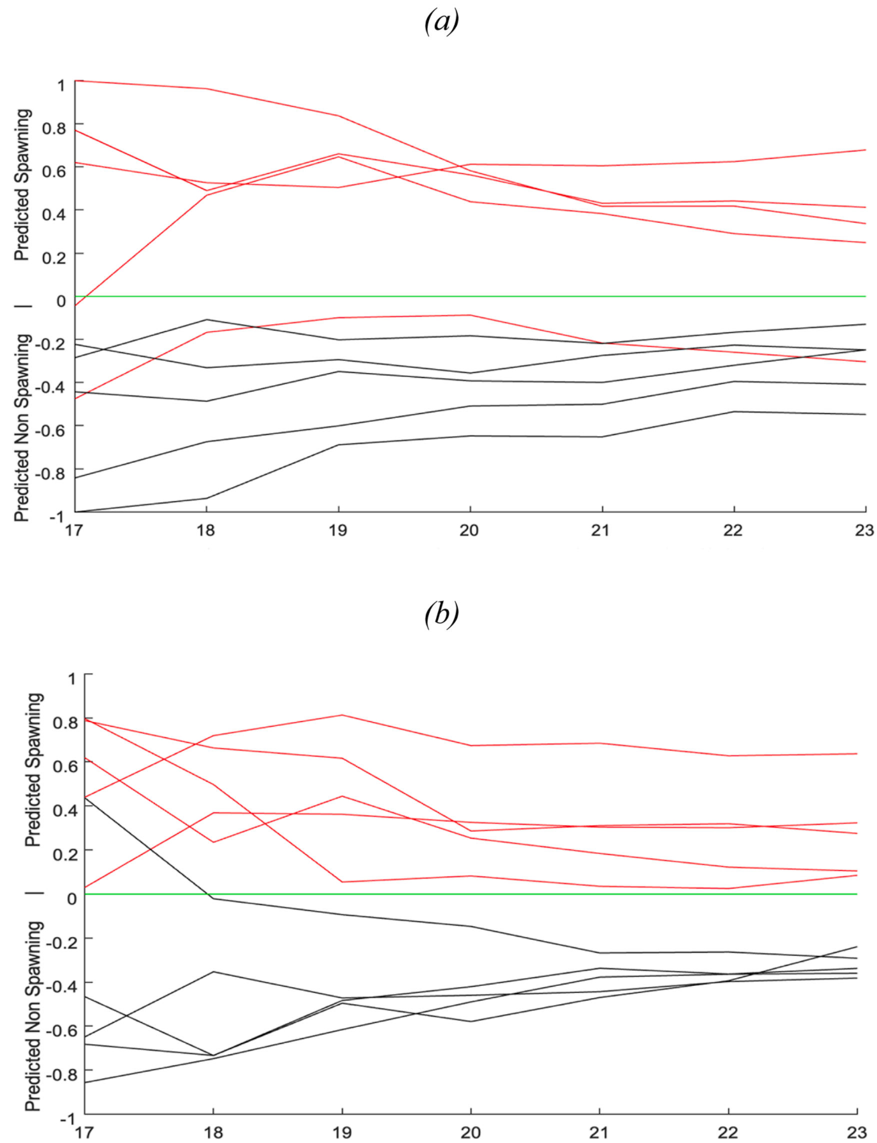

Fig. 7. Prediction of 5 spawning and 5 non-spawning nights in Mix1 (a) and Mix2 (b). Red lines on the positive axis indicate correctly predicted spawning nights, while black lines on the negative axis indicate correctly predicted non-spawning nights. When lines cross the axis (e.g., red below zero or black above zero), the prediction is incorrect. The x-axis represents time (17:00–23:00).

Follow individually showed lower predictive performance (Fig. 8a, b).

Combining predictors generally improved accuracy. The pairing of LA with RTH produced the highest performance among two-predictor combinations, reaching up to 82 % in Mix2 and over 70 % in Mix1 (Fig. 8c, d). Combinations that excluded LA consistently showed lower accuracy, confirming its central role in the prediction model. When all four predictors were included, accuracies reached 90 % in both groups (Fig. 8e, f).

Overall, these results indicate that LA, particularly when integrated with static behaviours such as RTH, is a strong indicator of spawning probability, while Guardian and Follow contributed less to prediction accuracy.

The combinations of three behavioural variables or all four variables gave high accuracies for prediction of spawning (Fig. 8e and f). In group Mix1, combinations incorporating all three behaviours consistently showed strong predictive capabilities, with accuracies ranging from 70 % to 90 % (Fig. 8e). Particularly, the combination integrating RTH, Guardian, Follow, and LA with four actives variables, demonstrated the highest accuracy at 90 %. The combination RTH, Guardian and Follow had the lowest accuracies of 60 % and below, while the other combinations of three behavioural parameters achieved peak accuracies between 80 and 90 %. Interestingly, in Group Mix2, the combination of LA, RTH, and Guardian achieved 99 % accuracy by 23:00 and matched the prediction accuracy obtained with all four parameters (Fig. 8f). The combination involving the three behaviours RTH, Guardian, and Follow revealed consistent accuracies ranging from 55 % to 60 %.

Considering the spawning prediction accuracies from both groups Mix1 and Mix2, combinations excluding LA resulted in significantly lower accuracy (50–60 %) and the behaviour that had the lowest impact was Guardian. It consistently resulted in an accuracy of only 50 % in both groups across all hours. The regression model demonstrated high predictive accuracy when all behaviours were considered together, reinforcing its effectiveness in detecting complex behavioural patterns and predicting spawning nights. However, for paired and three parameters the highest accuracy of prediction (> 90 %) consistently appeared while using the LA and the three behaviours. The paired combination of LA and RTH achieved high accuracies of 100 % in Group Mix2, which was equal to prediction accuracies obtained with three or four parameters, while in group Mix1 the accuracies of this paired combination (LA and RTH) were similar to the accuracies obtained with three parameters

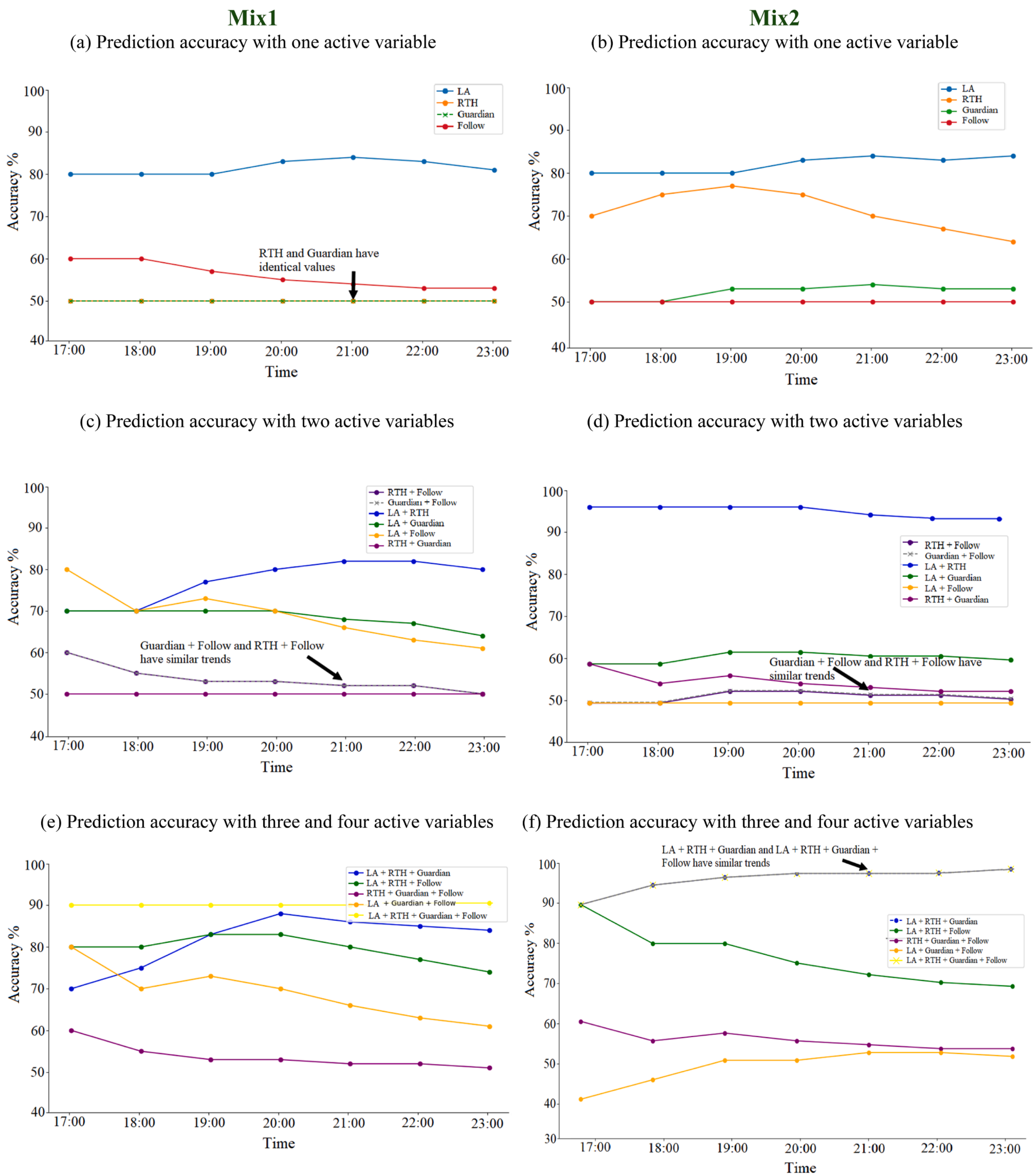

Fig. 8. Group Mix1(a, c, e) and Mix2(b, d, f) accuracy in %, to predict spawning and non-spawning nights using the combinations of RTH, Guardian, Follow, LA over Time in hour. Figures a and b used one active behavioural variable, c and d used two behavioural variables, and e and f incorporated three or all four behavioural variables—RTH, Guardian, Follow, and LA.

and close to the accuracies with all four parameters.

4. Discussion

The automated model developed and tested in the present study quantified specific behaviours (RTH, Guardian, and Follow) common in courtship preceding spawning, and measured LA, which increases in association with spawning in Senegalese sole [6,10,18]. The model achieved high accuracy and precision (>89 %) while eliminating the need for time-intensive manual video analysis. In addition to facilitating video analysis, the model incorporated a linear regression–based module that predicted spawning events from behavioural data, achieving >90 % accuracy. In the present study, the behaviours RTH, Guardian and Follow were analysed for the first time over the entire night, including both spawning and non-spawning nights. This study revealed a general increase in these behaviours during the initial period of spawning nights, with higher periods of activity between 17:00–19:00 (Mix1) and 21:00–22:00 (Mix2).

A key advantage of automated behavioural analysis is the substantial reduction in manual effort. Previously, Fatsini et al. [10] were required to manually observed 180 min from five nights per tank for courtship behaviours (RTH, Guardian, Follow, and Coupled) and 3120 min per tank per season for LA. In addition, to correctly score videos, multiple observers would repeatedly watch these minutes of videos. Our automated system processes videos and identifies behaviours with minimal human intervention, enabling continuous tracking over extended periods and generating larger datasets. Researchers can now focus on data interpretation rather than manual observation, allowing more comprehensive behavioural studies. This extended analysis was facilitated by automated methods. Similarly, Wang et al. [24] developed a deep learning model combining RGB and optical flow data for fish behaviour detection, demonstrating that automated approaches can analyse full recording periods continuously. Together, these studies show that automated methods enable comprehensive behavioural analysis across entire nights, capturing detailed patterns impractical to quantify manually. This approach support more extensive studies without the resource demands of manual observation

The automated model achieved accuracy >90 %, consistent with other machine learning approaches in aquaculture [14,28]. Recent YOLO-based studies demonstrated comparable performance for fish behaviour detection [24,25], while deep learning approaches proved effective for breeding behaviours [8] and feeding patterns ([16]: 80 % accuracy). Our results (accuracy 87.49 %, precision 82.16 %, specificity 96.31 %) demonstrate similar performance levels, indicating that automated systems can effectively replace manual observation across diverse aquaculture applications.

Despite of this high accuracy, model performance depends on environmental factors including camera position, image quality, lighting, and tank characteristics. Retraining may be required when applying the model to different settings. When comparing model outputs to manual annotations by Fatsini et al. [10], the main error sources included misclassification between similar behaviours (e.g., RTH vs Guardian), false detections from environmental artefacts (Fish Nest – a fish shape left in the sand by a fish), and reduced detection accuracy for distant or partially occluded fish (Partial Images, Blurred Images, Reduced Features). These limitations largely reflect the natural class imbalance in video data, where most frames contain no target behaviours. Detailed descriptions of each error type are provided in Supplementary Text S1.

For Follow, the model was tested over five spawning nights, on average the model exhibited a low FP rate of 1.33 %, indicating a good level of precision. However, the FN rate was higher at 8.92 %, suggesting that the automated model may miss some instances of Follow (Fig. 4). The model generated FP due to certain movements, such as bubbles from aeration, that could mislead detection. These discrepancies arose from factors like movement artifacts and occlusions in the video footage, as well as limitations in the tracking algorithms. For the Follow specifically, a single model was trained because the dataset was consistent, with instances of fish appearing mostly similar. The performance of this model was satisfactory, achieving an accuracy of 89.75 %, a precision of 86.57 % (Fig. 4) and correlation coefficients $R = 0.99$. These results demonstrate that the model effectively detected and analysed Follow behaviours, given the uniform nature of the dataset.

However, there are some limitations in applying such automated methods to dynamic breeding environments. Factors such as varying breeding densities and interference from environmental conditions face significant obstacles to behaviour recognition accuracy. Therefore, further research would be necessary to enhance the algorithm's adaptability and reliability in real-world aquaculture settings. In contrast to the work of Du et al. [8], the present study focused on the reproductive behaviour of a flatfish species, Senegalese sole. Flatfish present unique challenges and advantages for behaviour recognition due to their benthic nature and the subtlety of their behavioural cues. The method developed to validate automated results by comparing them with manual observations ensured accuracy, an approach that has been applied across different species and behavioural studies [14,25]. One significant advantage of this approach is its ability to handle the complexities of flatfish behaviour. Senegalese sole exhibit less pronounced and more nuanced behaviours compared to the species studied by Du et al. [8]. For example, our study identified specific reproductive behaviours such as RTH, Guardian and Follow each with varying levels of accuracy. RTH and Guardian behaviours are very similar and require careful training of data; hence, five different models were trained and compared their performance with manual observations. On the other hand, Follow involves tracking multiple fish, which presents a significant challenge due to interactions and overlapping movements, but the present study achieved reliable tracking and analysis.

Several studies have focused on using fish behaviour to predict feeding and welfare or disease problems. For example, Måløy et al. [16] introduced a Dual-Stream Recurrent Network designed to automatically capture the spatiotemporal behaviour of salmon during swimming. This innovative method validated on a dataset comprising underwater salmon videos, yielding a prediction accuracy of 80.0 % on the test dataset. In the study by Liu et al. [15], a computer vision-based method was applied to measure the feeding activity of Atlantic salmon in recirculating aquaculture systems (RAS), utilizing a camera mounted above the rearing tank. This method specifically targeted feeding activity by analysing the intensity summation of difference frames due to fish motions. In contrast, the present study focuses on automated behaviour detection and tracking for Senegalese sole in RAS. For this purpose, machine learning algorithms were used to analyse various aspects of Senegalese sole behaviour, including reproductive behaviours, locomotor activities, and the prediction of spawning nights. In comparison to previous studies, our model not only detects and tracks behaviours but also provides predictive insights into spawning events, offering a practical tool for aquaculture management. The study by Iqbal et al. [13] introduced an automated method utilizing CNN for early detection of changes in fish behaviour patterns, crucial for aquaculture productivity. The study focuses on an automated method for early detection of changes in fish behaviour patterns, our research extends beyond behaviour pattern detection to encompass the prediction of spawning nights in addition to analysing reproductive behaviours and tracking LA. This broader scope allows for a more detailed understanding of Senegalese sole reproductive dynamics, improving the utility of automated methods in aquaculture management.

In addition to effectively capturing behaviours and activity, our model incorporated a linear regression approach to predict spawning and non-spawning nights through four key parameters: three behaviours and LA. The regression model was tested across both groups, Mix1 and Mix2 (Fig. 8). Predictive performance was strongest when LA was considered alongside the primary behaviours RTH, Guardian, and Follow, highlighting the importance of combining both dynamic and static behaviour indicators for accurate spawning predictions. Using recent advancements in machine learning, similar studies have showcased their effectiveness in different fish-related tasks, such as automated recognition of breeding behaviour in broodstock [8], real-time detection and tracking of abnormal behaviour [25], and automated analysis of zebrafish behavioural responses in controlled experiments [5]. These approaches pave the way for long-term behaviour monitoring systems that, like ours, provide actionable predictions to support aquaculture management.

Interestingly, LA emerged as the strongest predictor across both groups, with accuracies ranging from 80 % to 84 %, higher than any other individual parameter. This can be attributed to the fact that LA captures elements of RTH, Guardian, and Follow behaviours. The model registered any movement lasting more than three seconds, which incorporated both the smaller movements associated with RTH and Guardian, and the longer movements typical of Follow. While RTH and Guardian were less important predictors on their own, their contribution became more useful when combined with LA. The relatively low weight of Follow in the regression model might be explained by its overlap with

LA, suggesting that much of its predictive power is already captured by the latter. The model also demonstrated high predictive accuracy across different time slots, particularly between 20:00 and 23:00, when spawning behaviour intensified. Among the behavioural parameters, LA and RTH were easier to incorporate and analyse in the model, compared to the Follow, which required tracking in association with algorithms to determine that individual fish movements were within speed and distance thresholds. These findings highlight the importance of LA and RTH as primary spawning predictors, suggesting that future models could focus on these parameters for simplified yet effective prediction systems.

5. Conclusions

In this study, the behaviours RTH, Guardian, Follow, and LA were analysed in Senegalese sole, and their application in predicting spawning and non-spawning nights was investigated. By integrating these behavioural indicators into a linear regression-based predictive model, the study successfully identified periods of increased spawning activity and achieved high predictive accuracy across different time slots. Among the parameters, LA and RTH were the most informative, highlighting their potential as key predictors for simplified future models. The automated approach presented here not only reduces the reliance on time-consuming manual video analysis, but also allows continuous monitoring of behaviours over extended periods, providing detailed insights into reproductive patterns. This capability is particularly valuable for aquaculture operations, as it enables better planning of breeding strategies, improved resource allocation, and informed decision-making.

Future research could extend these methods to other flatfish species or adapt them for different aquaculture environments. Efforts may also focus on integrating additional behavioural indicators, optimizing model efficiency, or applying the approach to larger-scale production systems. This research establishes automated behavioural analysis as a viable solution for reducing dependence on wild-origin breeders while enabling more efficient and sustainable Senegalese sole aquaculture.

Ethics statement

Not applicable: This manuscript does not include human or animal research.

If this manuscript involves research on animals or humans, it is imperative to disclose all approval details.

If Yes, please provide your text here:

CRediT authorship contribution statement

Abdul Qadir: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Data curation, Conceptualization. **Neil Duncan:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Wendy Ángela González-López:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Data curation. **Elvira Fatsini:** Writing – review & editing, Validation, Resources, Investigation, Data curation, Conceptualization. **Francesc Serratosa:** Writing – review & editing, Supervision, Software, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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Signed by

Abdul Qadir

Tarragona, Spain, June 6th, 2025.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.atech.2025.101668.

Data availability

Data will be made available on request.

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