ASEAN Engineering Journal

Full Paper

APPLICATION OF YOLO MODELS IN THE DETECTION OF FISH BEHAVIORAL CHANGE UNDER ACUTE EXPOSURE TO SYNTHETIC ESTROGEN IN THE ENVIRONMENT

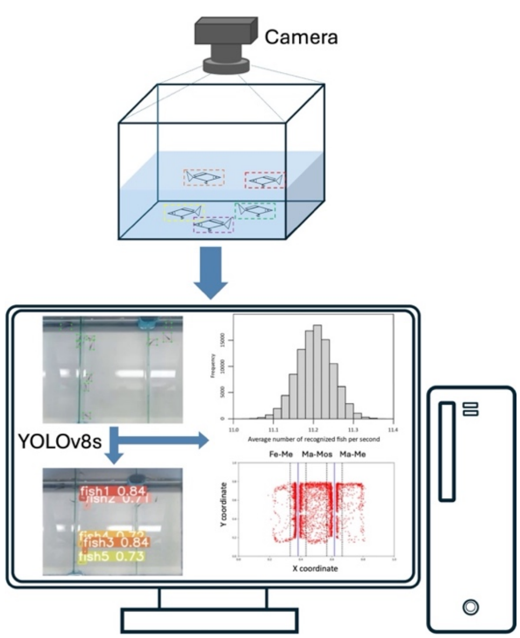
Bui Huy Kien[a], Nguyen Duc Hieu[a], Nguyen Van Thang[a], Nguyen Hoang Oanh[a], Vu Hai[b], Dang Minh Hieu[a]*

[a]Vietnam Japan University, Vietnam National University, Hanoi, Vietnam
[b]School of Information and Communication Technology, Hanoi University of Science and Technology, Hanoi, Vietnam

Article history
Received
03 June 2024
Received in revised form
28 August 2024
Accepted
31 August 2024
Published online
31 May 2025

*Corresponding author
dm.hieu@vju.ac.vn

Graphical abstract



Abstract

Changes in the behavior of small fish have recently been commonly used in assessing the impact of water pollutants, especially those of the group of endocrine disruptors. Behavioral studies mostly use visual observations, which can introduce bias and inconsistency in observational results. Recent studies have developed computer vision tools for tracking fish movements that allow automatic detection of small fish movements with high accuracy and consistency. In addition, computer vision combined with machine learning can help analyze, identify, and predict changes in fish behavior, easily integrated into environmental and ecological monitoring systems. This study uses YOLO (You Only Look One) algorithm models to detect fish in video data. Comparing the effectiveness of YOLO versions with the training data set shows that the YOLOv8s model has the highest efficiency and is selected for detecting and analyzing fish behavior in the environmental impact assessment model. The amount of image data for training the YOLOv8s model is also determined to be approximately 800 images. The training results show that YOLOv8s has high detection efficiency with a high frequency of detecting 11 fish in video frames. Results from detecting and analyzing fish positions in video data using the trained YOLOv8s model showed that the males of both mosquitofish (*Gambusia affinis*) and medaka (*Oryzias latipes*) species were affected following a two-day acute exposure to the estrogenic stressor, 17α-ethinylestradiol, in the aquatic environment at a concentration of 5 ng/L. While male mosquitofish when not exposed to estrogen tended to pay more attention towards the tank compartment containing female medaka, when exposed to estrogen, they increased their tendency towards the compartment containing male medaka. Additional research is needed to increase the accuracy and effectiveness of the YOLO algorithms in fish detection for behavior evaluation in an environmental impact assessment model.

Keywords: 17α-ethinylestradiol, YOLO, computer vision, fish behavior, object detection.

1.0 INTRODUCTION

Water quality has become one of the main topics discussed on a global scale in recent decades [1]. Among aquatic pollutants, a group of substances known as endocrine-disrupting chemicals (EDCs) is thought to have major impacts on the health of humans and other animals, especially aquatic animals [2]. These substances possess the ability to affect the normal activities of the endocrine glands and thereby cause changes in the physiological activities, reproduction, and development of animals [3, 4].

Small fishes have long been widely used subjects in research on the environmental impact of EDCs because they are easily biologically exposed to these substances through various routes [5, 6]. Recent studies used small fishes, such as zebrafish, medaka, minnow, mosquitofish, etc., as potential and effective research models for neurological, pharmacological, genetic, and aquatic toxicology studies [7-11]. These studies are based on observing small changes in fish behavior when exposed to environmental chemicals that can induce behavioral changes. Orger and Polavieja (2020) have described quite comprehensively and in detail the classification of behaviors in zebrafish as the basis for studies on fish behavior [10]. Melvin et al. (2017) describe factors such as acclimatization and observation time as well as experimental design to the effectiveness and reliability of assessment of fish behavior in a laboratory setting [8]. Fish behavior studies so far have been largely based on naked-eye observations done by researchers and technicians in laboratory settings. This work is often tedious, time-consuming, and requires trained laborers. In addition, such observations create uncertainty and inconsistency in results between observations done by different researchers/technicians.

Computer vision is one of the fields of artificial intelligence that uses deep learning methods to create computer vision models with the ability to acquire, recognize, process, and analyze digital images, including biological images [12, 13]. Using computer vision techniques to automatically study the characteristics and behavior of one or more fish individuals has recently received increasing interest in the research community [14, 15]. Computer vision typically involves collecting 2D and 3D images of research objects, processing and analyzing the images with various computer vision models, and making decisions. By replacing the human eye and brain with digital cameras and computers, computer vision techniques can make the analysis of fish behavior in the aquatic environment easier, more accurate, and more consistent [16]. Computer vision techniques can also make data collection for environmental impact assessments easier, faster, and less costly and labor intensive. Combined with automated decision-making tools that allow for a deeper understanding of the interlinkages and complexities of ecosystems and likely future impacts, automated data collection and detection tools such as computer vision can contribute to improve data analysis and predictions to inform environmental impact assessment decisions [17].

You Only Look One (YOLO) is a computer vision technique first introduced in 2016 by Redmon and colleagues that uses a Convolutional Neural Network (CNN) architecture to detect the position and shape of objects [18, 19]. It presented for the first time a real-time end-to-end approach for object detection. This approach significantly improved detection speed compared to previous methods, making real-time object detection feasible. Since its introduction, YOLO has evolved through multiple versions, each enhancing performance and accuracy [20]. YOLOv2, introduced in 2017, has included batch normalization, anchor boxes, and a higher-resolution classifier, leading to accuracy improvement and faster training times [21]. YOLOv3 further refined the architecture by incorporating Darknet-53, a deeper feature extractor, and multi-scale predictions, which enhanced its ability to detect objects at different scales [22]. YOLOv4 kept the same YOLO philosophy with characteristics of real-time, open source, single shot, and a darknet framework [23]. YOLOv5 gained popularity due to its implementation in PyTorch, making it more accessible and user-friendly. YOLOv5 provides five sub-versions: YOLOv5n (nano), YOLOv5s (small), YOLOv5m (medium), YOLOv5l (large), and YOLOv5x (extra large). The most recent version, YOLOv8, was released in January 2023 by Ultralytics, the company that developed YOLOv5 [24].

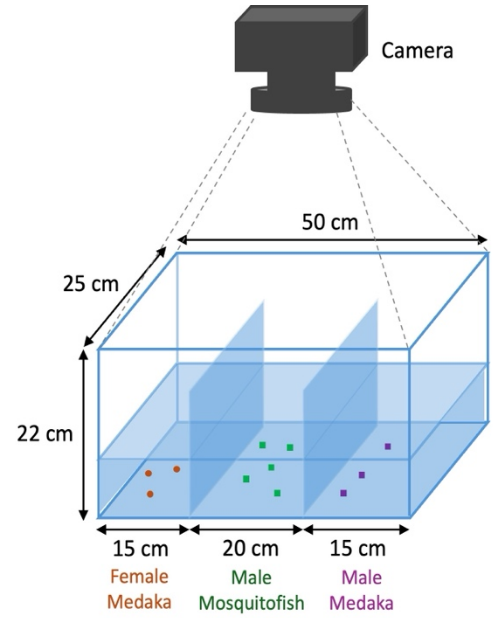


Figure 1 Setup of fish behavior model for environmental impact assessment.

Following the current trend, YOLOv8 is anchor-free, reducing the number of box predictions and speeding up Non-maximum Suppression (NMS). This simplifies the detection process, leading to faster and more accurate predictions. In addition, YOLOv8 uses mosaic augmentation during training, enhancing the diversity and robustness of the training data, which improves the model's ability to generalize and accurately detect small objects. This makes YOLOv8 particularly adept at detecting small and intricate objects, a crucial capability for monitoring aquatic environments where precise identification of small fish species like medaka fish and mosquito fish is necessary.

YOLO has so far developed through many versions. The technique has been widely used and proved efficient in smart agriculture to identify different types of plants [25-28]. This study evaluated the use of different versions of the YOLO model to identify and locate small fish species, such as medaka and mosquitofish, in a fish behavior assessment model. In addition, the study also examined the use of the appropriate YOLO model in assessing behavioral changes in medaka and mosquitofish when exposed to synthetic estrogenic contaminants in the aquatic environment.

2.0 METHODOLOGY

2.1 Fish Behavior Assessment Model Setup

The fish behavior assessment model is built with a glass tank with dimensions of length × width × depth of 50 × 25 × 22 cm, respectively. The tank is divided into three compartments because the inner glass wall is fixed but not airtight, allowing chemical interactions between the fishes in the compartments, if any, to still take place. The small fish used in the study included two fish species commonly found co-living in shallow, freshwater areas, including medaka (*Oryzias latipes*) and mosquitofish (*Gambusia affinis*). Fish were caught directly from

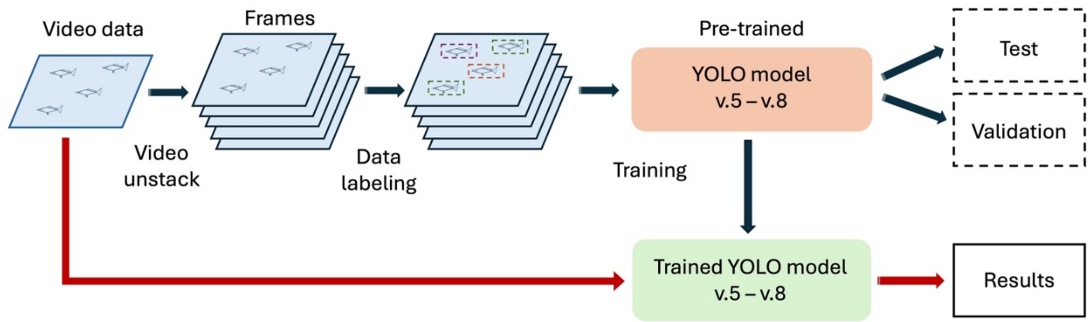


Figure 2 Training procedure of YOLO models for fish detection in video data.

the environment and acclimatized in laboratory conditions for two weeks before being selected according to a uniform size, which are 3.5±0.05, 3.4±0.05, and 2.5±0.05 cm of body length for male mosquitofish, male medaka and female medaka, respectively, and distributed into compartments in the tank as shown in Figure 1. The water temperature was maintained at 23±0.5°C, dissolved oxygen remained at 8±0.3 ppm, and pH was 7.2±0.1. A camera (Fujifilm, Tokyo, Japan) was mounted above to record fish movements in the chambers vertically from above.

In this model, fish were exposed to a synthetic estrogen, 17α-ethinylestradiol (EE2) (Sigma-Alrich, Germany), at a concentration of 5 ng/L. Ninety percent (90%) of the water in the aquarium will be changed daily to ensure that the EE2 concentration in the water is always maintained at 5 ng/L throughout the experiment. Fish in the compartments are fed a sufficient amount once a day. Laboratory conditions were maintained at an ambient temperature of 24 - 26°C, and the light-dark circle was 12-12 hours.

2.2 Data Preparation

At 2 and 4 days after initial exposure to EE2 in the water, the camera was turned on and recorded the movements and positions of the fish in each chamber. The camera was operated at a frame rate of 60 frames per second (fps) with a resolution of 1280 × 1024 pixels. The data collected are 10-minute videos. A ten-minute video recording of the movements of the fish in a separate tank without EE2 was also recorded as control data.

For training preparation, frames were extracted at a rate of 1 frame per second from the collected videos. The images were randomly sampled from the dataset and divided into data packages consisting of 50 to 1200 images. Each image was manually labeled with a single class, and bounding boxes were drawn around each fish. For each data package, the data was standardized to a size of 640 × 640, which is the input size for YOLO models. Subsequently, 80% of the data was used for training, while the remaining 20% was used to evaluate the model during training.

2.3 Training Procedure For Fish Detection With The YOLO Architectures

In this study, different versions of the YOLO algorithm model, versions 5 to 8, were used and trained for fish recognition on the acquired video dataset. For each version of YOLO, there are different corresponding sub-versions named with the characters x, l, m, s, and n. The process of training and analyzing video data is described in Figure 2. Different numbers of frames were used to train different versions of the YOLO models and compare their performance in fish detection from the video data to select the

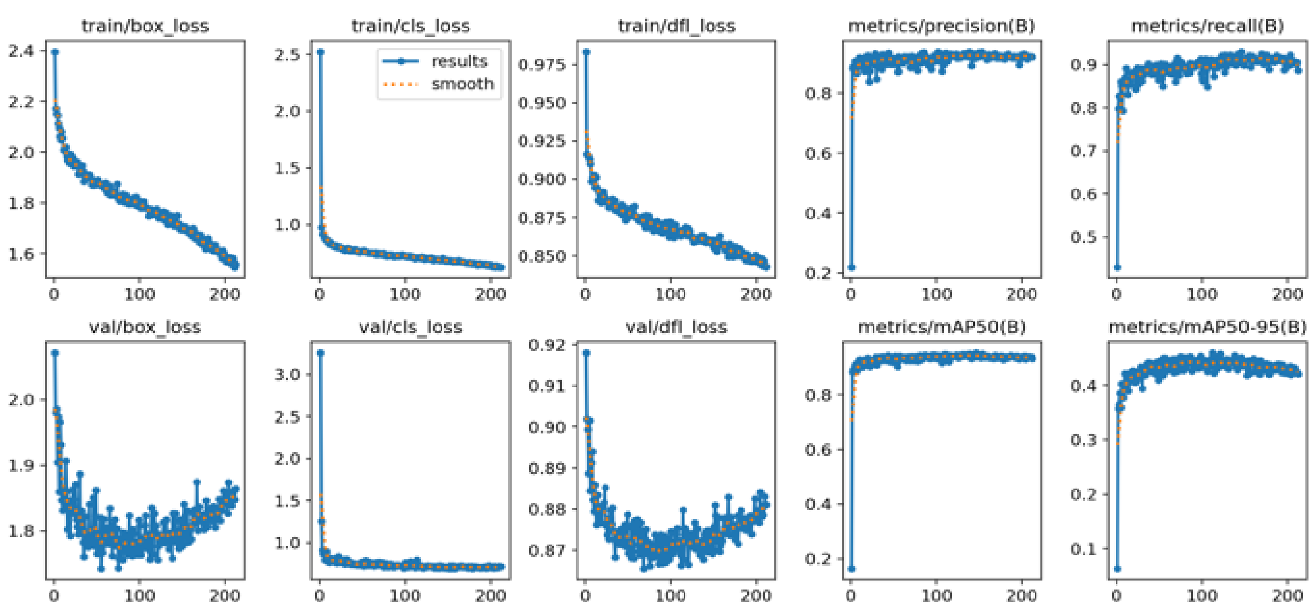


Figure 3 An example for training procedure of YOLOv8s with 800 images.

best version. The final selected version is used for fish detection in the acquired video data.

The model training took place on the Windows operating system, within the Anaconda environment, using the PyTorch framework. All tasks were carried out on a computer with a 13th generation Intel Core i9 processor, 32 GB of RAM, and utilizing Nvidia RTX A4000 graphic card. The YOLOs were trained for 400 epochs, and the model with the best-performance was selected. Figure 3 shows an example of the training results for the YOLOv8s model with 800 images. The model converges quickly after about 50 epochs. Although the model shows signs of overfitting after around 100 epochs, as indicated by the validation in box loss and distribution focal loss, the metrics (precision, recall, mAP) remain stable and high, suggesting that the model still performs well on the validation set.

2.4 Metrics

Several metrics have been used to evaluate the performance of object detection models, such as precision, recall, average precision, etc. In evaluating fish behavior detection models, it is essential to select appropriate metrics that provide a comprehensive understanding of the model's performance. Two key metrics - mean Average Precision (mAP) and Intersection over Union (IoU) - are chosen for this purpose due to their ability to address critical aspects of object detection: classification accuracy, detection accuracy, and position estimation. For the fish classification, the training data includes one base class labeled "Fish". Once an object is detected, it must be classified as a fish. For the fish detection accuracy, the model should detect exactly 11 fish in the tank, reflecting the consistency of the experimental conditions and we will assess this aspects in section 3.2. And for the fish position estimation, this study uses two metrics, mean Average Precision (mAP) and Intersection over Union (IoU), to evaluate and compare the performance of different versions of the YOLO algorithm in fish detection on an environmental impact assessment model.

Mean Average Precision is often used as a standard metric to analyze the accuracy of an object detection model. mAP values are calculated over recall values from 0 to 1. Average Precision (AP) is calculated as the weighted mean of precisions at each threshold; the weight is the increase in recall from the prior threshold. mAP is the average of the AP of each class that can be calculated as:

$$\text{mAP} = \frac{1}{N}\sum_{i=1}^{N} AP_i$$

where, APi is the AP of class i, and N is the number of class.

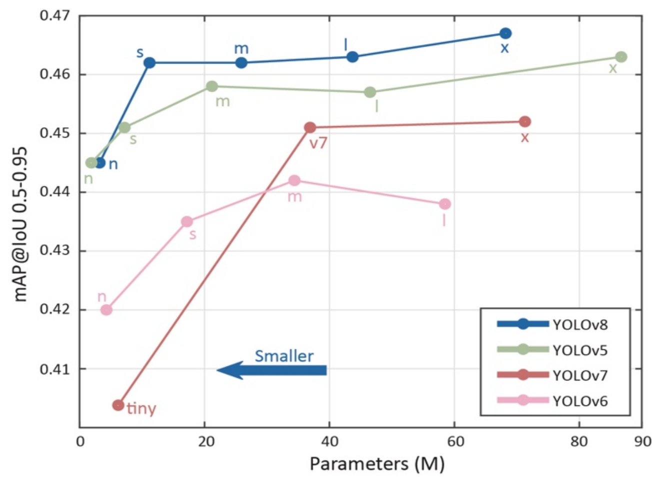


Figure 4 Comparison of YOLO versions on fish detection performance.

Meanwhile, Intersection over Union indicates the overlap of the predicted bounding box coordinates to the ground truth box. Higher IoU indicates the predicted bounding box coordinates closely resembles the ground truth box coordinates. IoU is among crucial indicators for assessing the model's accuracy and can be calculated as follows

$$IoU = \frac{Area\ of\ Overlap}{Area\ of\ Union}$$

3.0 RESULTS AND DISCUSSION

3.1 Comparison of YOLO Versions On Fish Detection Performance

In this experiment, versions 5 to 8 of the YOLO model have been tested and compared for object detection performance. Each version comprised several sub-versions named with characters n, s, m, l, and x. The mAPs of each version at the IoU threshold from 0.5 to 0.95 are shown in Figure 4.

As indicated, in comparison to previous versions like YOLOv5, YOLOv8 exhibits notable improvements. However, YOLOv8 improves upon YOLOv5 with a more advanced architecture featuring the C2f (Cross-stage partial bottleneck with two convolutions) module, an anchor-free model, and a decoupled head for better task-specific processing. It utilizes enhanced loss functions (CioU - Complete Intersection over Union and DFL - Dynamic Feature Learning) and advanced activation functions (sigmoid for objectness, softmax for class probabilities), leading to higher accuracy, especially for small objects. YOLOv8 also offers a state-of-the-art semantic segmentation model (YOLOv8-Seg) and achieves superior performance metrics, such as a 53.9% AP on the MS COCO dataset, compared to YOLOv5's 50.7%. Additionally, YOLOv8 is flexible with CLI and PIP package options for easy deployment [29].

Compared to sub-versions of YOLOv8, the YOLOv8s model with 11.2 million parameters is slightly larger than the YOLOv8n with 3.2 million parameters but achieves significantly higher accuracy. Meanwhile, the YOLOv8m, YOLOv8l, and YOLOv8x models have larger architectures with more than 25.9 million parameters but do not offer much higher accuracy. Therefore, for a compact sub-version that balances cost savings and low network weight with improved accuracy, the YOLOv8s is chosen for the fish detection model.

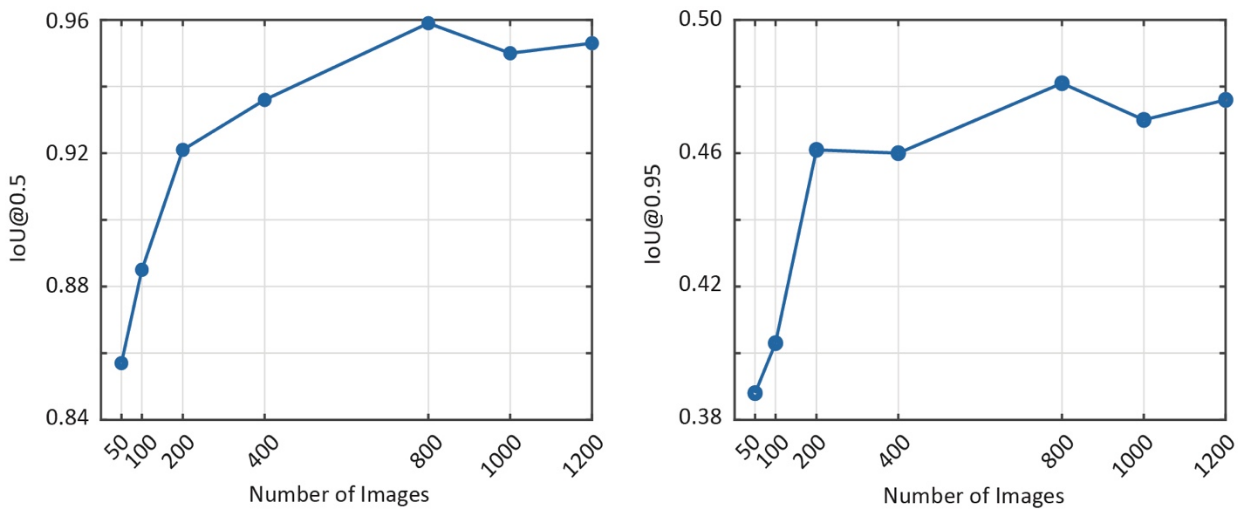


Figure 5 The accuracy of YOLOv8s when traning on different training datasets with the two IoU thresholds of 0.5 (left) and 0.95 (right).

3.2 Traning and Evaluation of YOLOv8s for Fish Detection

The YOLOv8s training process was first tested and validated with a training-to-validation ratio of 0.8 to 0.2. The accuracy of YOLOv8s in detecting fish during training is illustrated in Figure 5, with two IoU thresholds of 0.5 and 0.95. The data shows that fish detection accuracy increases with the size of the training datasets. Dataset packages containing 400 to 800 images are sufficient for training. However, even if the number of images in the training datasets exceeds 800, the accuracy of the model does not improve significantly.

The effectiveness of the YOLOv8s model after training with 800 images was evaluated based on the number of fish detected per second during the experiment. This is an important measure to ensure the model operates properly, effectively, and with high accuracy in detecting fish. In this environmental impact assessment model with small fishes, the total number of fish in all compartments is 11 fishes. However, at each time, due to the fixed position of the camera and capturing angle, lighting, and movement of the fish in overlapping areas of each other from a top-down view, the YOLO model cannot perform the task of distinguishing and detecting each fish. This causes the number of fish detected in some frames to be either lower or higher than the actual number of fish in the tank, which is 11 fish in total. Figure 6 shows the frequency of detecting the full number of fish in the experimental tank by the trained YOLOv8s model. In the 10-minute experiment, 600 images were used to detect fish with YOLOv8s. Nearly 200 images were detected as containing 12 fish. This issue arose due to the fish's shadow being cast onto the tank's wall, resulting from suboptimal experimental conditions. Additionally, over 100 images showed only 10 fish because the fish moved into a corner where light reflected onto the camera. In future experiments, we will aim to improve the experimental environment to maximize YOLO's performance in fish monitoring. Overall, the results show that the fish detection efficiency of the YOLOv8s model after training is high, as the number of fish detected in the tank is most often 11.

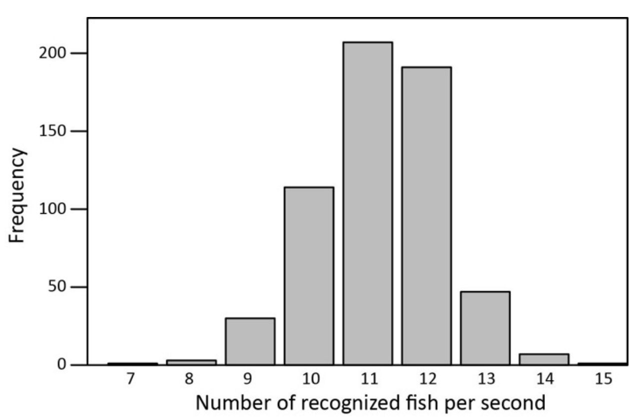


Figure 6 Frequency of numbers of fish recognized per second.

3.3 Assessment of Change In Fish Behavior In Respondents To The Appearance Of An Estrogenic Stressor In The Environment Using the YOLOv8s Model

In this experiment, the trained YOLOv8s model was used to analyze 10-minute videos capturing the movements of fish in an environmental impact assessment model. The position of the fish at each detection time is marked. Figure 7 shows the frequency of fish appearing at different locations in the experimental tank.

Male medaka and male mosquitofish showed significant behavioral changes after two days of environmental exposure to EE2, while female medaka did not show significant behavioral differences. When comparing the fish detection results between the experimental model (exposed to EE2 at a concentration of 5 ng/L) and the control model (not exposed to EE2), the frequencies of appearance of female medaka (in the left compartment) in the area near the mid compartment where

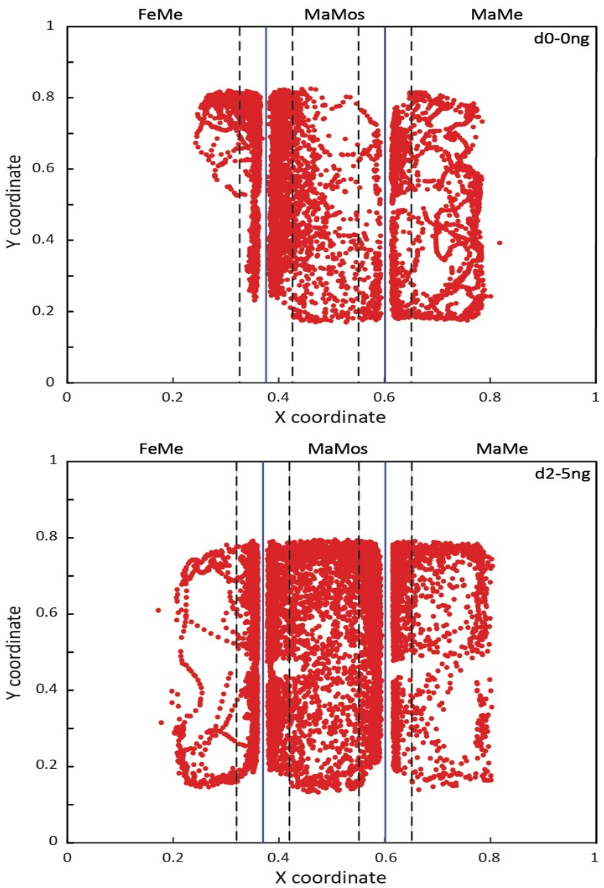


Figure 7 Fish behavior assessment under the presence of estrogenic stressor at different concentrations: 0 (upper) and 5 (lower) ng/L, using the YOLOv8s model. Solid lines in the middle of two dotted lines denote transparent fences, dividing the experimental tank into separated compartments. Dotted black lines were added to describe the proximity areas of approximately 4 cm around the fences. FeMe, MaMos, and MaMe denote female medaka, male mosquitofish, and male medaka, respectively, in their

holds the mosquitofish are not much different. Meanwhile, the frequencies of appearance of male mosquitofish in the experimental model in different areas within the compartment changed when their frequency of appearance in the areas near the right compartment containing male medaka increased significantly. In the control model, male mosquitofish appeared mainly in the areas near the compartment containing the female medaka fishes. The presence of male medaka fish in the control model was distributed fairly evenly throughout the experimental compartment. However, after two days of exposure to EE2 at a concentration of 5 ng/L, these fish appeared mainly in the areas near the middle compartment containing the male mosquitofish.

The above results show that the behavior of fish in the environmental impact assessment model changed after exposure to estrogenic stressor EE2 at a concentration of 5 ng/L. Males of both medaka and mosquitofish were more affected by the presence of EE2 in the environment compared to females of medaka. EE2 has been reported to impact the behavior of small fish in the environment. At concentrations from 5 ng/L, EE2 can affect anxiety and shoaling behavior in adult male zebrafish (*Danio rerio*) [30]. Acute exposure to EE2 has been reported to reduce the boldness behavioral syndrome in female Siamese fighting fish [31]. Another study showed that EE2 had different effects on male and female guppies (*Poecilia reticulata*) [32]. Males decreased their risk-taking behavior, while females increased their risk-taking with increasing EE2 levels. There are only a few studies on the effects of EE2 on behavior and interactions between different species in the environment. Dang et al. (2017) reported an increase in the aggressive behavior of mosquitofish toward medaka when acutely exposed to EE2 in the environment at a relatively low concentration from 0.5 ng/L [11].

Over the past two decades or so, there has been increasing interest in a group of estrogenic compounds known as endocrine disrupting agents (CDCs) that are often present at trace or low concentrations in the environment and can mimic the function of estrogens in animals and humans [33, 34]. Examples of this group include natural and synthetic estrogens, contaminants from pharmaceuticals, personal care products, etc. Because the environmental concentrations of such chemicals are often very low, this makes it difficult for environmental regulators and governments to determine or predict their impacts on human and animal health and to prepare regulations for their production and use.

A commonly used method to determine the impact of estrogenic pollutants such as natural and synthetic estrogens in the environment is to use small animals such as juvenile fish to assess changes in their social, reproductive, and metabolic behaviors when exposed to pollutants [35-38]. Estrogenic substances can cause changes in the synthesis and metabolism of hormones in the animal body, thereby causing behavioral, reproductive, and various other abnormalities in exposed animals. This study suggests a tool that allows rapid, accurate, consistent, and automated determination of behavioral changes in fish when exposed to environmental pollutants, supporting the analysis, assessment, and prediction of impacts on the environment and ecosystems of pollutants. Replacing visual observations with cameras allows for extended observation periods to assess changes in fish during long-term exposure to pollutants and the long-term effects on fish. However, both the YOLO model and the experimental model need further development to allow for more accurate fish detection and to be able to track complex fish movements in the aquatic environment.

4.0 CONCLUSION

This study developed a computer vision technique using the YOLOv8s model to detect small fish, medaka, and mosquitofish, in 10-minute video data obtained from an environmental impact assessment model. The technique was demonstrated to be effective and highly accurate in detecting small fish in the video dataset. The results from training the YOLOv8s model showed a high frequency of detection of 11 fish in the impact assessment model. The results of fish recognition from the video data showed behavioral changes in males of both medaka and mosquitofish after acute exposure (2 days) to the estrogenic stressor, 17α-ethinylestradiol, introduced into the environment. Both the YOLO model and the impact assessment model need further development to increase the accuracy and efficiency in detecting fish and their complex movements. Computer vision techniques using YOLO models promise to be effective tools in supporting the analysis, assessment, and prediction of the environmental impacts of estrogenic pollutants, in particular, and behavioral-affecting chemicals, in general, in the aquatic environment.

Acknowledgment

This study was supported in part by the grants VJU.JICA.22.04 and VJU.JICA.23.03. The authors acknowledge the kind support from VNU Vietnam Japan University and JICA Vietnam.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

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