

Bioindication of Surface Water Supported by Automatic Image Analysis Using Deep Learning Neural Network – *Cyclotella* Case Study

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ABSTRACT

Bioindicative methods involving the identification and counting of indicator organisms (e.g. algae) are widely used methods in the assessment of surface water quality. For this reason, the purpose of this paper was automatic image analysis using the YOLO v8 deep learning neural network, directed at the detection of freshwater algae *Cyclotella*. Changes in the number of these organisms can indicate changes in the water quality and the trophic status of the reservoir, which makes automating their detection an important task. Traditionally, the detection and counting of objects in microscope images was done manually, but by using machine learning and especially neural networks, the process can be automated. YOLO (You Only Look Once) is an example of a network that, after proper training and validation, is capable of performing image detection in real time. In this study, the Roboflow object tagging tool was used to create a dataset divided into training, validation and test sets. Training of the network, validation of the model and evaluation of its metrics were carried out. The paper presents the obtained metrics of the YOLO v8 network on the validation set, such as *Accuracy* = 0.960, *Precision* = 0.964, *Recall* = 0.995. The presented results confirm the effectiveness of the applied method in automatic analysis of microscopic images containing algae and thus the high application potential of the method in supporting bioindication studies of surface water quality.

Keywords: bioindication, freshwater algae, surface water, microscopic samples, object annotation, automatic image analysis, computer vision, deep learning neural network.

INTRODUCTION

Currently, biological indicators, particularly species composition and abundance of algae, are considered an important element in assessing the ecological status of water bodies [EU WFD, 2000; Charles et al., 2021]. Diatoms are widespread unicellular organisms that play an important role in the functioning of aquatic ecosystems, producing a significant proportion of primary production [Serôdio, Lavaud, 2020]. Many diatom species are highly sensitive to changes in the quality of the aquatic environment, so they are

used as indicators of organic pollution, trophicity and acidification [Sladeczek, 1986; van Dam et al., 1994; Kelly et al., 2008; Stevenson et al., 2010; Charles et al., 2019; Çelekli et al., 2021].

The peculiarity of diatoms is that they have an armor (shell) made of two parts (lamellae) and composed of silicon dioxide. Taxonomic identification of diatoms is based on the morphology of the silica carapace and is a rather difficult task, as it requires a detailed study of the structure of the silica coverts [Kociolek et al., 2015]. Accurate identification of species allows a detailed biological analysis of the parameters of surface

water influenced by anthropogenic pressure, for example, the impact of storm sewers collecting rainwater of urban areas [Babko et al., 2019; Babko et al., 2020]. On the other hand, modern studies have shown that siliceous crust is subject to considerable variability under the influence of environmental conditions such that some species identified on the basis of morphological characteristics may represent ecomorphotypes (ecological variants) of the same species [Kociolek et al., 2015]. Given the difficulties in identifying species, for practical purposes of ecological assessment of the status of water bodies, the use of taxonomically coarse biotic data may be allowed, in particular the identification of algae to genus [Rimet, Bouchez, 2012; Edwards et al., 2020]. While such data will not allow an accurate determination of the saprobity or trophicity of a reservoir, it can be used to assess biodiversity based on the presence of so-called morphospecies – morphologically distinct representatives of diatoms. In turn, the use of information on individuals correctly assigned to a number of accepted sets (species or genera) allows assessing the situation based on the analysis of the values of quantitative biocenotic indices, for example, the entropy-based Shannon index and similar. Such an approach makes it possible to collect and use quantitative data in the analysis of the quality of the aquatic environment or the impact on it of urban technical infrastructure such as stormwater drains. Nevertheless, using quantitative data classifying individuals to a particular species is also useful in this type of analysis [Kozłowska et al., 2023]. Given the above, the present work addressed the study of *Cyclotella* objects and aimed to improve the work of hydrobiology researchers by automating the detection of *Cyclotella* objects in a microscopic sample.

Diatoms of the genus *Cyclotella* have drum-shaped short-cylindrical cells with circular lamellae 6-80 µm in diameter, the disk ornamentation consists of radial striations or ribs in the marginal part and a different-looking central field: smooth, punctuated, with striations or mottling; there may be insertions between the edge of the lamina and the peripheral stripe [Picińska-Fałtynowicz, Błachuta, 2012]. Species of this genus are found in rivers, streams, and lakes. The taxa belonging to this group are considered important ecological indicators in a wide range of environments, from oligotrophic to hypereutrophic [Kociolek et al., 2015]. They can also be one of the datasets

analyzed using ecological indices (e.g., the Shannon index and similar) that quantitatively describe community structure [Kozłowska et al., 2023].

Thus far, the process involved a skilled biologist manually counting objects from a microscope image or photograph. Such a person had to spend a lot of time reviewing the images, during which task he had to recognize and count these objects manually, each time he prepared a new sample for testing the whole lengthy and laborious process started from the beginning. In order to speed up and automate this process, an automatic image analysis method using deep learning neural networks was used.

Deep learning using neural networks is an advanced and extended version of classical machine learning which is mainly based on classification and regression tasks using simpler models compared to neural networks, such as decision trees, random forests, support vector method, etc. The idea of deep learning, which is part of artificial intelligence in the broadest sense, is to teach a neural network based on the available data so that it performs its task in the most optimized time and with the highest possible efficiency [LeCun et al., 2023]. In the case at hand, the task is to correctly detect *Cyclotella*.

Neural networks are characterized by a structure intended to resemble the operation of the human brain and are based on a neuron-like structure, depending on the type of layers and its application, several types are distinguished, characterized by different degrees of structure and a different architecture depending on the task for which they were created [Rocco et al., 2017]. The components of the basic network architecture are the layers responsible for each task. The first component is the input layer into which data is input in the form of, for example, images, where the information that a single pixel from an image has been represented by a single neuron of the network [Krizhevsky et al., 2024].

After the data is loaded into the network, it is processed by hidden layers (the complexity of the network depends on their number) each hidden layer can have a different number of neurons depending on the specifications of the network. In these layers the processing of data takes place with the help of appropriate mathematical procedures, thus the most important part of the operation of the network, i.e. the learning of patterns and dependencies by the network, occurs in the hidden layers. The last type of layers present in

the network is the output layer, where the final prediction of the network is generated, depending on the type of prediction it is supposed to make, for example, in classification tasks it corresponds to the number of classes the model is supposed to predict [Anand et al., 1993].

In automatic image analysis, CNN convolutional networks in particular have found application due to their complexity and the use of convolutional layers in which various types of filters are applied to the image to extract from it identifiable features, such as shapes, colors, texture, or other special features that can aid prediction. CNNs also have pooling layers; their task is to reduce the dimensionality of the data that has been fed into the model [Gu et al., 2018]. This results in a reduction in the number of network parameters, thus reducing the probability of network overfitting, which is an undesirable phenomenon, the situation is analogous to the phenomenon of network underfitting.

The first applications of neural networks in image analysis date back to the 1980s, when Kunihiko Fukushima first used a convolutional neural network to recognize handwriting by computer. His new approach is considered a milestone in research on image analysis and deep learning [Fukushima, 1980] it was the first attempt to teach a machine to “look” and a major step towards the development of artificial intelligence. In his project called “Neocognitron”, Fukushima used an innovative network architecture which consisted of multiple convolutional layers which enabled the processing of information in a specific hierarchy, Fukushima’s network was capable of learning without human supervision (so-called unsupervised learning), which helped to improve the process of image analysis and was considered a breakthrough discovery of those years, the idea behind it was that the network should mimic the operation of the human visual cortex as much as possible. Since the first application of CNNs, many things have changed, deep learning researchers have developed the concepts proposed by Fukushima improving the time of the calculations performed and the accuracy, networks have been created which deal with the tasks of image detection included in automatic image analysis much more efficiently, an example of such a network is the YOLO (You Only Look Once) network which was created by Ali Farhadi, Santosh Divvala, Joseph Redmon, and Ross Girshick in 2016

[Redmon, et al., 2016]. The YOLO network can be used for real-time detection and does not require the user to have a ready-made model of high computing power [Hussain, 2023]. YOLO, as the name implies “looks at a photograph only once” i.e. a single image passes through the network only once which causes the network to reduce the learning and prediction time. The first stage of processing by the YOLO network is to divide the image into a grid of squares. For each square, it determines the probability of the appearance of a rectangle (bounding box) indicating the location of the searched object. The probability results at the end are unified and the dimension of the rectangle is given, along with the class to which it points. In prediction mode, the YOLO network offers a bounding box outlining the object being searched for and indicating the probability of a given result. Another advantage of the YOLO network is that it can detect multiple objects simultaneously without losing too much efficiency, which can help biologists who care about identifying multiple species simultaneously on a microscopic sample, leading to a significant acceleration of the generally time-consuming research. In such a situation, all clearly visible individuals in the field of view can be identified and counted automatically, requiring the attention and intervention of a trained biologist only in a few, more complicated cases. An important advantage of the discussed networks is also the possibility of implementing the algorithm for real-time detection of objects directly in the microscope and using other capabilities of the YOLO network, such as image segmentation which will allow more accurate extraction and identification of the components of the searched object, analysis of the dimensions of the searched organisms on the basis of data about the frame surrounding the object, owing to which the network will be able to count the area of the searched object. This can be helpful in further accurate quantitative analysis of the searched organisms and creation of sets of descriptive statistics to complement the research [Aly et al., 2021]. Hence, the purpose of this paper was to test the hypothesis of the probable high efficiency and speed of the applied method in the automatic analysis of microscopic images containing *Cyclotella* algae, and thus to confirm the high application potential of the method in supporting bioindication studies of surface water quality.

MATERIALS AND METHODS

The study tested the feasibility of using neural networks to recognize microscopic images of centric freshwater diatoms of the genus *Cyclotella* (Figure 1). The dataset used for this task contains 1000 microscope images taken at x40 magnification of main lens with an Olympus CX4 microscope. These images contain a total of 1203 *Cyclotella* specimens. The dataset was divided into 3 parts: 70% of the dataset was randomly allocated to the training set, 20% was allocated to the validation set and 10% went to the test set. Dividing the dataset according to such proportions ensures that there is enough data on which the YOLOv8 network can learn, perform validation and check its fit on the test set.

The dataset was tagged in the Roboflow tool, which works closely with Ultralytics. This tool allows the processing of data in the form of images in a user-friendly way and does not require specialized knowledge [Ciaglia et al., 2022]. This tool and the functions it offers enable to accurately label objects in the image, a process that can be carried out one hundred percent manually, which, given the difficulty of recognizing microbial objects in a microscopic image, was used in this research and required consultation with a scientist specializing in hydrobiology, who made corrections and improved the quality of the dataset, during consultation on the accuracy of *Cyclotella* labeling. In the present work, the average number of objects per digital photograph is 1.2, the average size of the labeled *Cyclotella* object expressed in pixels –here it equals 0.79 mp. Once the photos were correctly labeled, the collection was divided into the aforementioned three classes using random balancing.

To train the YOLO v8 network for detection, the Python programming language supporting the use of libraries from Ultralytics was used, along with an environment that enabled the use of a free GPU –Google Colab. Learning of the model was carried out using a Tesla T4 GPU, which significantly accelerated the learning process of the network compared to the use of powerful personal computers. The YOLO v8 network was trained on 100 epochs. With the use of a T4 GPU, the process of training 100 epochs was relatively fast, with a calculation execution time of 0.49 hours. Learning began with initializing the weights randomly, then the image was passed through all three layers discussed earlier. During training, loss functions were calculated, very important during model evaluation because they are based on several errors the network can make. The most relevant for this research is an error that affects the precision in the final shape of the predicted box, this is the so-called localization error, which shows a comparison of the coordinates of the location of the predicted bounding-box with the actual coordinates that were marked in Roboflow. The next step in learning the network was to apply Backpropagation, an algorithm used to update the network weights and minimize the loss function [Rojas et al., 1996]. For this purpose, the study used the Adam (Adaptive Moment Estimation) optimizer with a learning rate of 0.002 and a momentum of 0.937 at the beginning of training, then the values of these parameters were updated on a regular basis.

The metrics calculated from the confusion matrix that were used to evaluate the network were *Accuracy*, *Precision* and *Recall*. Moreover, the *mean Average Precision* (mAP), derived from the Average Precision metric, plays a crucial role in evaluating the detection task performed by the



Figure 1. Examples of digital images detail containing *Cyclotella* used for training, validation and testing of YOLOv8 network

YOLO v8 network. The formulas describing the metrics using the afore-mentioned classes are shown below. In the referenced formulas, n denotes the number of classes while AP is the value of the Average Precision metric for the i -th class [Carvalho et al. 2019].

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \quad (1)$$

$$Recall = \frac{TP}{TP + FN} \quad (2)$$

$$Precision = \frac{TP}{TP + FP} \quad (3)$$

$$mAP = \frac{1}{n} \sum_{i=1}^n AP_i \quad (4)$$

After training the YOLO v8 network, the abundance of each class in the confusion matrix presented following training on the validation set is as follows. The respective class sizes are $TP = 245$, $FN = 1$, $TN = 0$, $FP = 9$. On the basis of on these values, the metrics $Accuracy = 0.960$, $Precision = 0.964$, $Recall = 0.995$ were counted, which indicate that the network performed Cyclotella detection almost perfectly. After model evaluation, the afore-mentioned metric $mAP = 0.991$ was automatically calculated. The model in

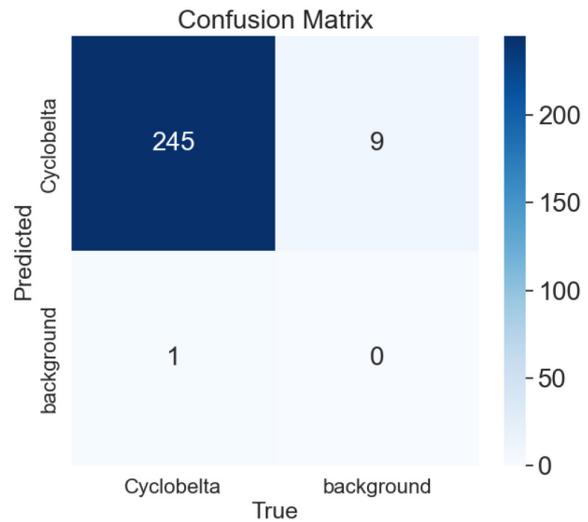


Figure 2. Confusion matrix for the YOLO v8

the detection task erred a total of ten times, once failing to recognize Cyclotella where it was present (FN) and nine times detecting it where it was absent (FP). In addition to the measures of model fit, YOLO also allows for a deeper analysis of the results obtained in the context of the width and height of the frames surrounding the objects, as well as the values of individual metrics for each epoch of network learning (Figure 2, Figure 3).

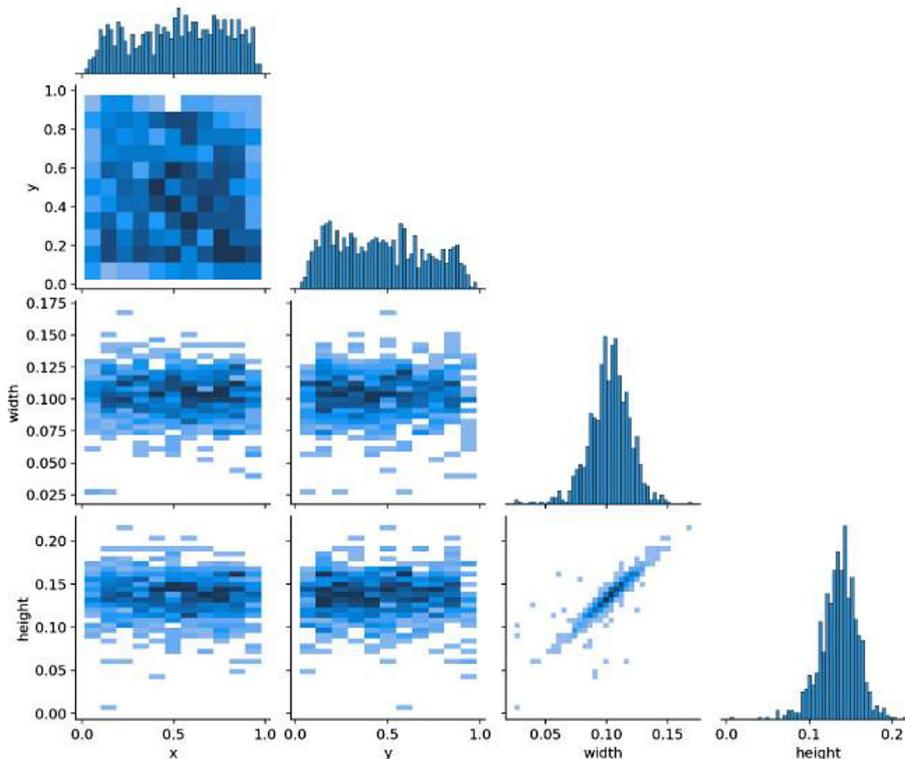


Figure 3. Height and weight distribution of rectangles after YOLO v8 predictions

From the graphs above, it can be seen that the network correctly predicted the shape of the object, most of the bounding-boxes were square, as indicated by the distribution of the height and width of the rectangles that surround the predicted object, the distribution is close to a normal distribution. After analyzing the results that the model achieved, tuning of the parameters of the best model retained after training was carried out, in terms of the greatest possible fit, the tuning was carried out for 50 epochs. The graphs showing the variation of the model's fit on the validation set during the tuning, and the parameters for which the model achieved the best fit on the set, are presented below (Figure 4).

The best fit the model obtained at epoch 47 and the metrics it obtained are: Precision = 0.990, Recall = 0.979, and mAP = 0.991. The variations of the most relevant model parameters during tuning such as learning rate (lr0 and lrf) and momentum, together with the value indicated with a + sign which turned out to be the best in the case of *Cyclotella* detection, are presented below.

This, it can be seen that parameter tuning in this case slightly improved the object detection capability, perhaps with the use of more computing power and parameter tuning for more epochs, a noticeable improvement can be achieved. The process of parameter tuning for 50 epochs required more time than just learning the network, the time was ~141 minutes despite using a cloud solution and using Tesla T4. This is almost five

times more than the time spent training the network for twice the number of epochs. This shows that the process is more time-consuming than just learning the network. On the best model that was obtained during parameter tuning, validation was performed on the basic settings offered by Ultralytics. This validation also did not result in a significant improvement due to the high metrics obtained during training and the high quality of the data in the set. The metrics between the original version of the model, the version after hyperparameter tuning and the one after validation differ by only a thousandth of a fraction in most cases. This shows the fact that the network has already performed admirably after training, and it can be difficult to achieve significantly better results without risking network overfitting, or without using data leakage. Finally, to perform detection prediction on the test set, the model that was created during tuning was retained due to the fact that it obtained the highest the metrics calculated from the confusion matrix.

The best model obtained during testing, after switching to prediction mode, made bounding-box predictions on 100 images presenting *Cyclotella* determining the exact probability of the appearance of a bounding-box edge in a given pixel.

The division of the dataset into training, validation and testing used during the study is not the only possible division used in deep learning an example of another division can be found in the work done by researchers, such as Plaksyvyi

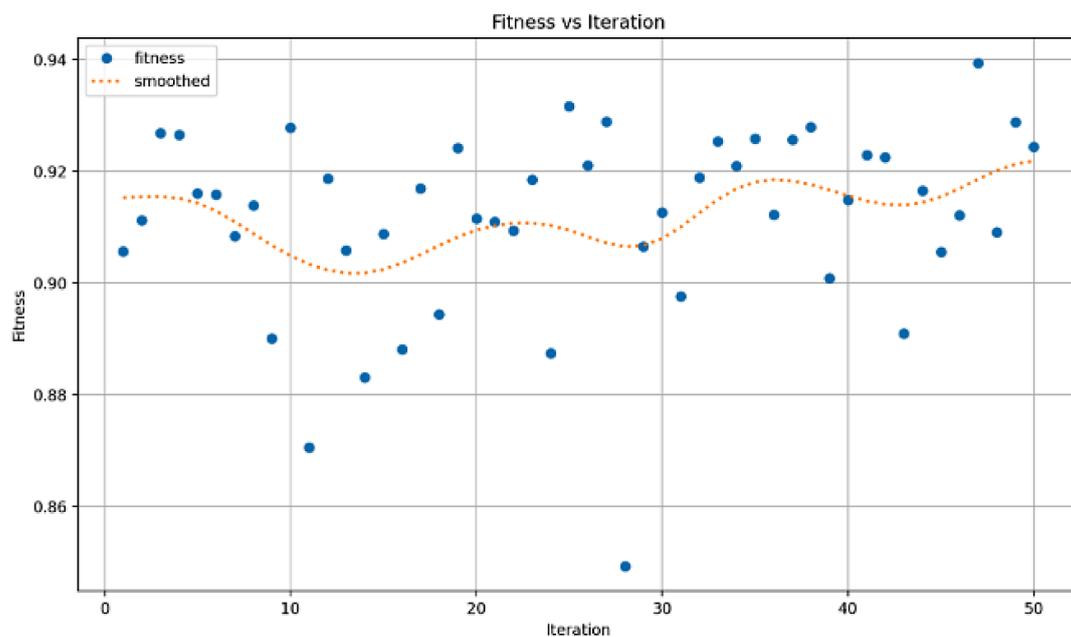


Figure 4. Model fitting during tuning

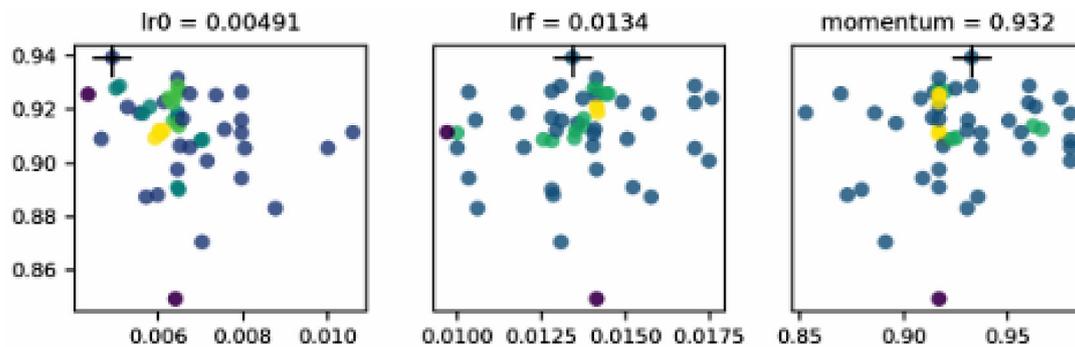


Figure 5. Parameters during the tuning of the YOLO v8 network

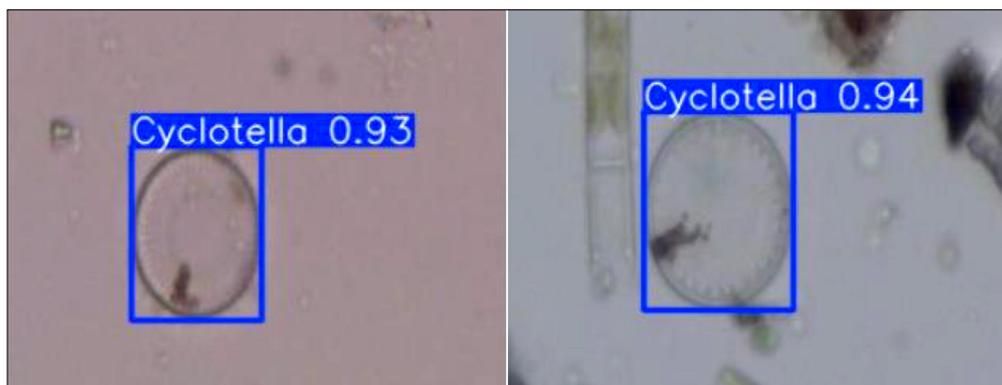


Figure 6. Two correctly detected Cyclotella objects

and his colleagues, who used a 6/2/2 division into training, validation and testing, respectively, for the study of deep learning networks, with the difference that the dataset was five times larger [Plaksyvyi et al. 2023]; thus, they could afford to use such a division without sacrificing the quality of the results and gaining less computation time when learning the network, in the context of research on a set of 1,000 elements the use of such a division would be pointless because the optimization of time that would be gained by training the network on 600 images instead of 700 would not be directly proportional to the efficiency of the network relative to the final results it obtained. The same division as that used in the Cyclotella object study was used by Dziadosz and colleagues [Dziadosz et al., 2024] in their study on activated sludge analyzed for detection of *Arcella vulgaris* objects by YOLOv4 and YOLOv8 networks. The dataset they operated with was similar in size to the one discussed in this article, amounting to 990 images. The use of such division resulted in excellent results by both networks, which shows the effectiveness of using deep learning networks in microbiology in detection tasks for circular objects visible in digital images.

From the viewpoint of learning the network, the randomness of the distribution of images in the way mentioned before is very important. This is a direct result of the way the images were acquired, the process took about a month by taking photographs of specific samples, digital images taken on a given day on a given sample are slightly different from images taken on another sample, so by teaching the network on images taken on, for example, the first sample and testing it on images taken on the last sample, there is a risk of over-learning the network and poor performance of the model on the test set. The images from each sample were characterized by different numbers of individuals, little change in the quality or appearance of the individuals and different levels of transparency of the sample under microscopic magnification, because they did not always come from the same sampling point on the river. Therefore, appropriate randomization of the collection during learning is very important.

The learning process of the YOLO v8 network, due to its architecture and complexity, is quite distinctive. The YOLO v8 network consisted of 3 main parts: the backbone, neck and head. Each of these parts performed a different task.

The backbone part was responsible for extracting the features of an object from a given image, which allowed the Cyclotella to be accurately determined. When the microscopic photograph was processed by the Backbone part, the network performed extractions of all Cyclotella's features from basic ones, such as its size at a given magnification to its specific structure, which resembles a coin and the radial stripes in the edge part leading from the center outwards and smooth or dotted (spotted) central field which are a distinctive feature of Cyclotella. This allows it to be distinguished from other "round" objects that are also present in the sample. The backbone layer consisted of multiple convolutional networks. After the image was processed through the first layer, the photograph went to the neck section where the data characterizing the Cyclotella object that had been extracted by the backbone section was aggregated using the PANet (Path Aggregation Network) which affected the accuracy of the prediction regardless of the scale of the object. The final stage was the passage of the photograph through the head part, where the final prediction of bounding-boxes on a given pixel was made in accordance with the previously discussed idea of CNN convolutional networks [Dziadosz, et al., 2024].

During network learning, the learning rate parameter in the optimizer was responsible for the length of the optimizer's jump to minimize the loss function, while the momentum parameter was responsible for the "timing" of the introduction of updated weights in the optimizer. As an alternative to the Adam optimizer, the Stochastic Gradient Descent (SGD) optimizer, which is often used in simpler tasks because it has fewer parameters for tuning and the static value of the parameters in it remains constant. However, Adam performs better than SGD in detection tasks. A feature of YOLO v8 network learning is that after each epoch it performs validations on the validation set to improve network performance and increase the quality of learning.

Evaluation of the quality of fit of the learned network was done using several key metrics. The standard metrics that are used to evaluate the model were calculated based on the confusion matrix, which distinguishes four states: true positive (TP) is the class of objects that were correctly identified; false negative (FN) is a class of objects that were in fact the object being searched for but the YOLOv8 network did not make a correct prediction and did not tag the object; false positive

(FP) comprises the cases in which, despite the absence of the object in the image, the network made a detection at the background location. The last class was true negative (TN); the size of this class in the considered case was 0, because the network was tasked with detection of only one object. The FN and FP classes were considered to be model errors.

The YOLO v8 network performed exceptionally well in detecting the Cyclotella object most probably due to its coin-like shape, but also the fact that the tuning and calibration of the network mentioned earlier was carried out. The near-circular geometry of this diatom contributed to a distribution of the height and width of the predicted bounding boxes that closely resembled a normal distribution. Objects with uniform and compact shape – in this case study even circular and symmetrical, are generally easier for neural networks to detect and learn because YOLOv8 tends to struggle more with irregular and asymmetrical shapes when generating accurate bounding boxes. To make credible this thesis, can be recalled authors' previous works, in which the compact, shell amoebae present in activated sludge were located and recognized with much greater precision, accuracy and recall than the more diverse in shape settled ciliates [Dziadosz et al., 2024; Staniszewski et al., 2024]. This happened with similarly sized training, test and validation image sets for both analyzed organism representatives. The way YOLOv8 segments the image into a grid likely makes it easier to predict the center of the bounding box for symmetrical objects, thereby improving the network's evaluation metrics.

CONCLUSIONS

The YOLO v8 network handled the task of detecting the Cyclotella object almost flawlessly, while the obtained metrics on the validation set were as follows: Accuracy = 0.960, Precision = 0.964, Recall = 0.995. Hence, it can be assumed that in the future, the use of neural networks and deep machine learning can significantly accelerate bioindication research and provide a useful tool for microscopic image analysis, while Cyclotella detection is one of many cases for which deep learning can be used. In the future, neural networks from the YOLO family can be used to detect other objects in a microscopic sample, as well as to segment the data parts of a biological

object. Performing research on this topic leads to the development of deep learning tools, and the improvement of the work of scientists in the field of assessment of surface water quality. The results presented here confirm the effectiveness of the method used in the automatic analysis of microscopic images containing algae and thus the high application potential of the method in supporting bioindication studies of surface water quality.

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