Real-Time Analysis of Hatching of *Folsomia candida* Eggs and Automated Counting of Emerging Individuals through Computer Vision

Exploring Computer Vision Techniques for Continuous Monitoring of *Folsomia candida* Development and Hatching Eggs

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***Abstract*— This study introduces a new approach to soil ecotoxicity assessment by utilizing *Folsomia candida* as a bioindicator through real-time monitoring of egg hatching and automated counting of emerging individuals. Leveraging computer vision techniques, this method accurately detects hatching moments, optimizing resource utilization, and enhancing testing efficiency. By adhering to standards, the system minimizes waste and reduces the need for constant manual supervision. Overall, this integrated system offers a streamlined solution for ecotoxicity evaluation and provides insights into chemical impacts on soil organisms.**

Keywords— Monitoring; Score; Digital Image Processing; OpenCV; Springtails (*Folsomia candida*).

I. INTRODUCTION

The current limitations associated with available sensors in biological research have underscored the necessity for the development of innovative instrumentation techniques. To address this demand, the adoption of living organisms as biological sensors has gained prominence, particularly within the realm of soil toxicity studies. These studies are focused on assessing organism behavior under diverse concentrations of chemical substances [1]. *Folsomia candida*, a representative member of the edaphic fauna, assumes pivotal roles in soil ecosystems, including nutrient cycling and fertility. Its exceptional sensitivity to environmental variations renders it a prime bioindicator species [2]. Notably, collembolans, such as *Folsomia candida*, have emerged as effective bioindicators for assessing the ecotoxicity of contaminated soils. Their ease of application and shorter test duration distinguish them from other approaches [3]. In this context, this investigation concentrates on analyzing the reproductive effects of *Folsomia* individuals aged between 10 and 12 days [4].

Following the guidelines of the ABNT NBR ISO 11267 (2019) standard, precise recording of the date and time of organism egg hatching is essential. Accomplishing this requires visual analysis to count and monitor hatching events [5]. The accurate identification of hatching moments offers the potential for real-time alerts to be issued to those overseeing *Folsomia* eggs at the Soil Ecotoxicity Laboratory (Laecos) - FT/UNICAMP. This optimized approach serves to prevent the waste of organisms below the age of 10 to 12 days and obviates the need for daily individual laboratory monitoring. Furthermore, it enables the estimation of the number of feasible tests based on average egg counts, enhancing the efficiency of soil ecotoxicity assessments and contributing valuable insights for environmental quality evaluation.

II. METHODOLOGY

To undertake this investigation, a monitoring system was devised to track the hatching of *Folsomia candida* eggs, aiming to count and discern the newly emerged organisms. The system was constructed utilizing the computer vision library OpenCV and was coded in the Python programming language. The comprehensive layout of the proposed system is delineated in Figure 1, providing an illustrative representation of its components and architecture.

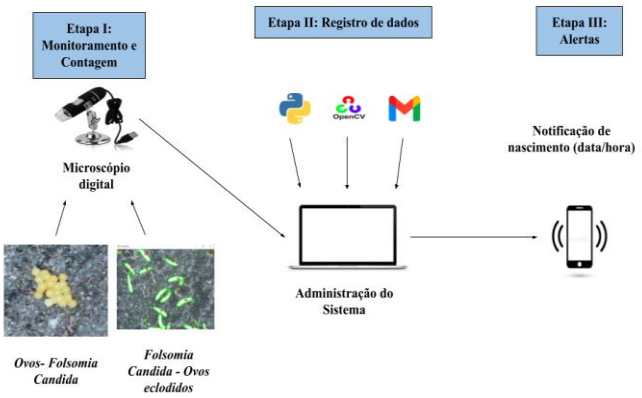

Fig.1. Implemented system diagram.

A. *Configuring the Monitoring System*

Initially, the research team introduced *Folsomia candida* eggs into a purpose-built glass container. This container featured an integrated LED lighting system and controlled resistors managed by a voltage regulator. This configuration se rved to prevent any potential fogging of the glass during recording sessions, ensuring the clarity of the images captured by the system. Figure 2 within the article provides a visual representation of this container, offering insights into its design and operational mechanism.

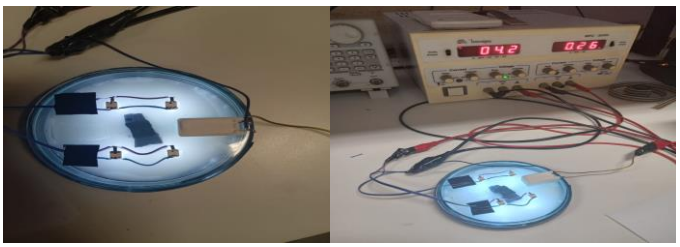

Fig.2. Container designed as an “incubator” for eggs.

Additionally, the glass container is situated within a controlled BOD (Biochemical Oxygen Demand) incubator, akin to a specialized refrigerator. The BOD incubator is designed to establish optimal conditions encompassing temperature, humidity, and light exclusion, essential for the meticulous examination of the samples. This strategic measure ensures the eggs remain in a stable state, maintaining a temperature range of 20°C to 22°C, aligning with the directives stipulated by the ABNT NBR ISO 11267 (2019) standard. This orchestrated environment provides an accommodating milieu for the eggs' developmental progress.

Incorporating resistors within the container design serves to harmonize the external temperature with that of the internal glass plate, effectively circumventing any potential moisture accumulation during the monitoring procedure. This considered approach is integral to preserving data integrity and the precision of the accumulated information.

B. *Egg Hatching Monitoring*

The monitoring algorithm involves real-time video frame capturing, motion detection utilizing thresholding and contour techniques, and automated email notifications upon egg hatching. To achieve this, proper positioning of the digital microscopic camera and the application of filters for noise reduction are essential to obtain a sharp egg image.

Image capture is executed using a strategically positioned digital microscopic camera, ensuring high-resolution images of eggs in separate cultures. The designed container in A) features a reduced diameter, purposefully narrowing the camera's focus area, facilitating egg monitoring.

The developed algorithm enables the detection of initial movements in newly emerged organisms, enabling the recording of their birth date and time.

C. *Count Of Hatched Eggs*

After movement detection, organism counting occurs a few days after hatching by employing a combination of Image Processing Filters (IPF) - cv2.Laplacian, cv2.GaussianBlur, cv2.threshold, and cv2.Canny. These filters are applied to attain sharp and clear images, facilitating the estimation of the quantity of hatched eggs through contour identification and numbering.

By applying thresholding techniques and contour processing, the algorithm effectively detects and counts the *Folsomia candida* organisms present within the image.

D. *Notification of Motion Detection and Count of Hatched Eggs*

Moreover, an email notification system was implemented to deliver pertinent information to researchers. Upon the first detection of hatching, the system automatically dispatches emails containing the birth date and time of the organisms, along with the count conducted after a 10-day interval. Following several days from hatching, a count is performed, and these data are also conveyed via email.

The decision to opt for email notifications was made over an initially proposed Android application due to its seamless communication and swift access to information. Email notifications emerged as more relevant for those in charge of segregating *Folsomia* cultures, enabling immediate awareness of organism birth without the necessity of accessing a database.

E. *Algorithm Flowchart And Main Functions*

The monitoring and counting algorithm was developed using the OpenCV (Open Source Computer Vision Library) for image and video manipulation, alongside Python's standard libraries such as 'datetime', 'smtplib', 'os', and 'email.mime' for managing date and time, email dispatch, and interaction with the operating system. Fig. 3 and Fig. 4 illustrates the comprehensive flowchart of the two algorithms combined, aiming for full detection. This integration is achieved through

the amalgamation of real-time *Folsomia* monitoring functionalities and their static image counting capabilities.

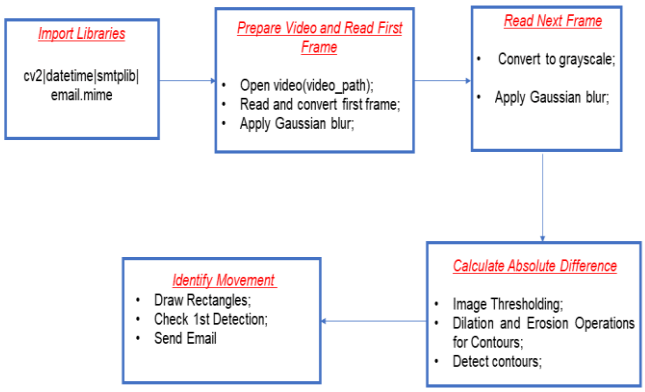

Fig.3. Flowchart of design algorithms - Monitoring. Source: authors.

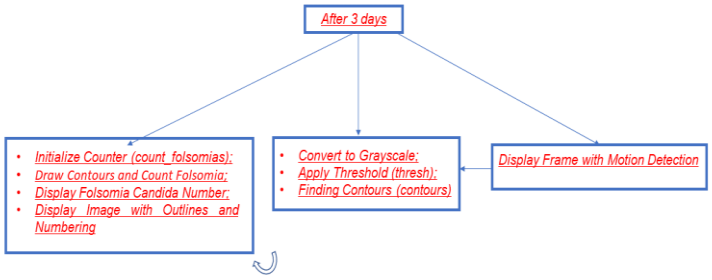

Fig. 4. Flowchart of design algorithms - Counting. Source: authors.

III. RESULTS AND DISCUSSION

Following motion detection, the system proceeds to outline the identified region, delineating it with a green rectangle, as depicted in Figure 5. Furthermore, the detection moment is archived as a .png image. This approach facilitates visual documentation of hatching events, enhancing comprehension, and establishing a comprehensive record of the outcomes observed during the monitoring period. Figure 4 provides an exemplification of this process, illustrating the storage of the image and preserving the exact detection moment for subsequent analyses.

Fig.5. *Folsomia candida* movement detection and archiving. Source: authors.

It is crucial to emphasize that archiving the moment of motion detection as an image serves the purpose of enabling the attachment of this image to the email notification, as depicted in Fig.6. This visual documentation substantiates the occurrence of *Folsomia candida* hatching. This measure reinforces the credibility of notifications sent to researchers, granting them direct access to visual evidence of egg hatching. This approach enhances transparency and accuracy in the monitoring process. By incorporating attached images within notification emails, the information conveyed is supplemented, ensuring the validation of results, and facilitating the comprehension of organism development throughout the study.

Fig.6. Notification of the birth of Folsomia with date/time and maximum period for removal of the culture (10 days). Source: authors.

Upon motion detection, a pivotal phase of the project ensued to accomplish the counting of hatched organisms from the eggs. This process took place several days following hatching detection and entailed the application of advanced Image Processing Filters (IPF), encompassing cv2.Laplacian, cv2.GaussianBlur, cv2.threshold, and cv2.Canny.

The amalgamation of these filters played a pivotal role in enhancing image sharpness and clarity, ensuring an accurate visual representation of the organisms. This IPF technique facilitated precise highlighting and outlining of each individual Folsomia born from the hatched eggs.

This approach reliably enabled the estimation of the quantity of effectively hatched eggs, unveiling indispensable information crucial for the execution of soil ecotoxicity tests. The *Folsomia* counting and contouring engendered a robust quantitative analysis, supplementing real-time monitoring and furnishing pertinent data for the meticulous selection of ideal cultures for testing purposes.

Figure 7 illustrates the outcome of this process, showcasing an image where the organisms are individually outlined and numbered after the application of the IPF filters.

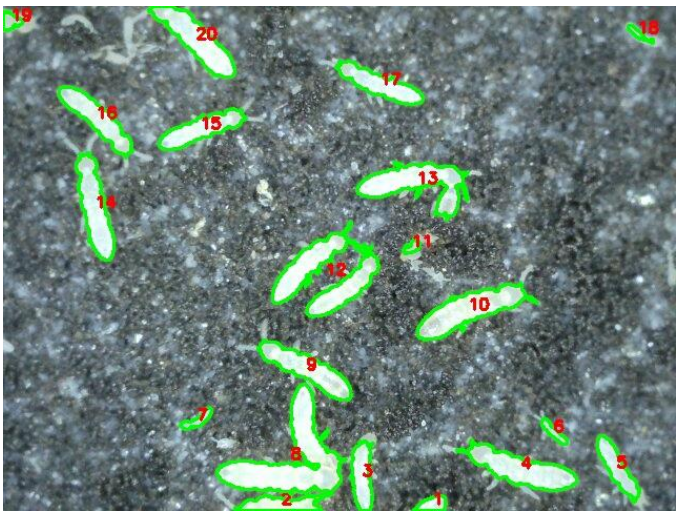

Fig.7. *Folsomia candida* count and outline after hatching. Source:authors.

The image from Figure 6 is sent via email as an attachment, accompanied by a message containing the total quantity of *Folsomia* generated by the culture after hatching.

IV. CONCLUSION

The implemented system demonstrated satisfactory outcomes in segregating cultures of *Folsomia candida* (aged 10 to 12 days) for soil ecotoxicity studies. The amalgamation of the real-time motion detection algorithm with *Folsomia* counting and contouring within static images facilitated precise tracking of hatching events and provided a dependable estimate of the hatched egg quantity.

The specially designed container, equipped with integrated LED lighting and controlled resistors, furnished an optimal environment for organism development, adaptable to diverse research settings and laboratories.

By centralizing the system on a local server and utilizing email notifications for researchers, swift communication of real-time hatching events was enabled, allowing for continuous monitoring of results. In summary, this project equips the toolkit for bioindicator organism analysis, enabling real-time monitoring of their development and post-hatching counting.

ACKNOWLEDGMENT

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