

Automated image-based analysis of developmental performance in *Ciona intestinalis*

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Introduction

Ciona intestinalis is an ideal model for EvoDevo studies (1). Developmental performance is quantified through manual observation, which is time-consuming because of the high number of larvae to analyze (Fig. 1). In our study we created a new pipeline to automatically estimate developmental performance under different perturbations.

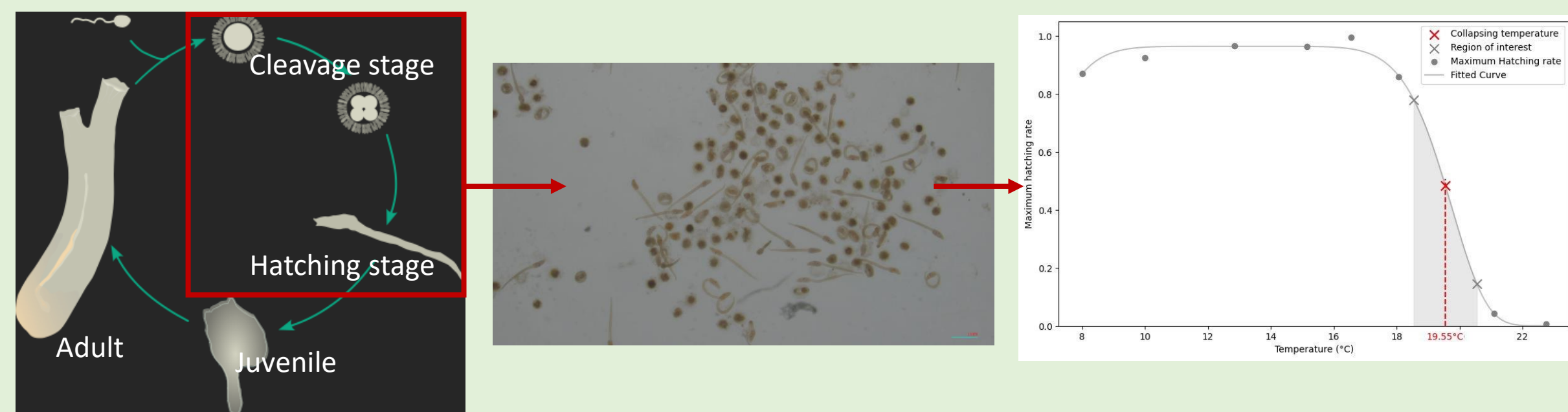


Fig. 1. Manual workflow for developmental performance. a) *Ciona* lifecycle, in the red box the hatching stage is used for the estimation. b) Raw image used for manual counting. c) Developmental performance at different temperatures.

Methods

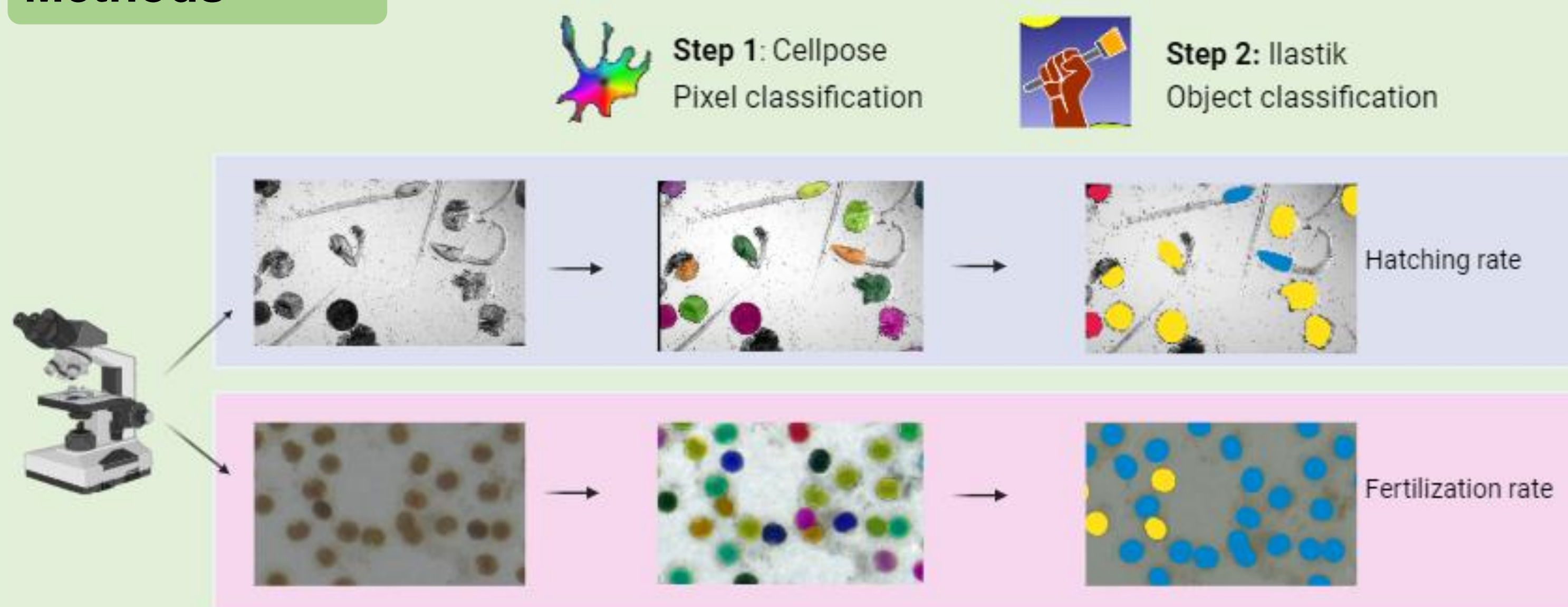


Fig. 2. Microscopic imaging and analysis workflow. In the top row the Hatching stage. In the bottom, the cleavage stage. *Ciona intestinalis* were fertilized in vitro. Brightfield images were acquired at two timepoints: cleavage and hatching stage (2). Images were processed using Cellpose and Ilastik. Training data was manually labelled to train the softwares. All the analysis was performed using Python.

Results

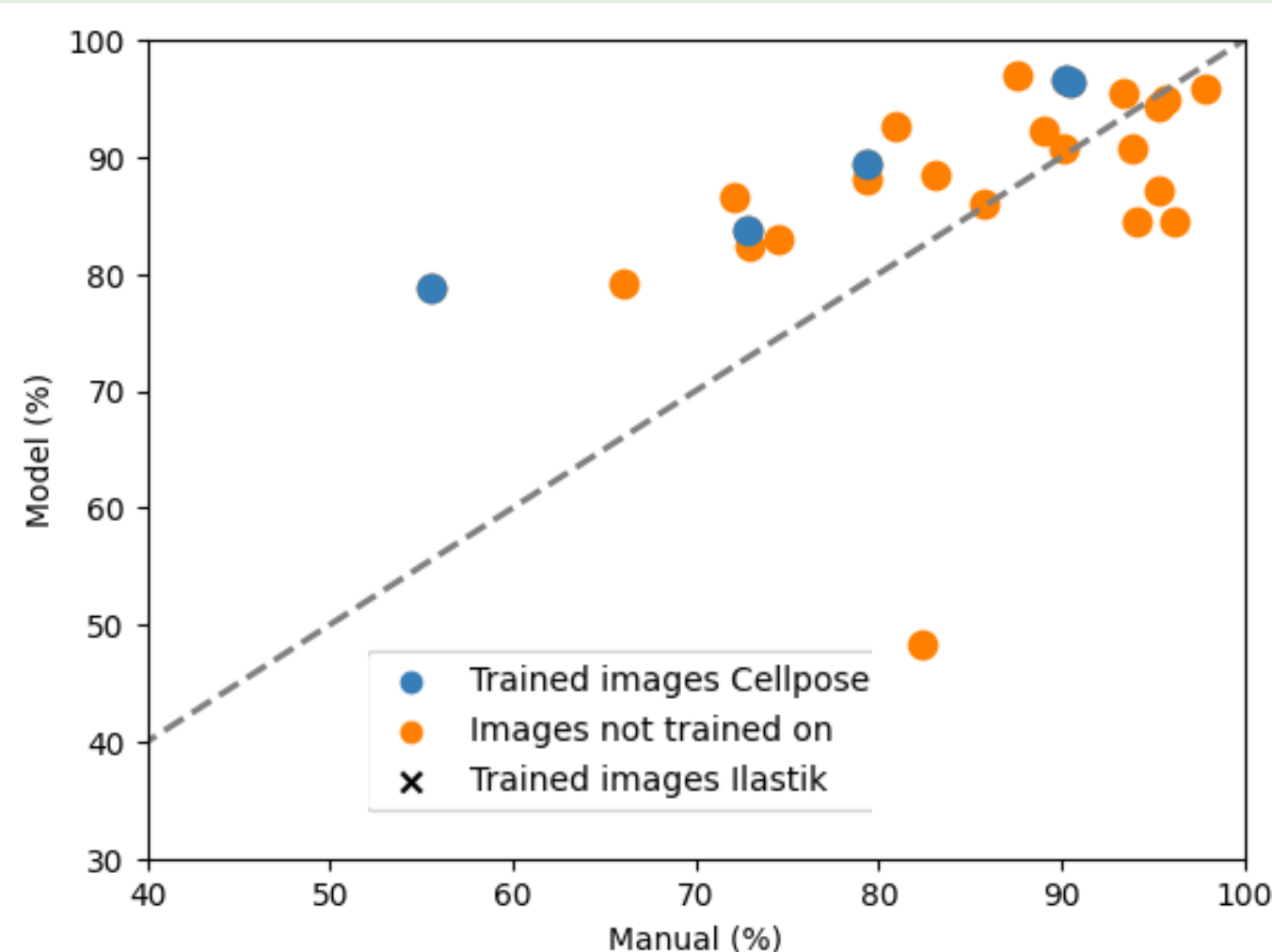


Fig. 3. Correlation plot of Manually curated data against the automated analysis performed for the fertilization rate.

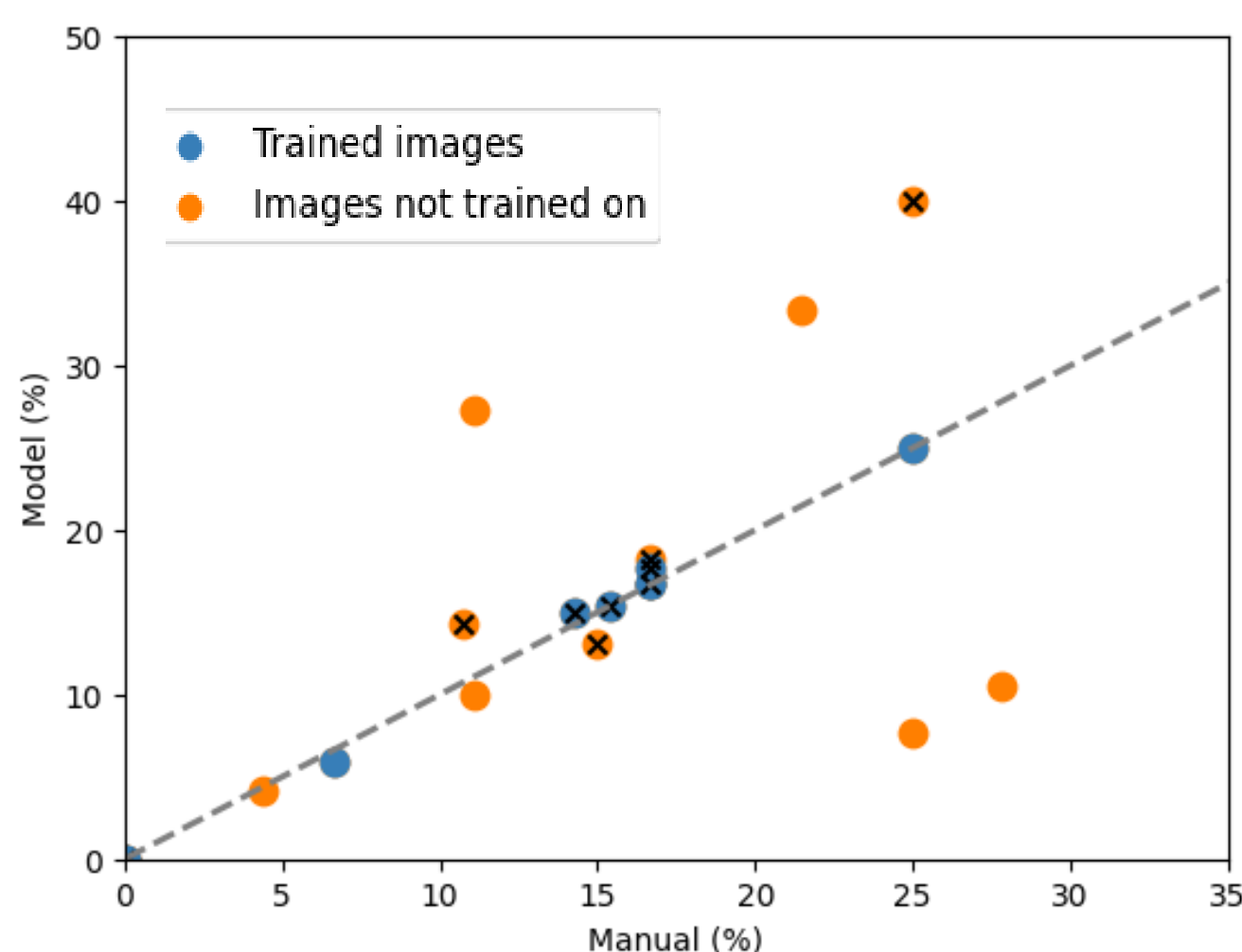


Fig. 4. Correlation plot of Manually curated data against the automated analysis performed for the hatching rate.

5 images were used to train Ilastik and Cellpose to distinguish embryos and unfertilized eggs.

Mean Fertilization rate:

Manual = 84.56%

Automatic = 87.50%

Mean Absolute error = 8.58%

10 images were used to train Ilastik and Cellpose to distinguish normal phenotype hatched larvae and all the rest of the objects (embryos and eggs)

Mean Hatching rate:

Manual = 13.98%

Automatic = 14.54%

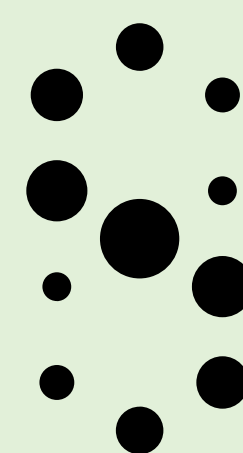
Mean Absolute error = 4.42%

Discussion

We have developed two new machine learning classifiers for automatic estimation of *Ciona*'s developmental performance. While further improvement is possible with a larger training dataset, our initial findings demonstrate the possibility of automating these analyses. Thus, the established pipeline holds potential for assessing the developmental performance of various perturbations and might be applicable to different model organisms.

References

1. Passamonek Y. J. & Di Gregorio A. (2005). *Developmental Dynamics* 233, 1. DOI: 10.1002/dvdy.20300.
2. Hotta K. et al. (2007). *Developmental Dynamics* 236, 7. DOI: 10.1002/dvdy.21188.



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