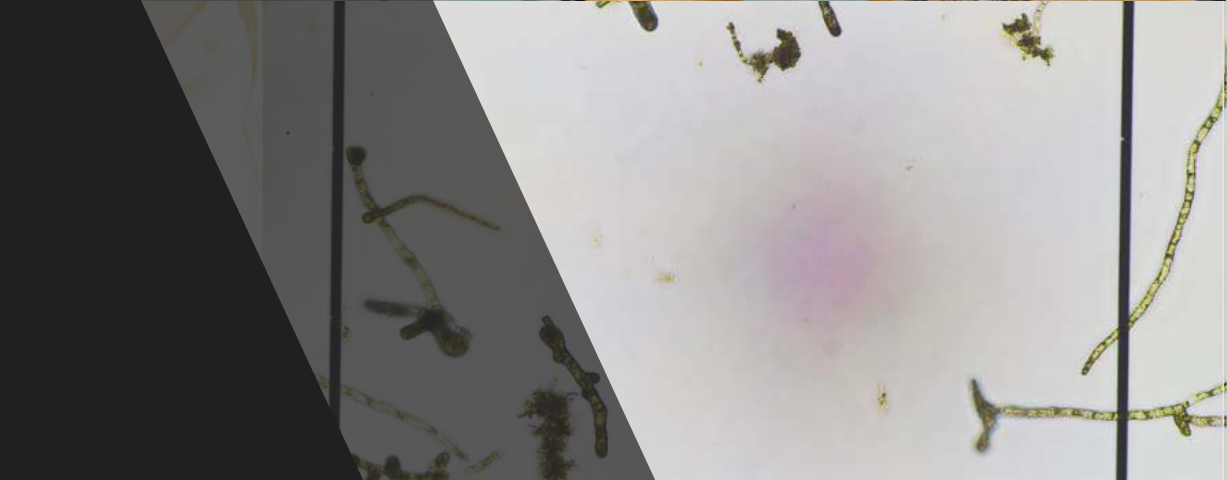
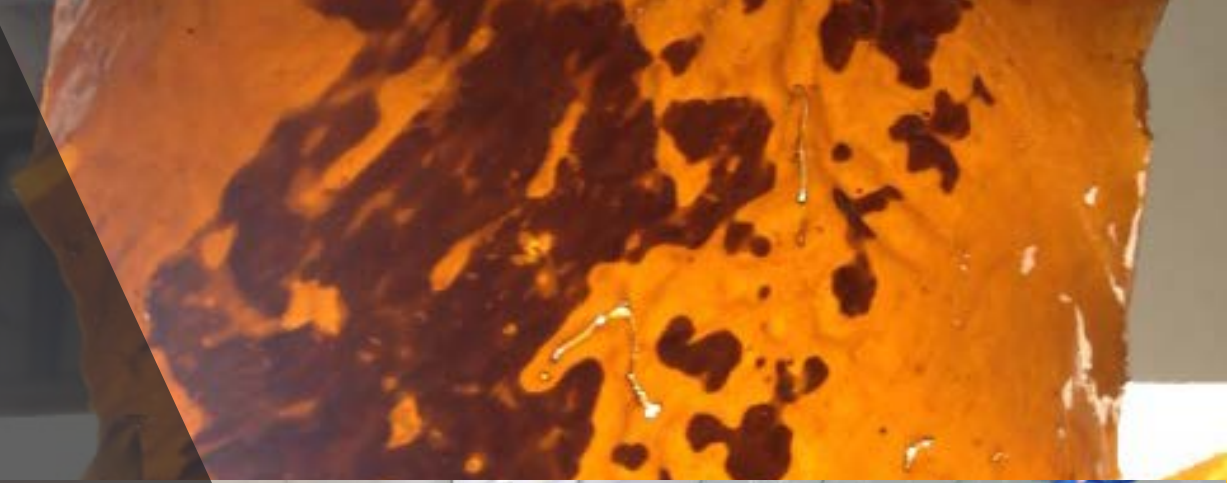


Jorunn Skjermo, Aires Duarte, Raven  
Cammenga, Kristine Steinhovden, Silje  
Forbord, Arne Malzahn, Aleksander Handå,  
Torfinn Solvang-Garten, Trond Størseth

# MACROSEA WP1

## Seedling Biology



# WP1 Objectives

- i. Establish quality parameters for use in seedlings production of *S. latissima*, *A. esculenta* and *P. palmata*
- ii. Characterize biological requirements and threshold levels for environmental variables
- iii. Develop a protocol for industrial production of *P. palmata*



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Initial studies of defined bottlenecks in the  
Palmaria seedlings protocol

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Compare different methods for monitoring of  
growth and quality of early stages of kelp

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Establish more knowledge about the seedlings  
production of *A. esculenta* (photoperiod and time  
for fertility induction, seeding density, fertilizer)

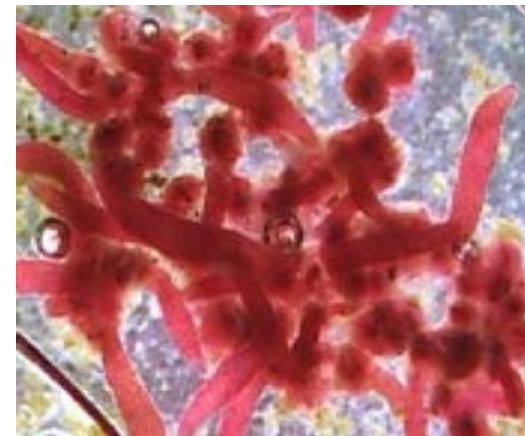
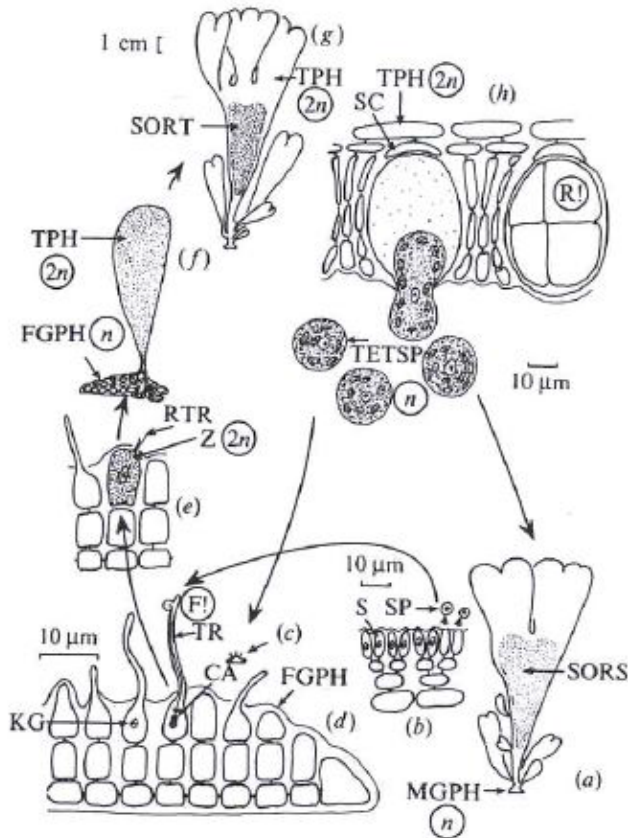
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Evaluate polyphenols as a possible indicator for  
quality or stress during the seedlings development

# Approaches

# Cultivation protocol for *Palmaria palmata*

- Disinfection
- Spore release
- Material
- Incubation conditions
- Contaminants
- Sea-phase

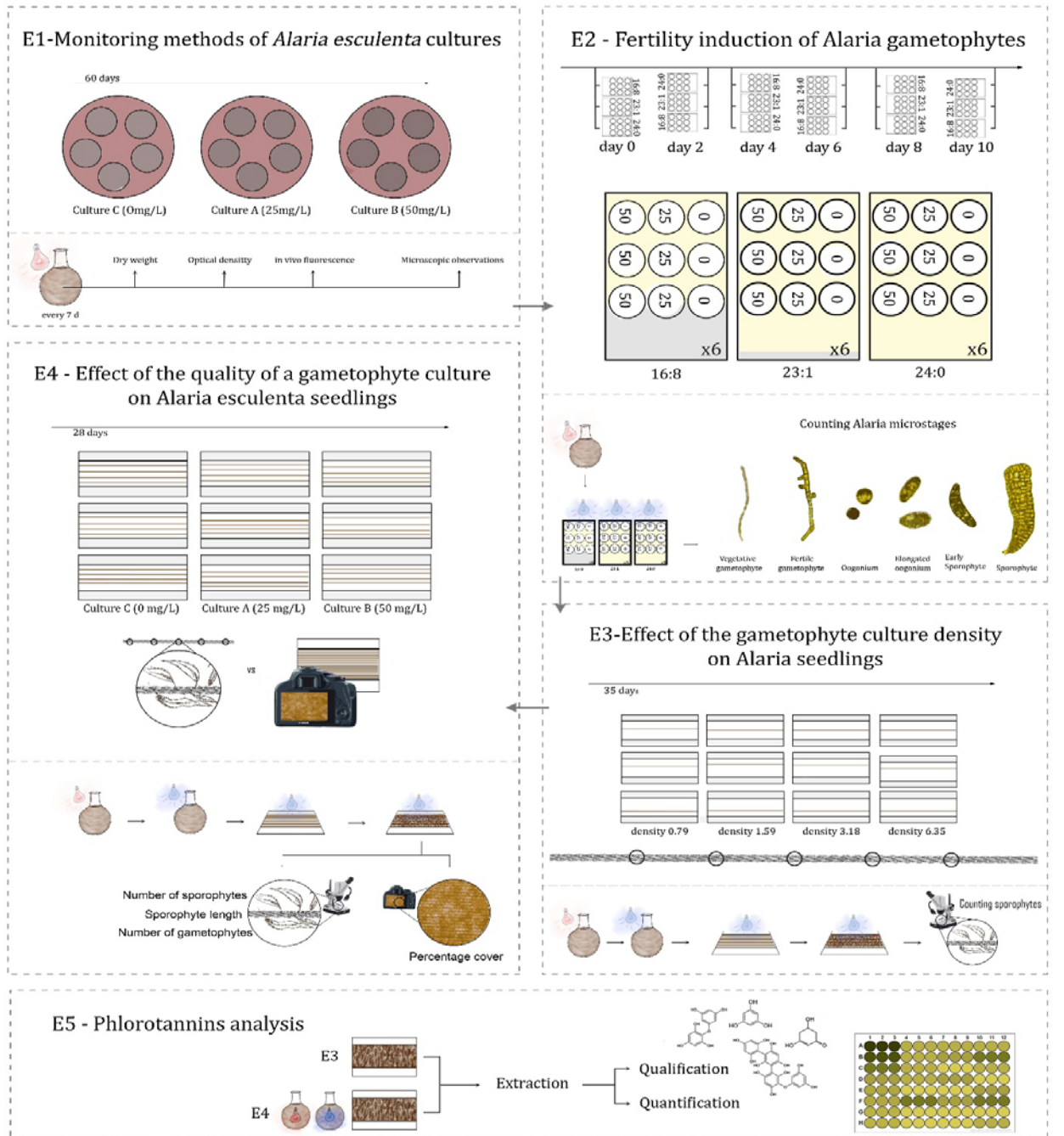


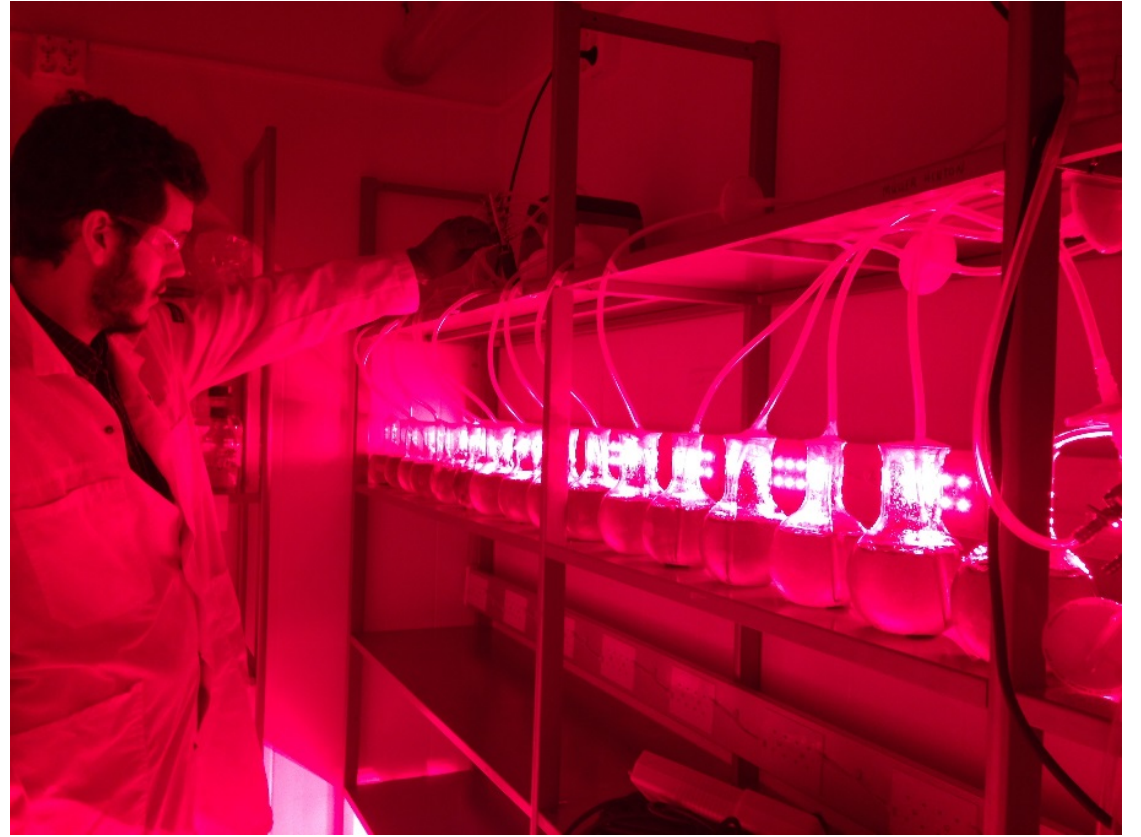
# Monitoring of growth and quality of early stages

- Gametophytes of Alaria
  - Effect of cultivation conditions on quality
    - OD
    - In vivo fluorescence
    - Density
    - Percentage distribution of life stages in the gametophyte culture
    - Dry weight
    - Seedlings size, number
    - Phlorotannins

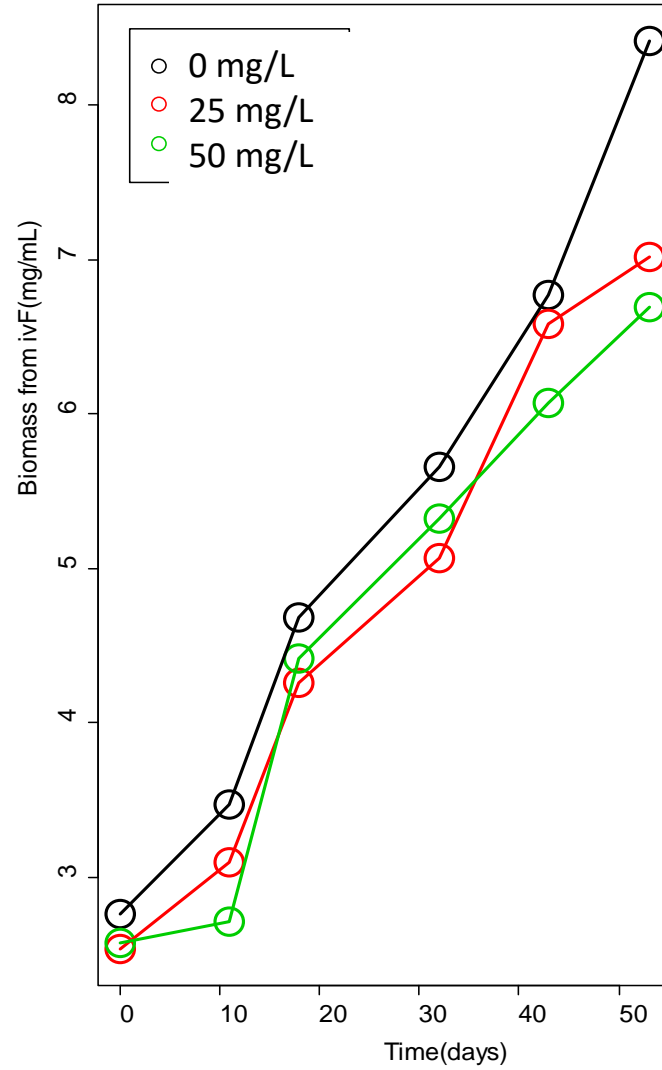
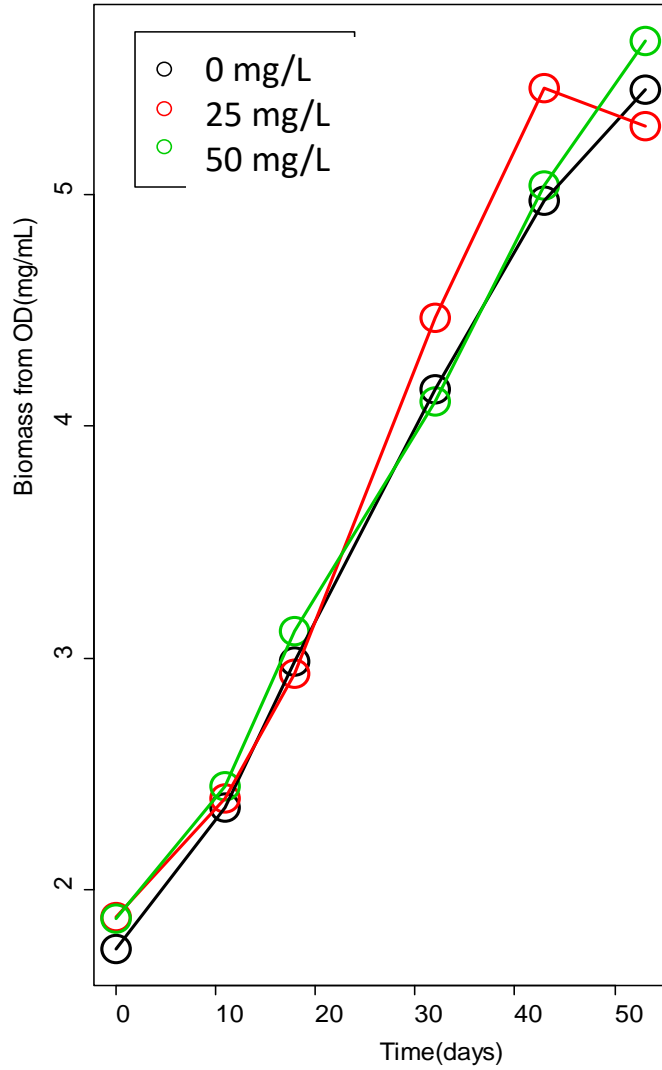
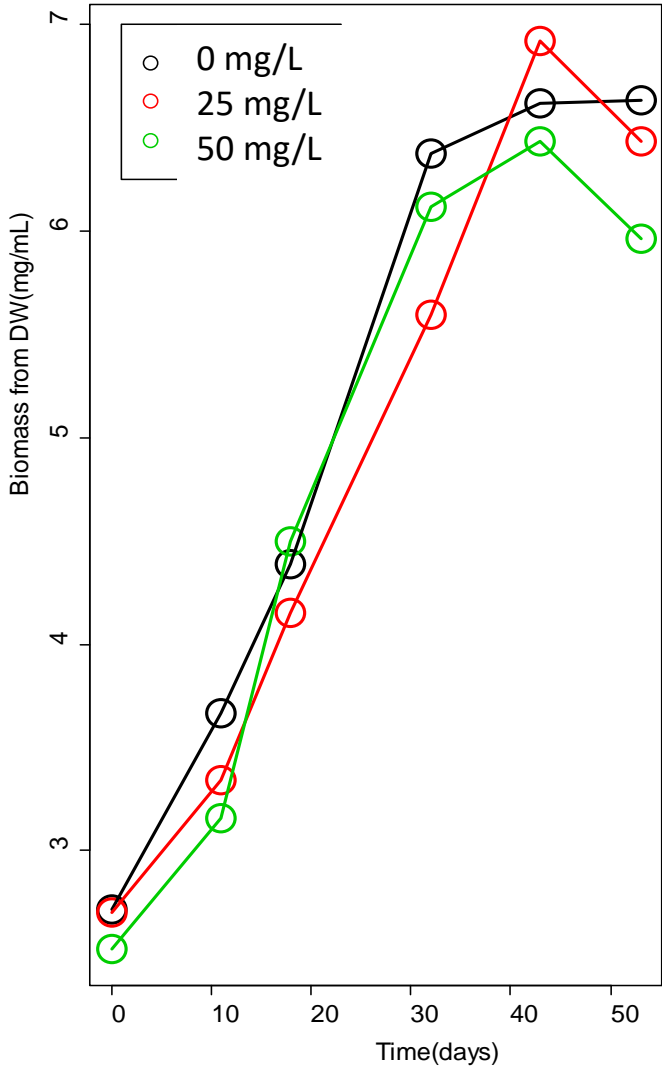
# Monitoring of growth and quality of early stages

MSc-student Aires Duarte  
University of Porto



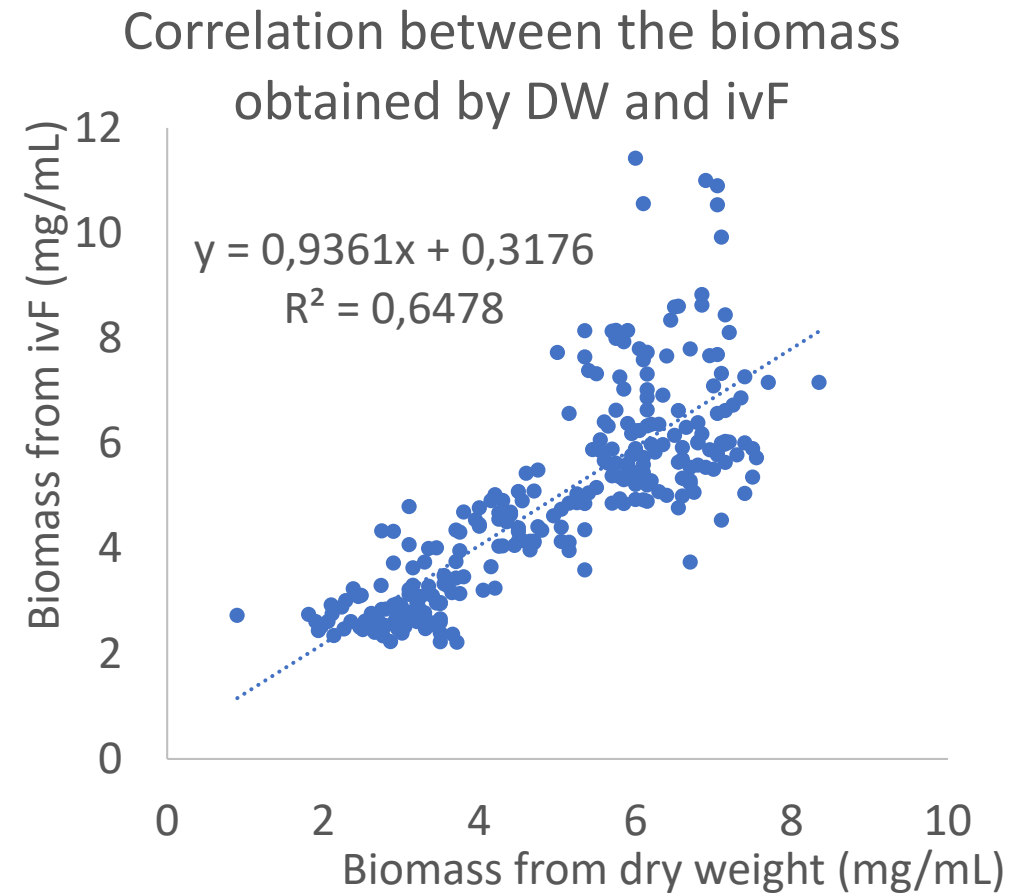
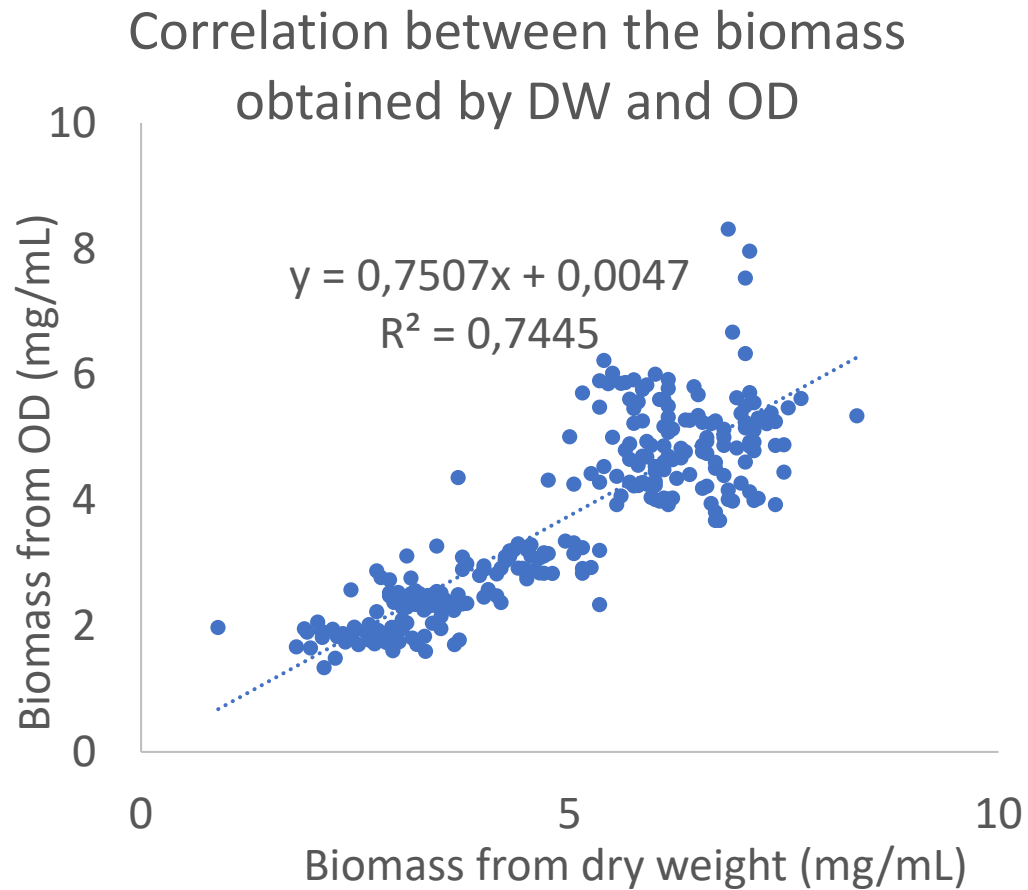


# Biomass of Alaria gametophytes from DW, OD

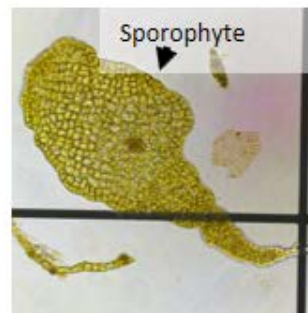
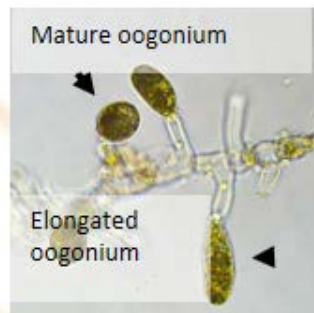
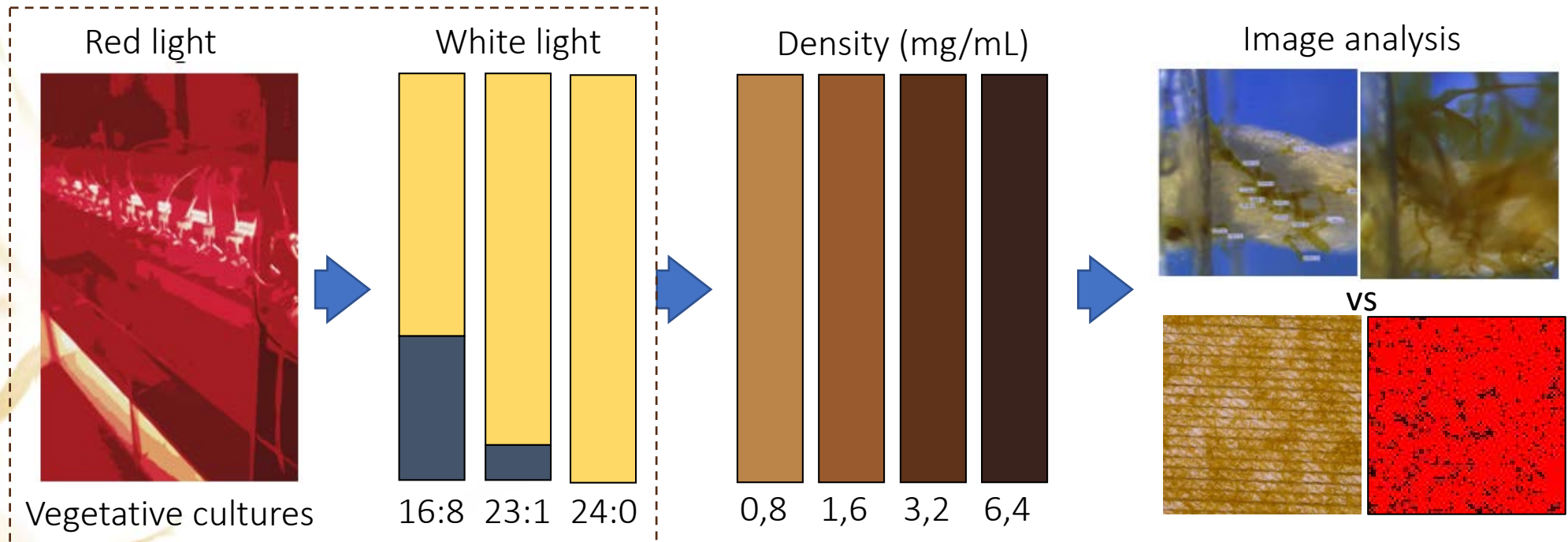




# Correlation between dry weight (mg DW/mL), optical density (OD) and in vivo fluorescence (ivF)



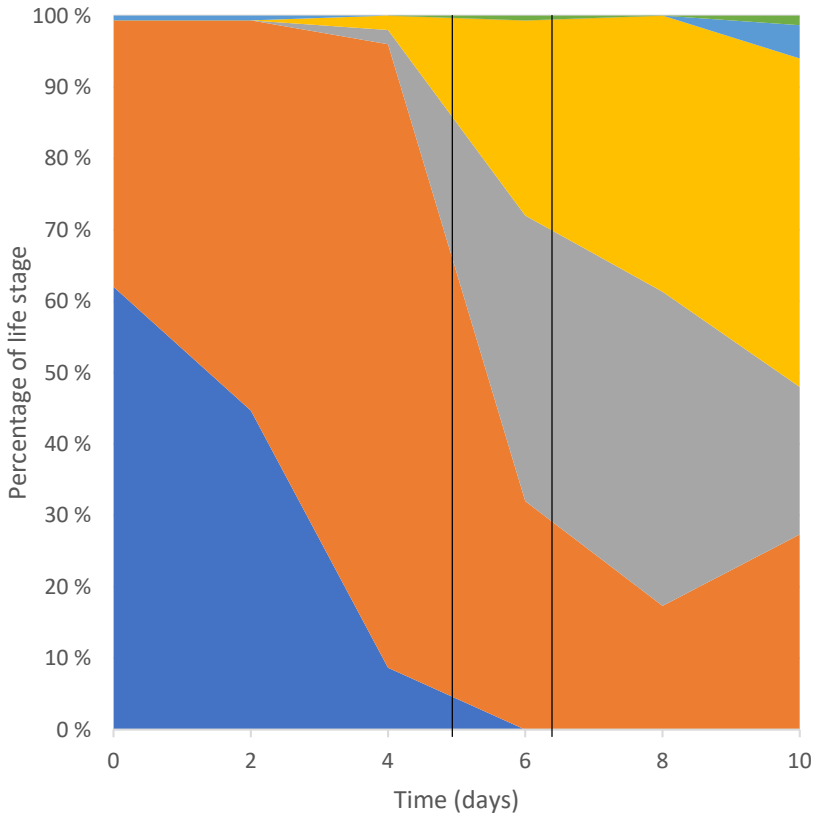
# Photoperiod and time for fertility induction - Experimental design



# Optimum photoperiod and time for fertility induction (*A.esculenta*)

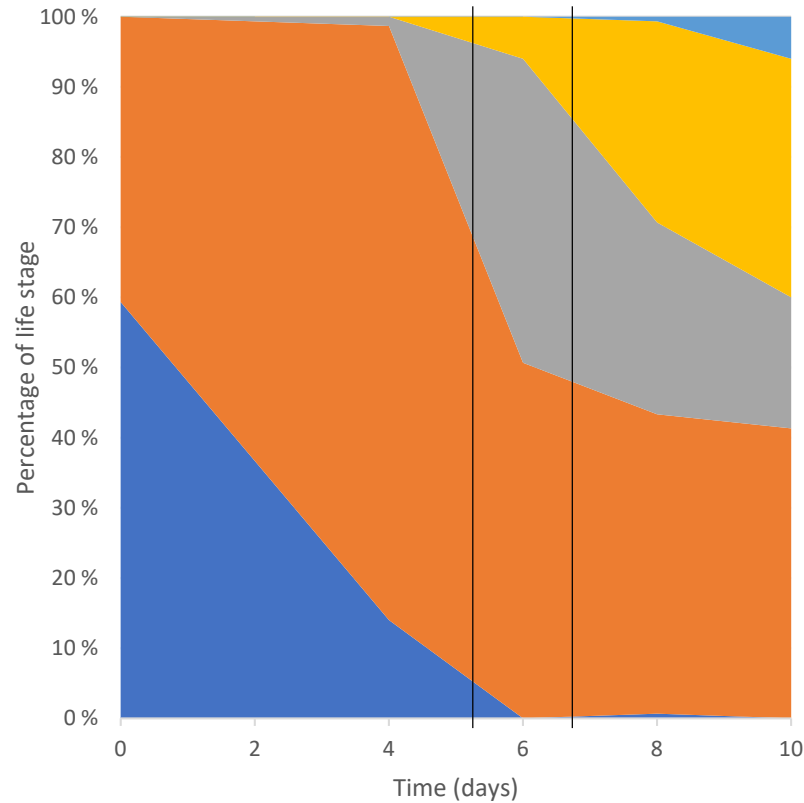
Life stages evolution at photoperiod

23:1



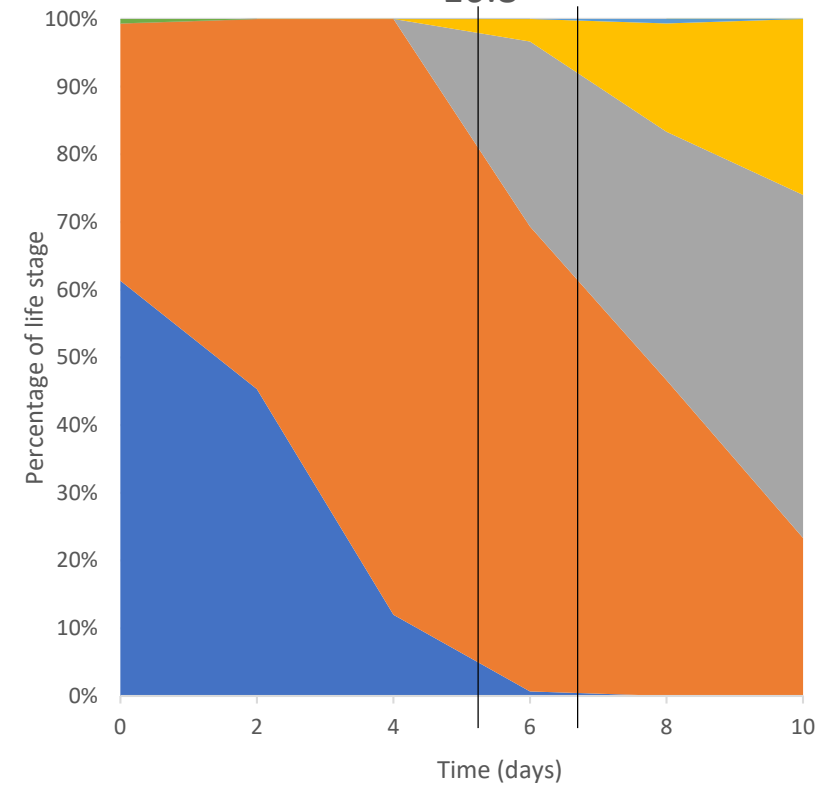
Life stages evolution at photoperiod

24:0



Life stages evolution at photoperiod

16:8



- vegetative gametophyte
- fertile gametophyte
- Mature oogonia
- Elongated oogonia
- Early sporophyte
- Sporophyte

- vegetative gametophyte
- fertile gametophyte
- Mature oogonia
- Elongated oogonia
- Early sporophyte
- Sporophyte

- vegetative gametophyte
- fertile gametophyte
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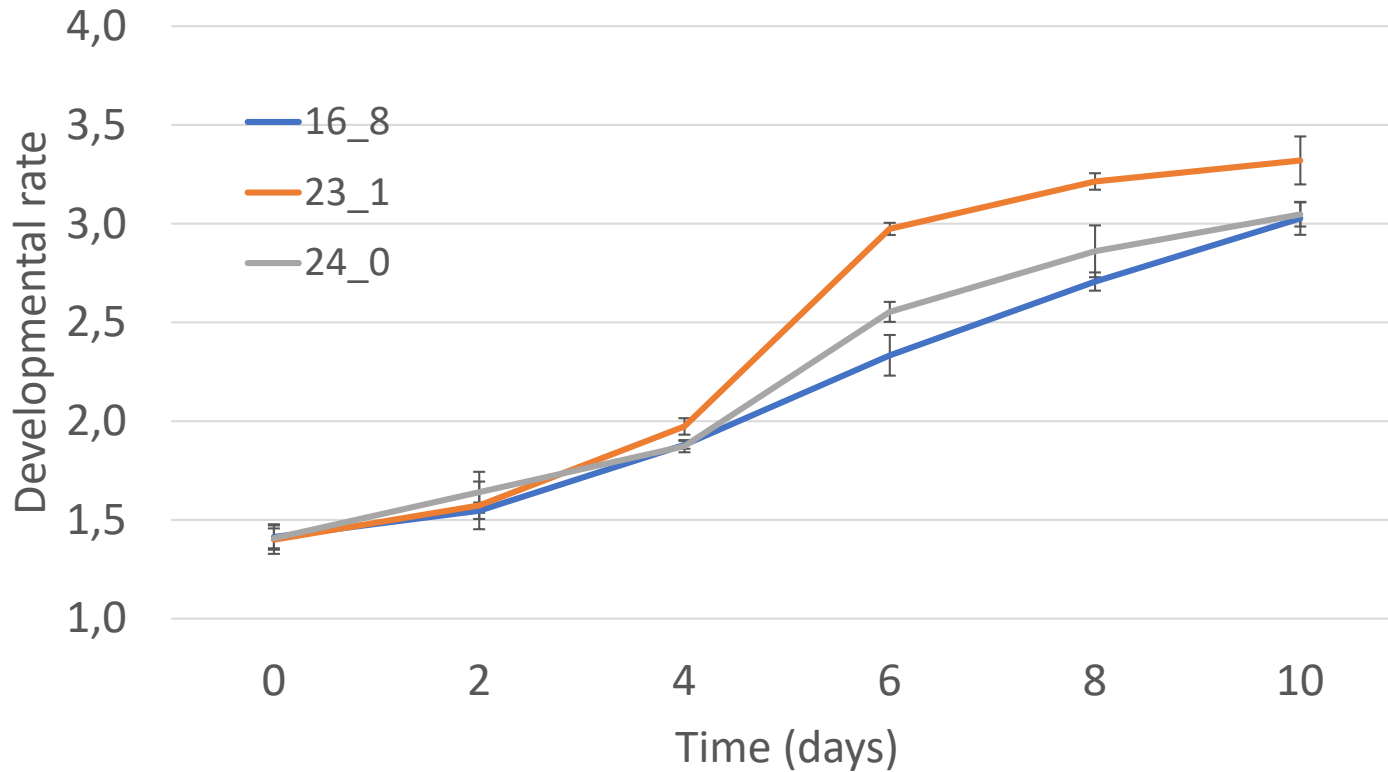
Photoperiod

Induction time

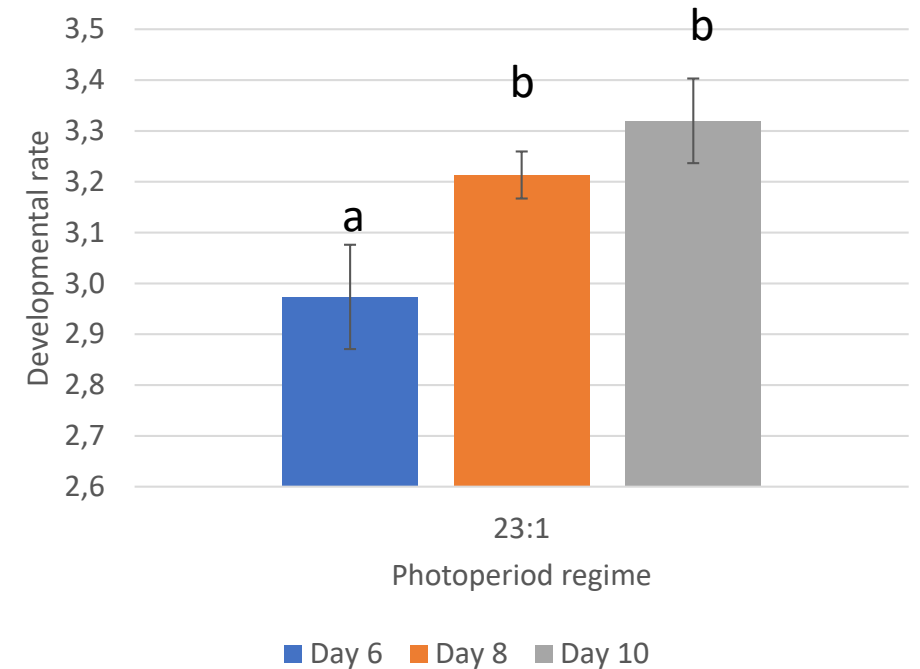
Density

# Optimal photoperiod regime for fertility induction

Developmental rate of *Alaria esculenta* under three photoperiod regimes over 10 days



Development rate of *Alaria esculenta* under 23:1 photoperiod on the day 6, 8 and 10

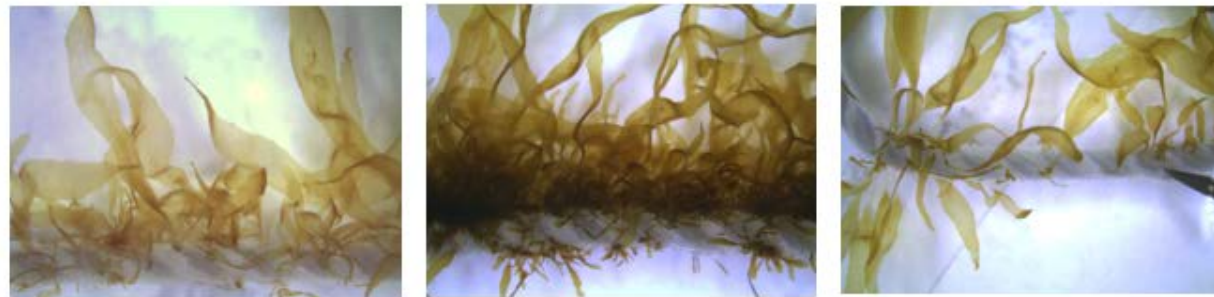
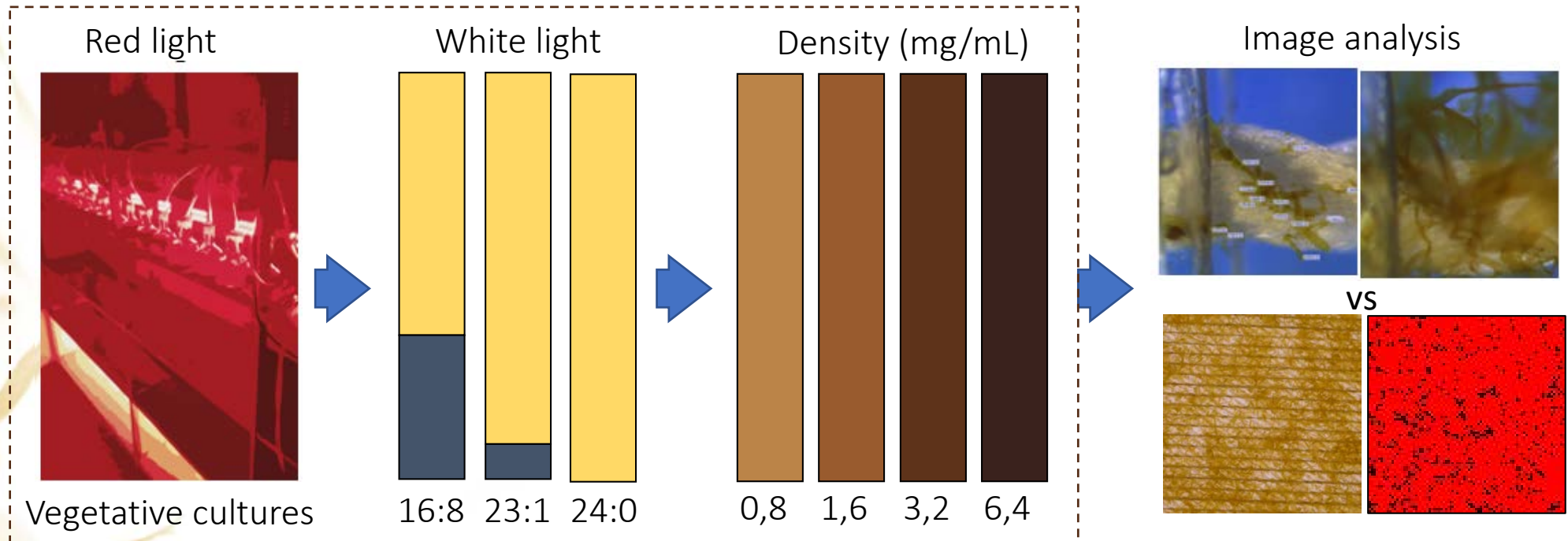


Photoperiod: 23:1 ✓

Induction time: 8d ✓

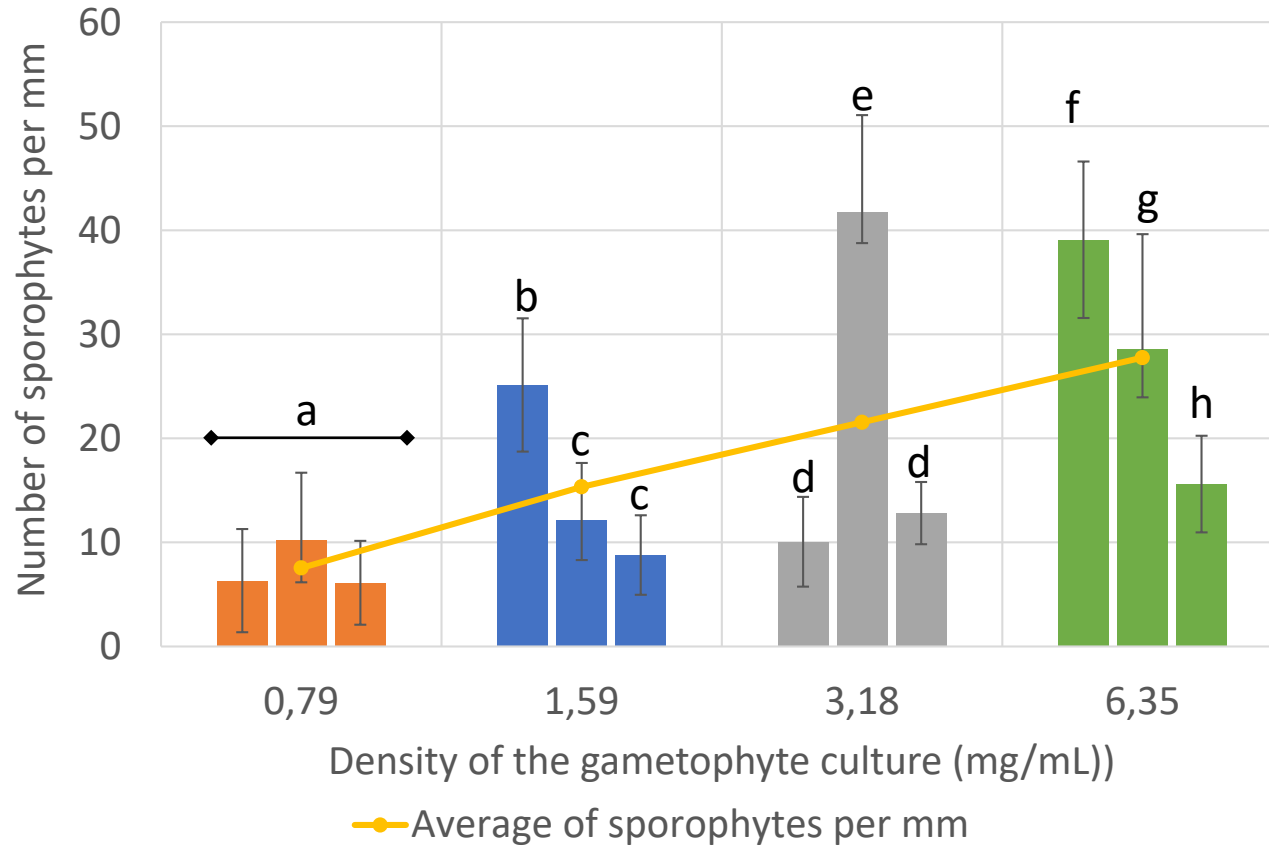
Density:

# Experimental design



# Optimal seeding density with gametophyte culture

Sporophytes per mm on the seedlings started with different gametophyte densities after 4 weeks



Densities (mg/mL)	Sporophytes per mm twine
0,8	7
1,6	15
3,2	21
6,4	27

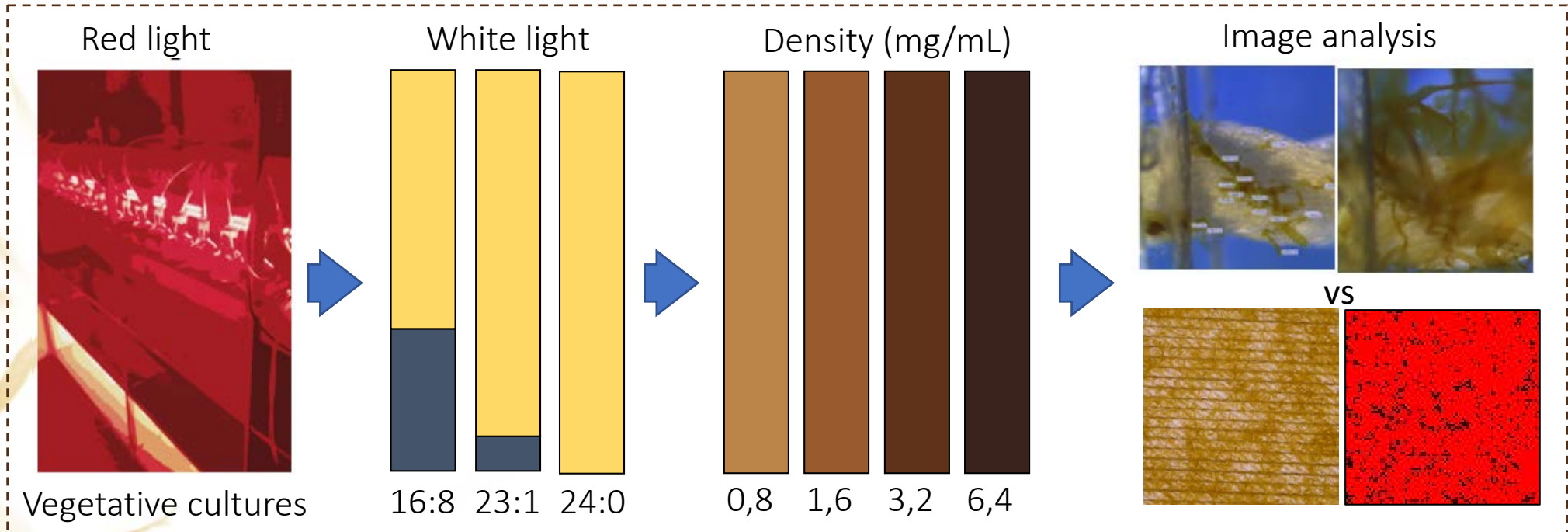
200 ml pr plate (34x40cm)

Photoperiod: 23:1 ✓

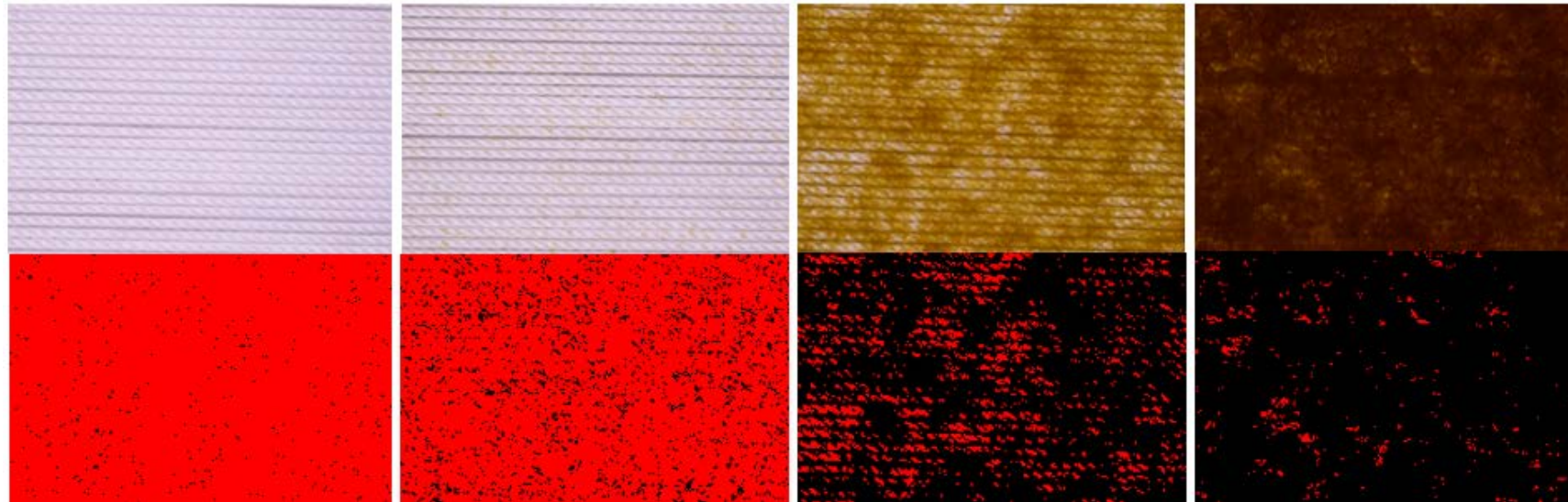
Induction time: 8h ✓

Density ✓

# Experimental design



# Image analysis to estimate growth Percent coverage (PC)



Day 7

Day 14

Day 23

Day 30



# Alternative way to estimate the seedling growth

The screenshot displays an image analysis software interface. The central window shows the original image of a seedling, labeled "Original Image" and "day\_15\_13 (1).JPG". The interface includes a toolbar with various tools, a file path field set to "E:\escritório\For analysis\Spools", and a save file path field set to "C:\Tare Aires\Tare Aires analysis.txt". The color mode is set to "HSV".

On the left, three histograms are displayed for Hue, Saturation, and Value. The Hue histogram is highlighted with a yellow box. The histograms show the distribution of pixel values for each channel, with the Hue histogram showing a peak around 180-200.

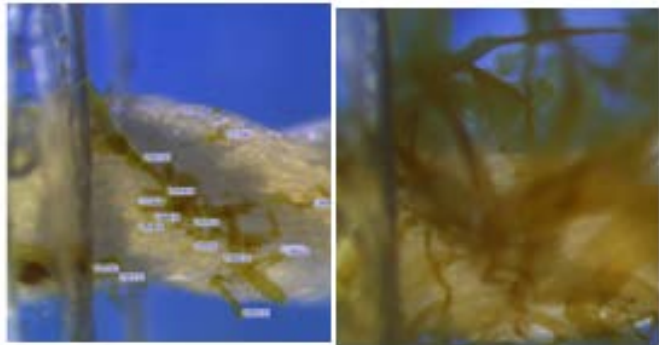
On the right, the analysis results are shown. The "BLACK PIXELS ARE COUNTED" section displays the following data:

Channel	Total # pixels	# bckgr pixels	% "growth"
Hue	36152320	32675596	10
Saturation	36152320	0	100
Value	36152320	16564176	54

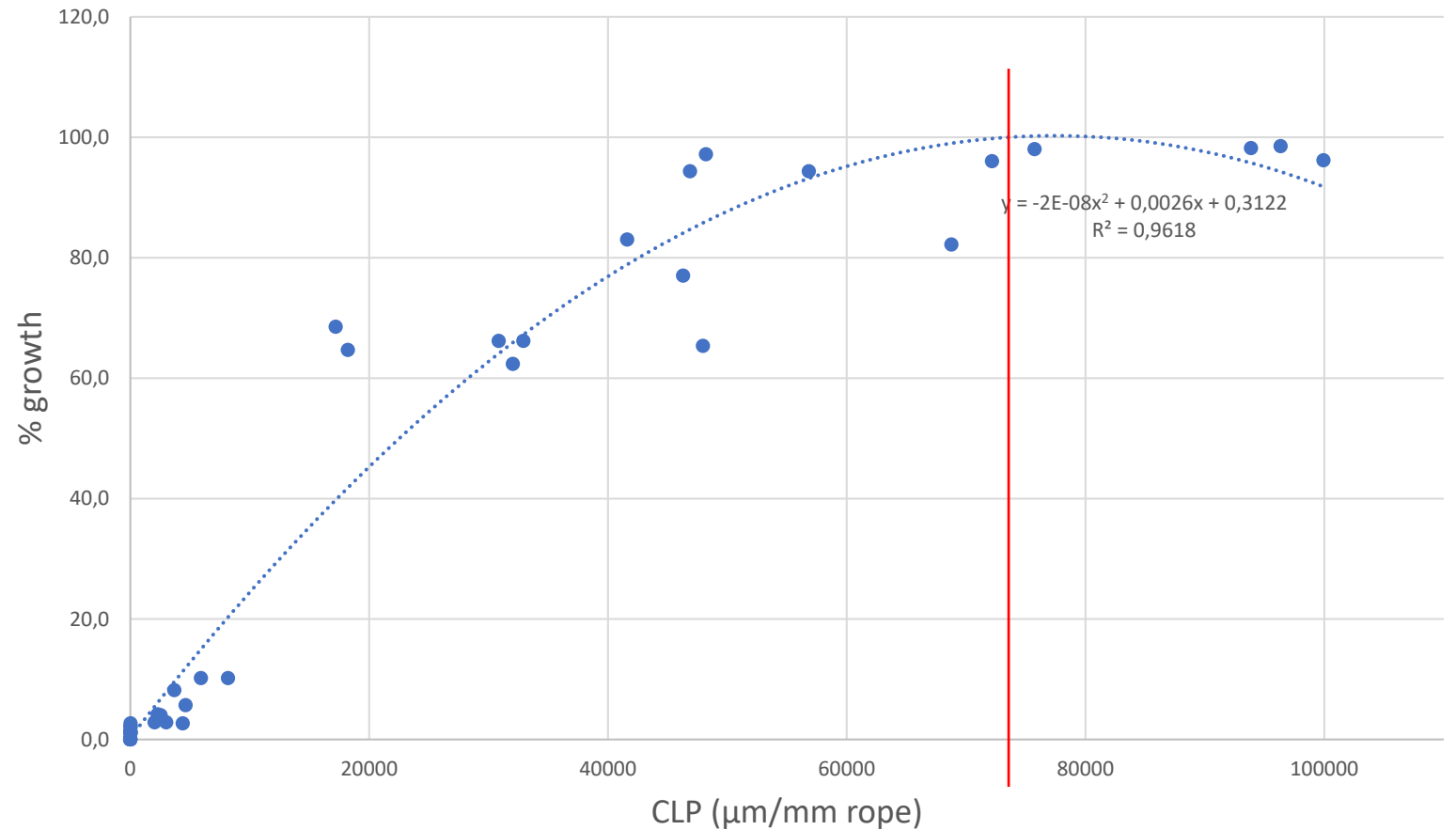
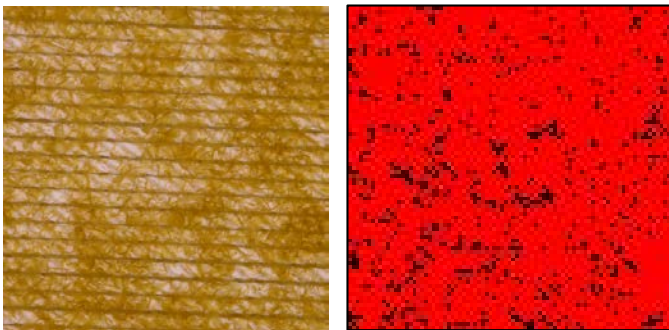
The Hue and Value results are also highlighted with yellow boxes. The interface includes buttons for "RECALCULATE", "DONE", and "STOP". The status bar at the bottom indicates the image dimensions and format: "7360x4912 0.10X 32-bit RGB image 201,188,206 (7010,1038)".

# Image analysis to estimate growth

Correlation between the seedling growth (%) obtained by image analysis and the counting and length product (CLP)



VS

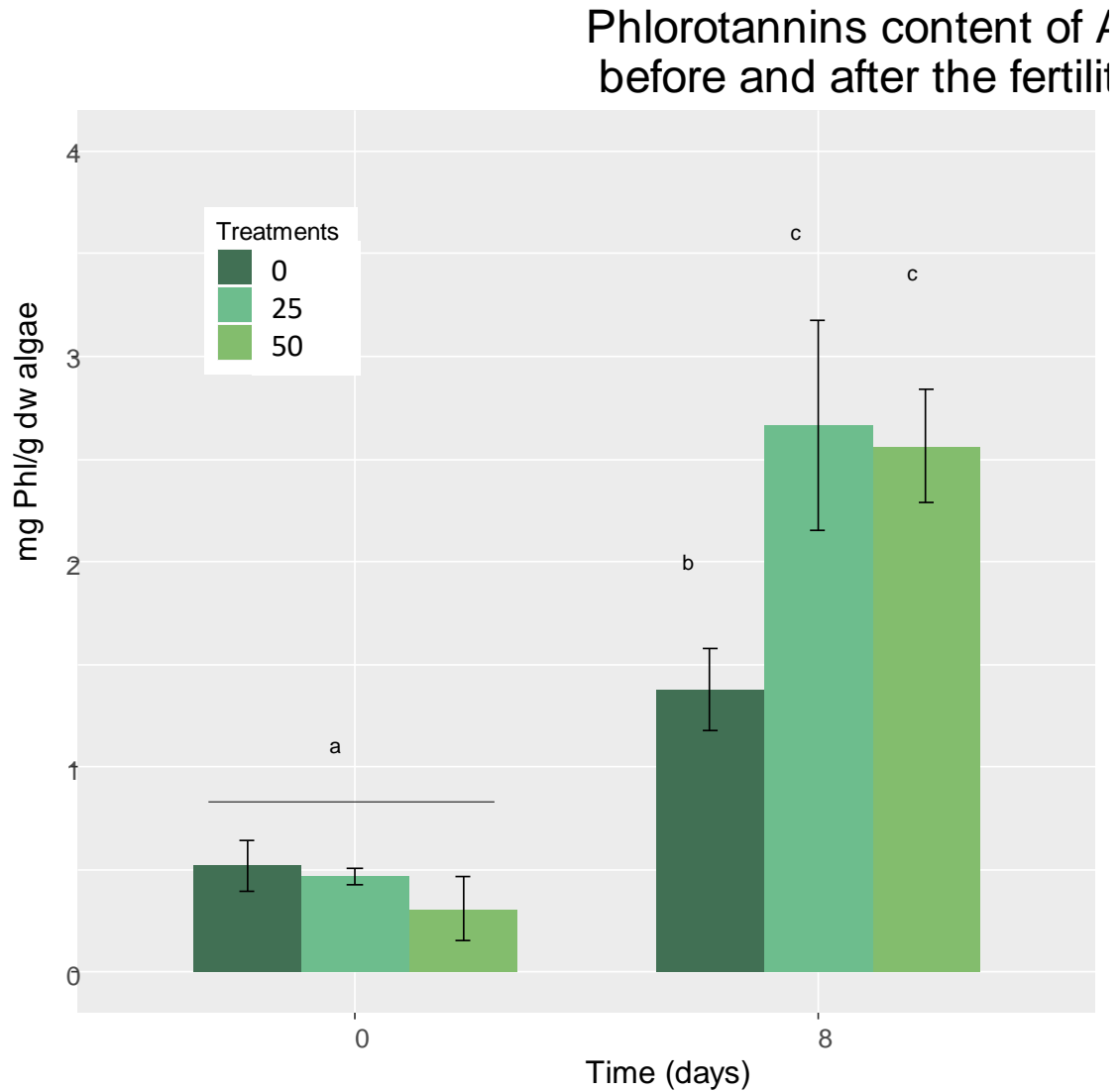


CLP = counting \* sporophytes length

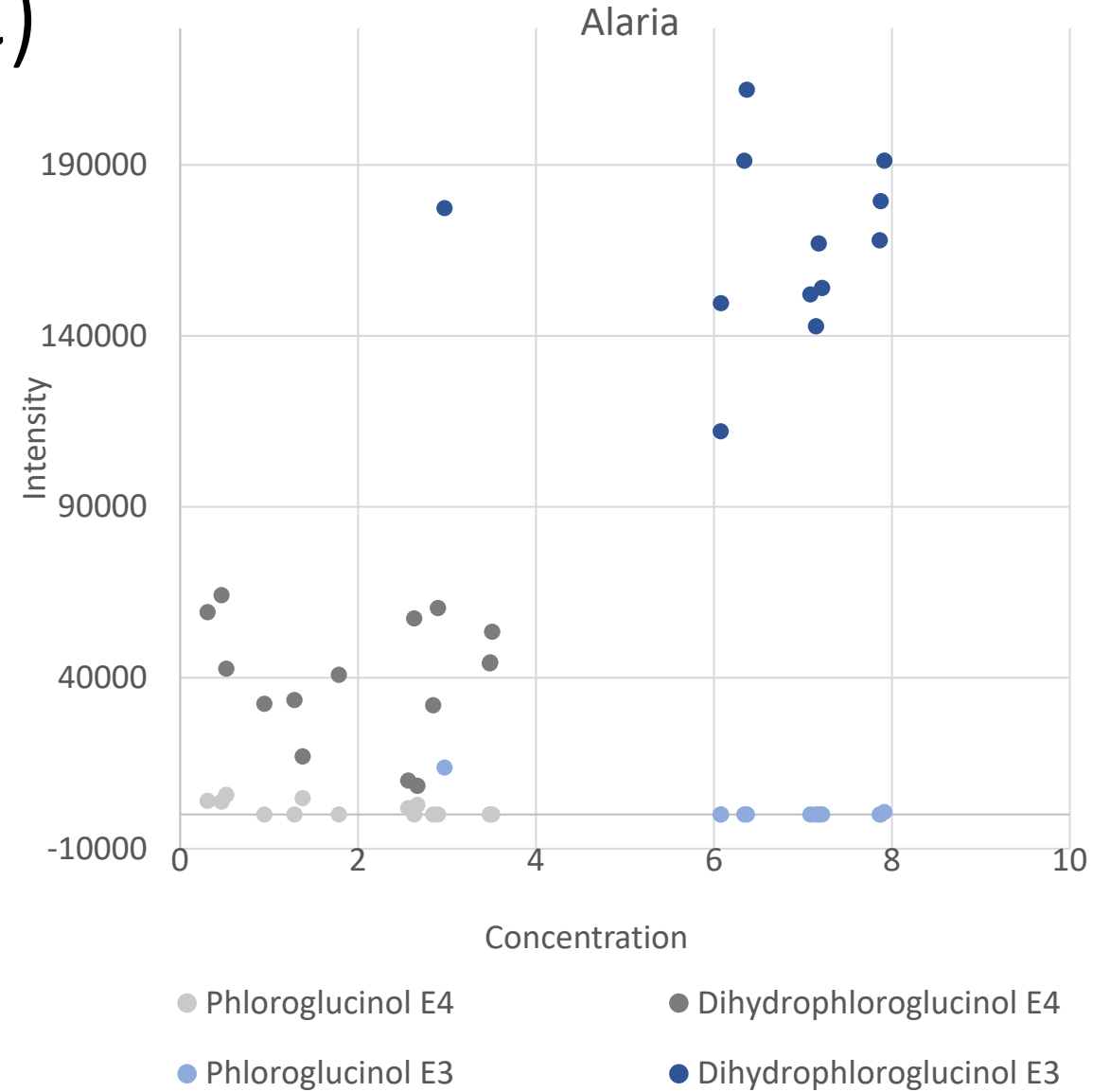
# Summary

- The optimal photoperiod for fertility induction was 23:1 (light:dark regime) during 8 days
- Of the tested culture densities, seeding with 0,8 mg/mL was most optimal (still too high?)
- Grow estimation of seedlings is possible by image analysis

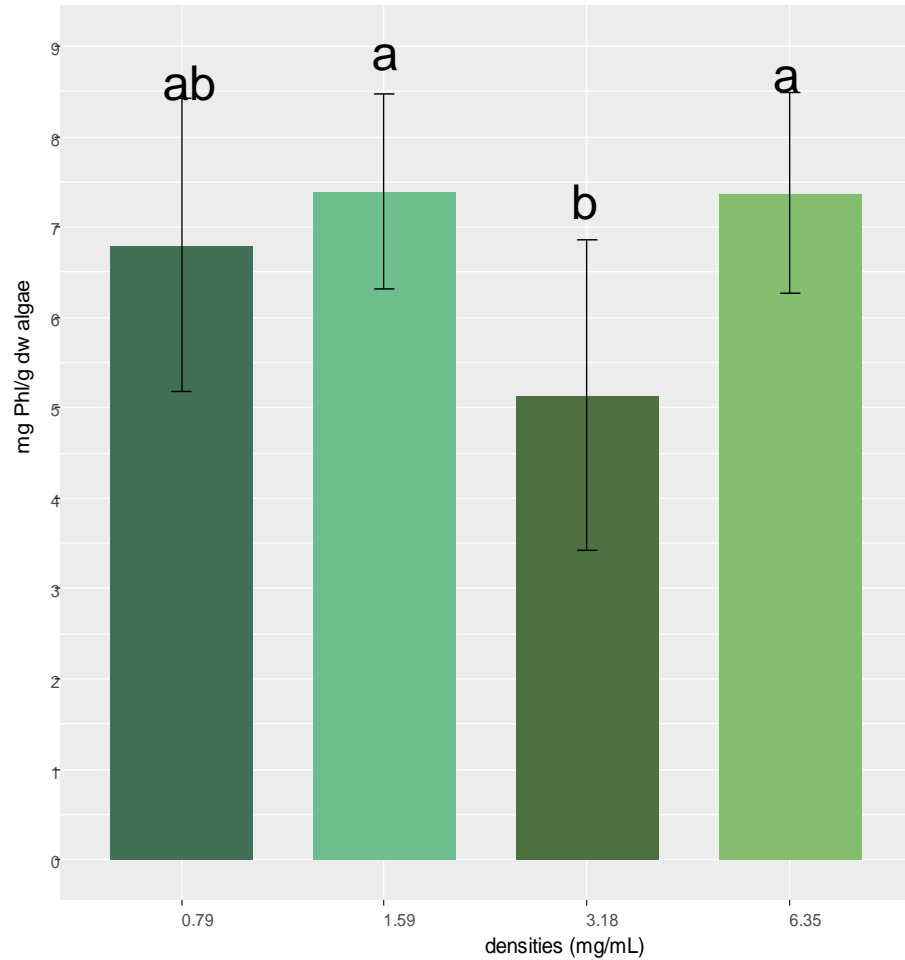
# Phlorotannin content (FC)



Correlation between intensity of phloroglucinol and dihydrophloroglucinol and concentration of



# Seeding density, no effect on total phlorotannin content



Densities (mg/mL)	Sporophytes per mm twine
0,8	7
1,6	15
3,2	21
6,4	27

## Conclusions so far

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Made progress in the methods for monitoring of growth and quality of early stages of *A.esculenta*

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Defined photoperiod and time fertility induction in *A.esculenta*

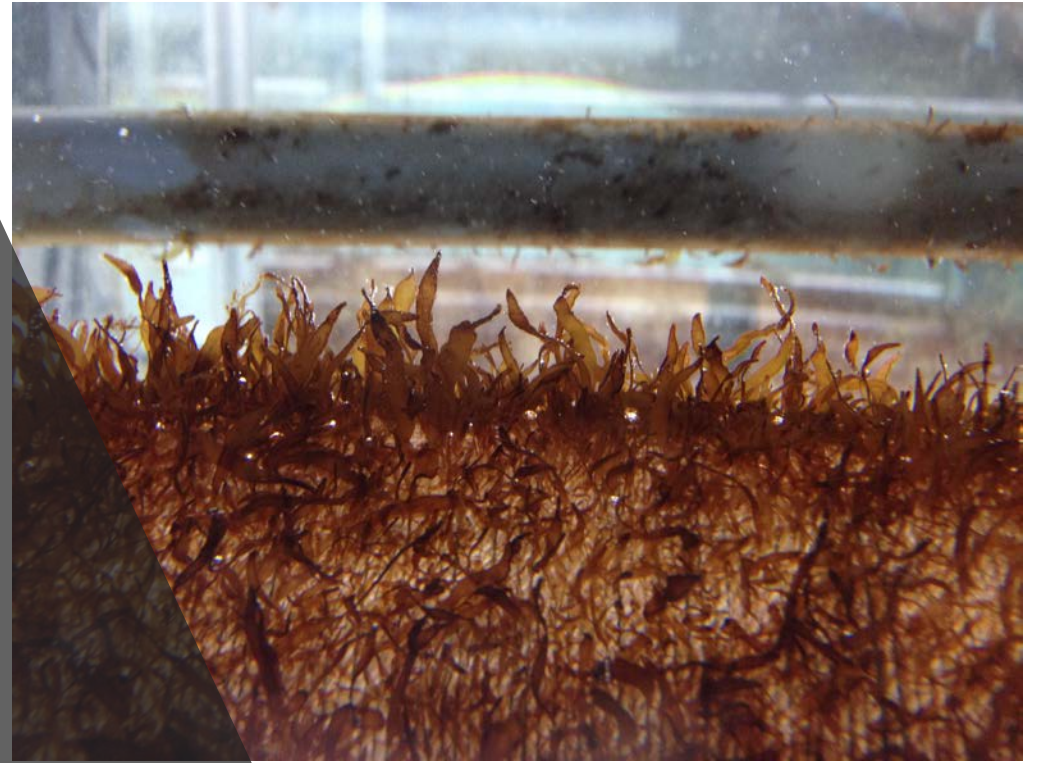
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Promising results from initial quantitative and qualitative analysis of phlorotannins

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Need more focus on *Palmaria*

Thank you!



MACRO**SEA**