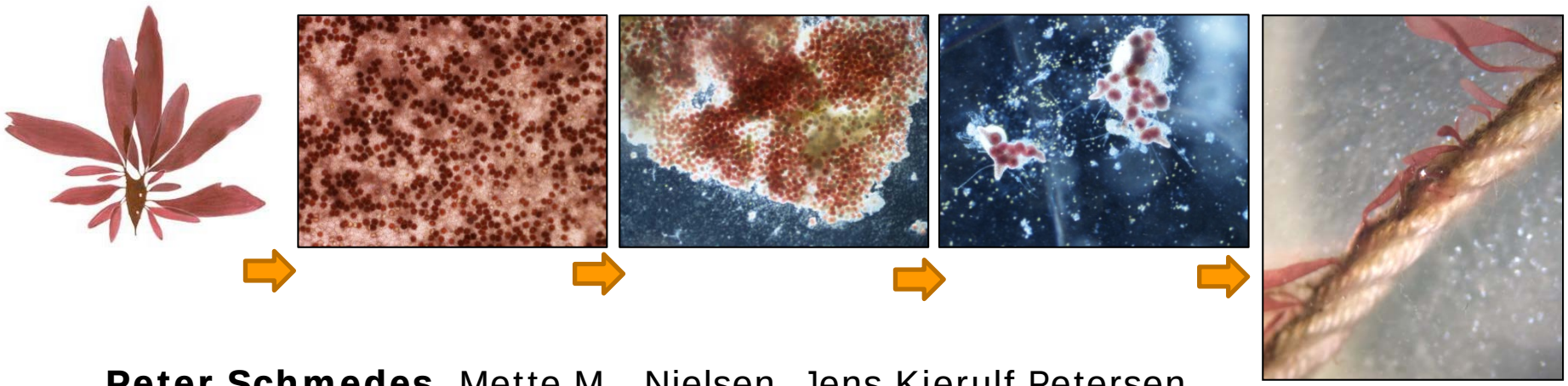
1. PhD project

***Improve Palmaria palmata* hatchery techniques**

- from tetraspores to seedling lines

NTNU
Norwegian University of Science and Technology



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PhD student

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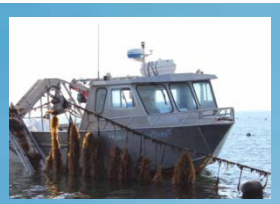
$$M2_i = \frac{\sum_j \frac{dR}{dt} N_j \frac{\varphi_{ji}}{\varphi_j}}{N_i \omega_i} \qquad \Delta \int_a^b \varepsilon \Theta^{\sqrt{17}} + \Omega \int \delta e^{i\pi} = \{2.7182818284\} \qquad \chi^2 \sum !$$

DTU Aqua
National Institute of Aquatic Resources

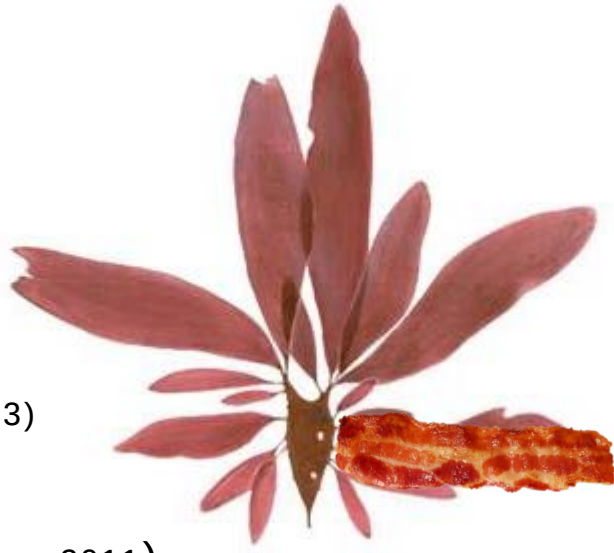
DTU Aqua

Danish Shellfish Centre
National Institute of Aquatic Resources

Experts in filter feeder cultivation and fisheries assessments
Dessimination Centre and Kitchen, Oyster Tours

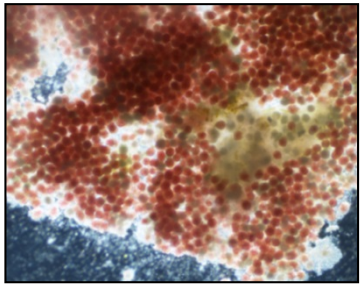



Palmaria in food
found in high valued end-products



- Snack Food or dried Dulse (prices in Denmark up to 3-6 Euro/25g)
- Composition of polysaccharides, aminoacids and minerals (Mouritsen, 2013)
- Candidate as salmon feed supplement (Moroney et al 2014)
- Cultivation is <u>not</u> feasible with current cultivation techniques (Werner&Dring, 2011)

In Nature
Palmaria is often epi-phytic



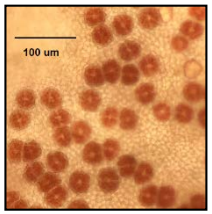
Cultivation Challenge:
High input = relative low yield

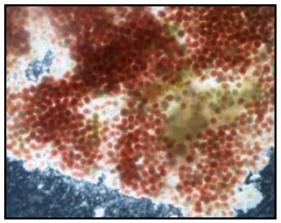
1 long-line of seeded nets
130 kg FW (1.8 t FW)

2-3 kg (2-5 t FW)

(Werner & Dring, 2011)

High percentage spore mortality at low agitation



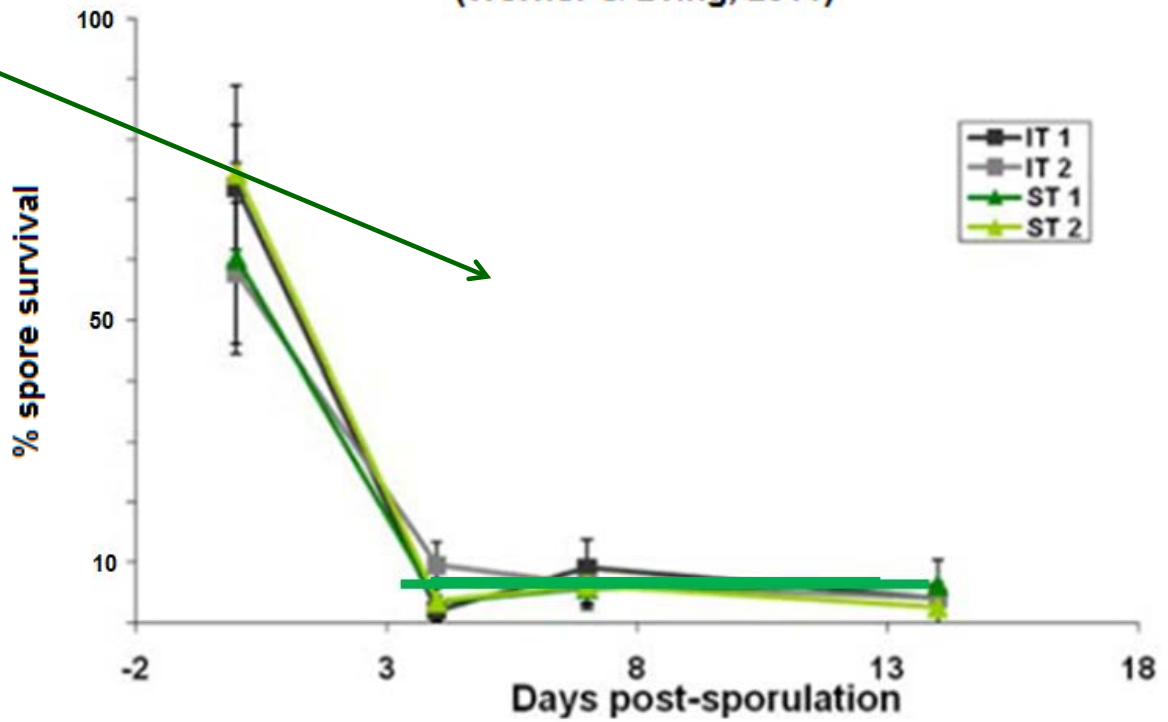


2n/n

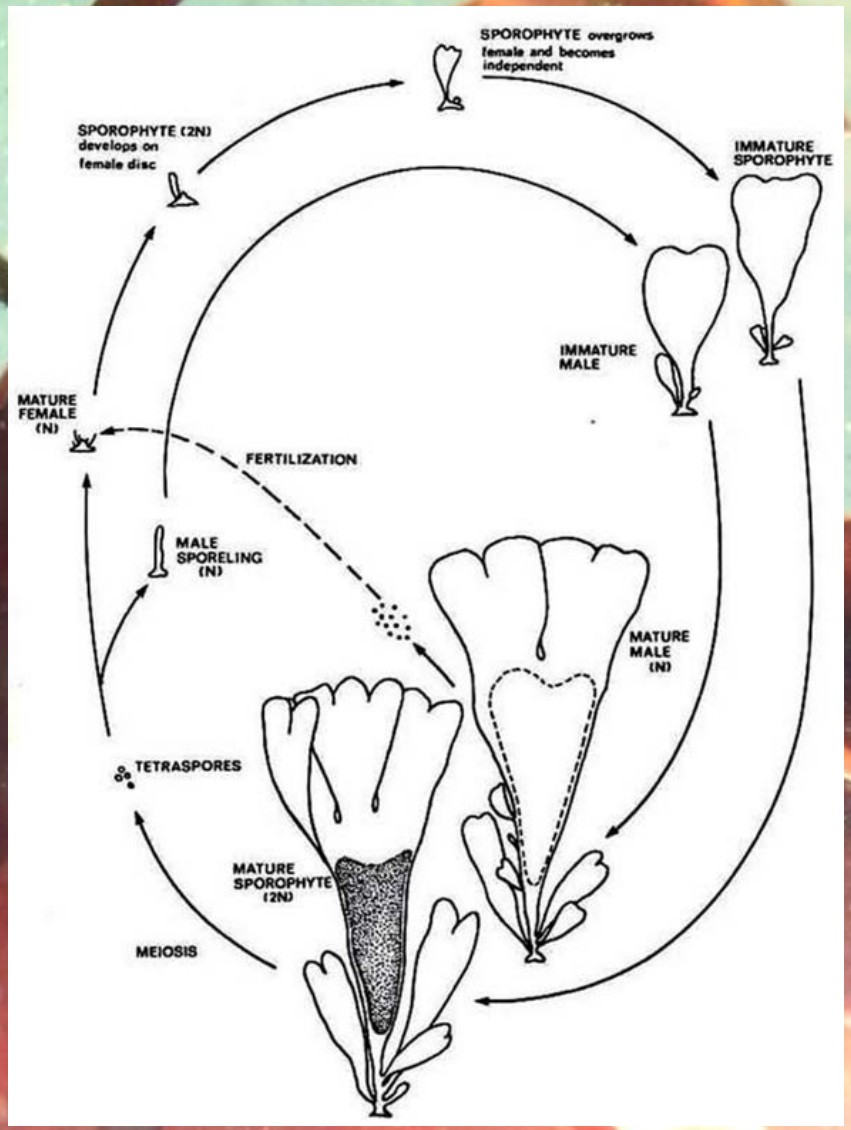
Fertile

tetraspores

(Werner & Dring, 2011)

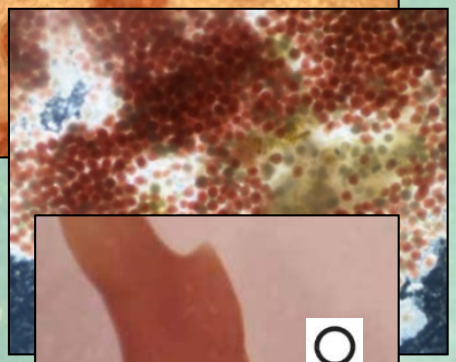


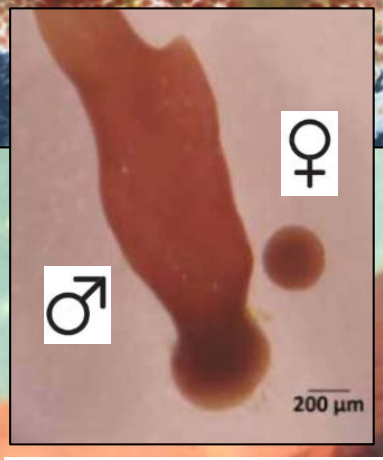
Hatchery steps based on life cycle of P. palmata



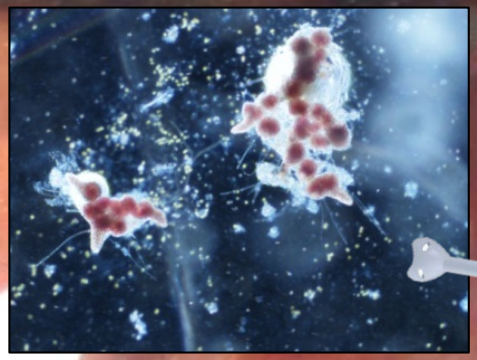
Tetrasporangia (fertility)

Can be found throughout the year
Peak season is Jan-April
(in Irish waters)
- 40.000 spores per gram tissue!





50/50% ->100?



My Objectives & Research Questions

- **Step 1. Prolong spore production.**

Can we induce fertility during summer or pre-natural occurence to expand the season?

- **Step 2. Can we optimise spore release?**

Release Experiment: What is the effect of spore release duration (time) and agitation?

- **Step 3. Investigate optimal use of spores.**

SporeUse1: Increase in seedling density by applying fertilisation step.

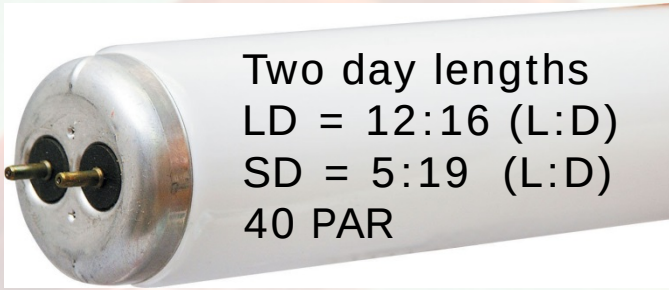
SporeUse2: Study surface preference of spores for settlement - effect of alginate coating and level of agitation.

SporeUse3:Q: Can we store, dispurse and get the spores to re-settle on substrate?

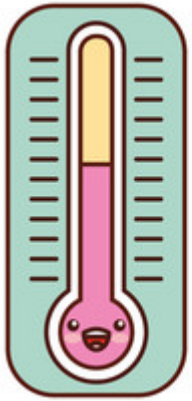
Results on the effect of blending spores and rope type

1. Induction of spore formation

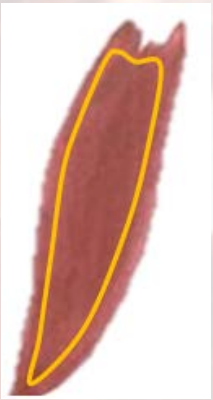
- 1. Attempt of pre-natural occurence
  - (69 days from 25.10.2016 to 04.01.2017)
  - Exposure of treatment combinations (= 16 treatments, n=5)



Two day lengths
LD = 12:16 (L:D)
SD = 5:19 (L:D)
40 PAR



10 C
5 C



F2-media
100 % (882 uM)
10 % (88,2 uM)

- Binomial data (fertile/non-fertile)
- Proc Glimmix SAS model

Class of variable:
Dates Replicate Fotoperiod Meristem Temp Nut Day
Fertility = Fotoperiod Meristem Temp Nut Day/ dist=binomial;random replicate*day ;
/* code for each container

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Fotoperiod	1	347	0.16	0.6912
Meristem	1	347	2.10	0.1482
Temp	1	347	0.86	0.3553
Nut	1	347	11.88	0.0006
Day	4	44	0.71	0.5890

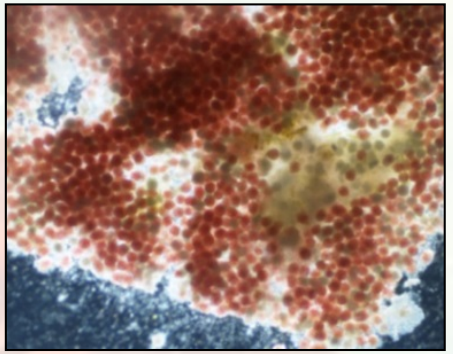
1. Induction of spore formation

off-season (summer)

- Collection
  - Palmaria collected and start of experiment in early July
  - Additional tissue collected in late August

- Laboratory treatments:
  - Edge removal, dessication, temp cycles, red light, connection to basal frond
  - Allantoin and Spermidine addition (metabolites) to medium

➢ Sporangia has yet to be induced!

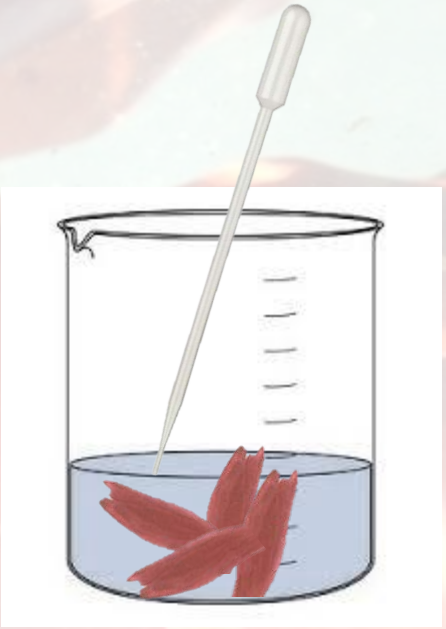


2. Spore Release Experiment

- Pre-treatment of triplicate plants (4 hours vs 24 h dehydration)

- 4h: Spore release at 10 ° C for 98 hours with no agitation
- Same amount of tissue

Pre-treatment



3 subsamples per replicate mean

2. Release Experiment

- Pre-treatment
  (4 h dehydration)

- Spore release at 10 ° C for 98 hours with no agitation
- Same amount of tissue (n=3)

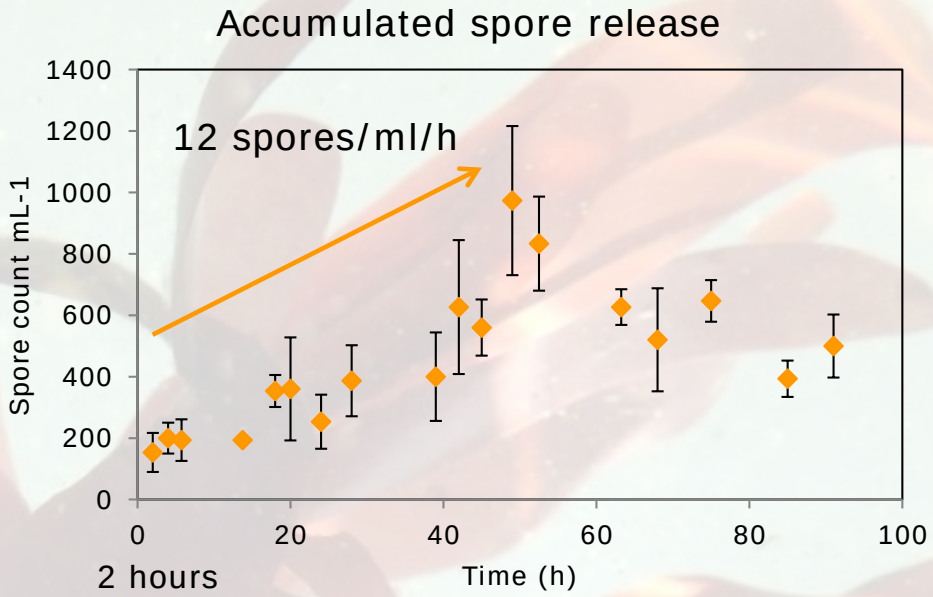
24 h dehydration

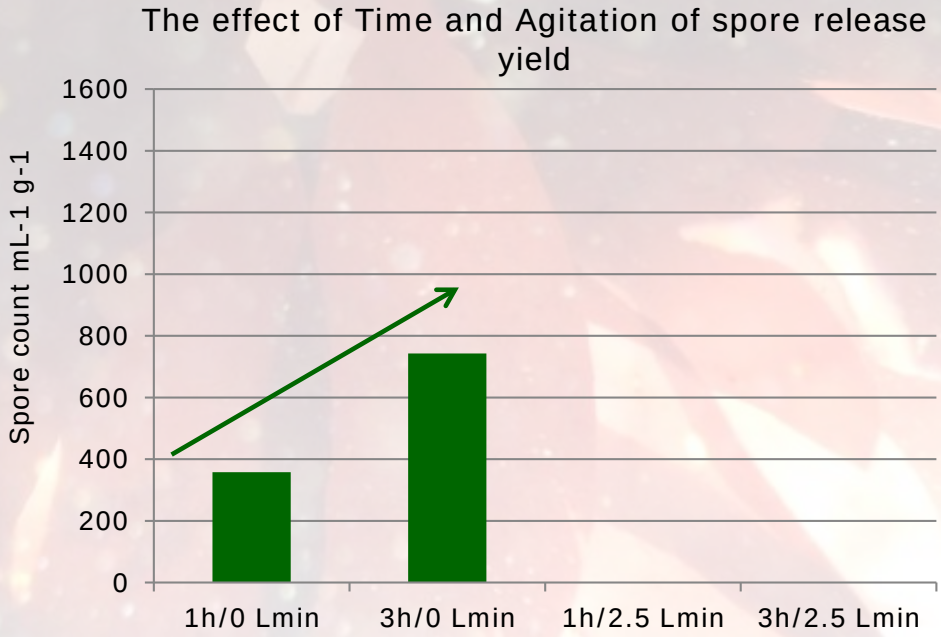
➢ show positive effect

- <u>No agitation</u>

- ~ 195 spores/ml/g/h

➢ Higher release rate compared to 4 hours pre-treatment.





n=3±SD

2. Release Experiment

- Pre-treatment
  (4 h dehydration)

- Spore release at 10 ° C for 98 hours with no agitation
- Same amount of tissue (n=3)

24 h dehydration + agitation

➢ show positive effect

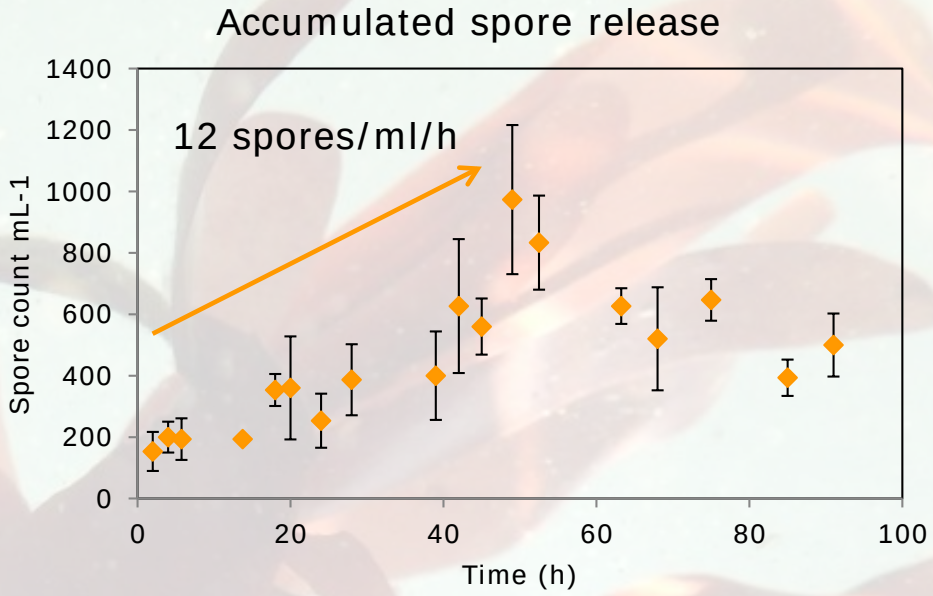
- No agitation

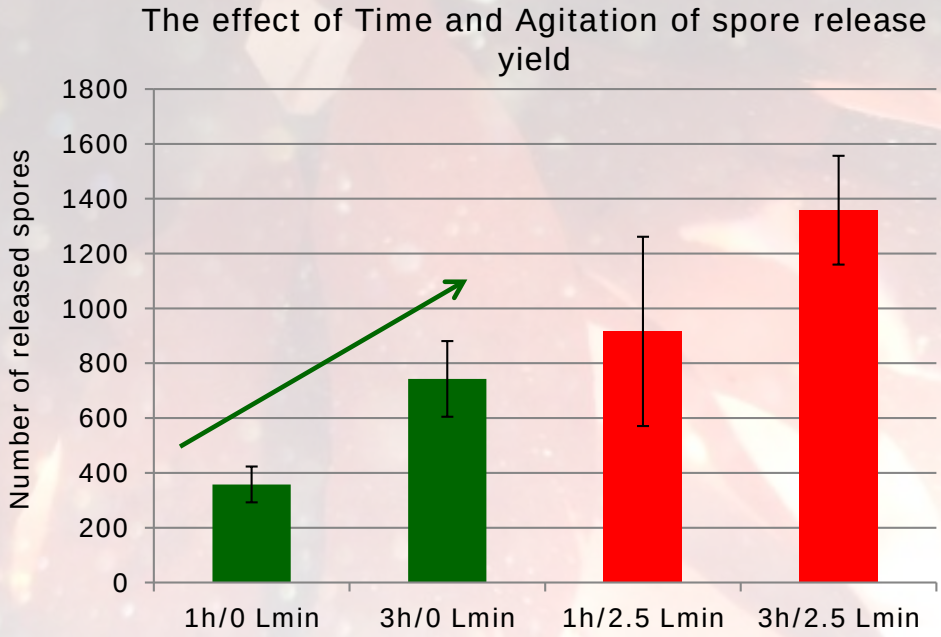
- ~ 195 spores/ml/g/h

➢ Higher release rate compared to 4 hours pre-treatment.

- With agitation

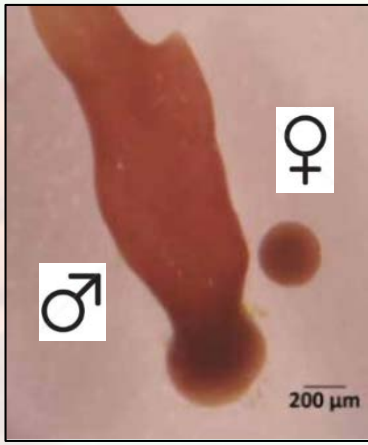
- Higher concentration
- Higher release rate
  ~ 202 spores/ml/g/h





n=3±SD

3. Applying a fertilization step (SporeUse1)

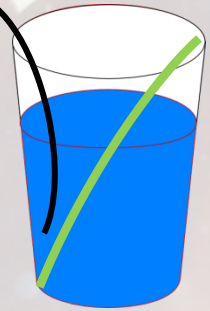


x 6

12 h drying

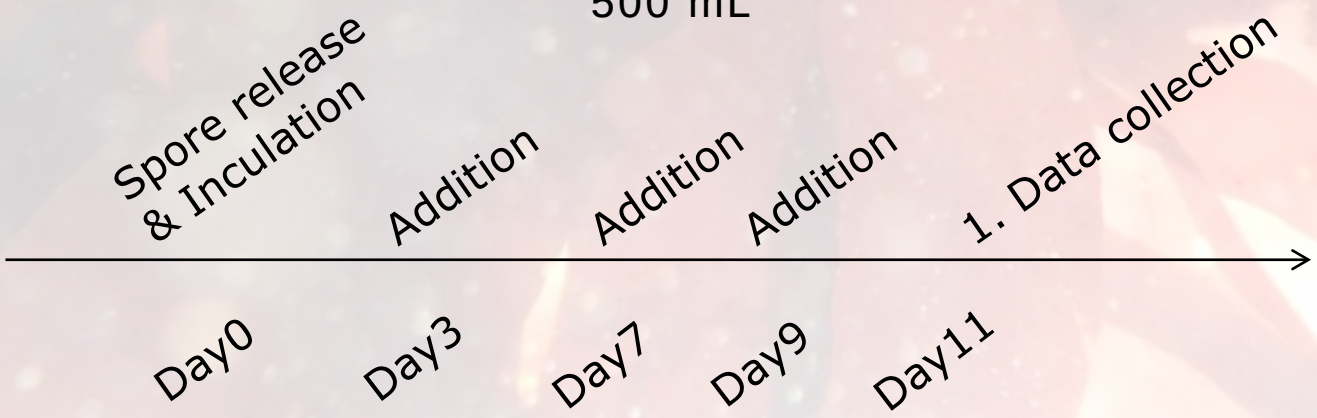
15 mL

Air

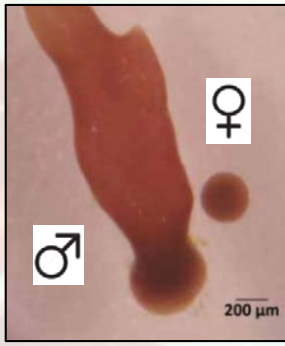


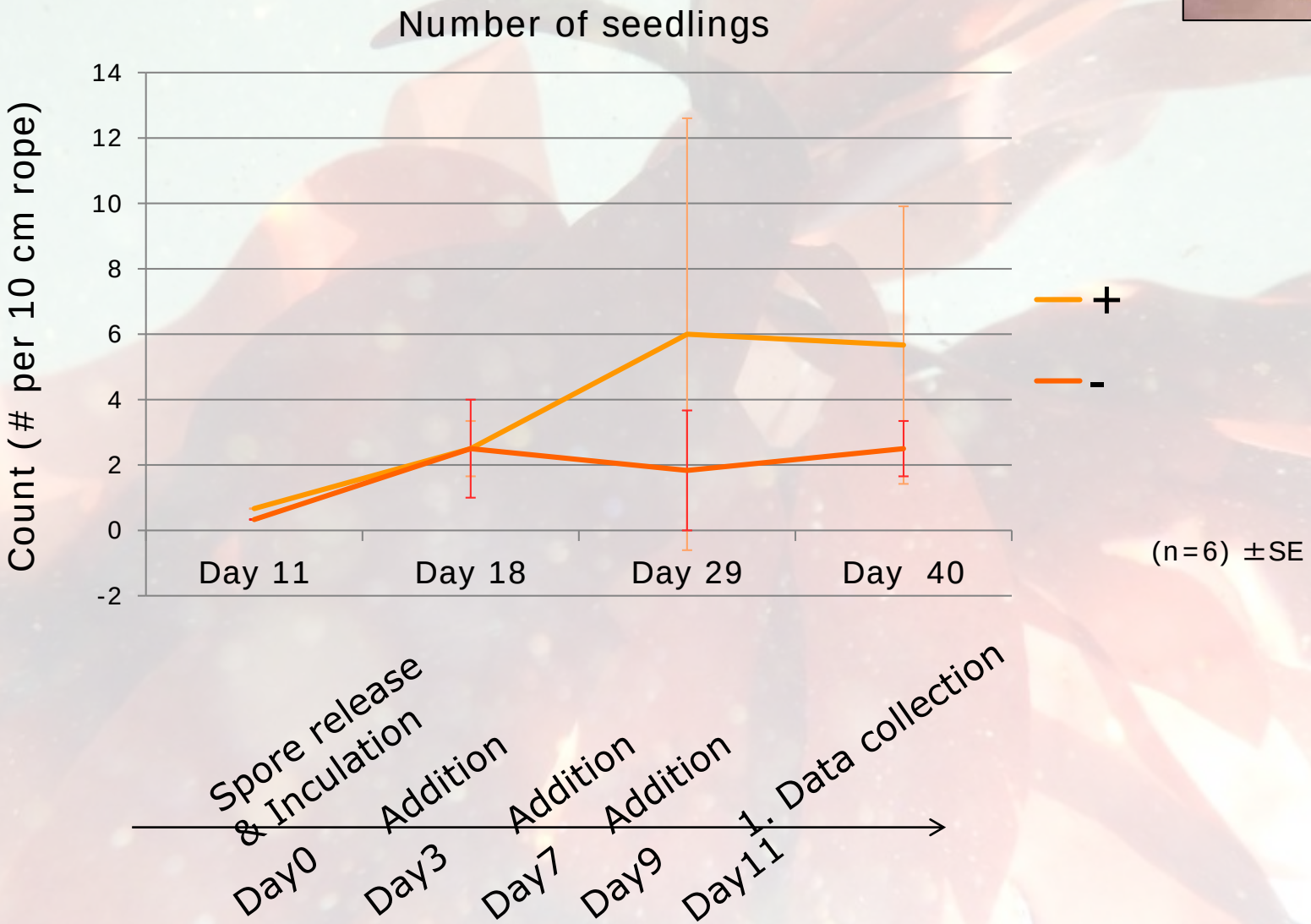
500 mL

+ : addition of 10 mL ♂
- : addition of 10 mL water



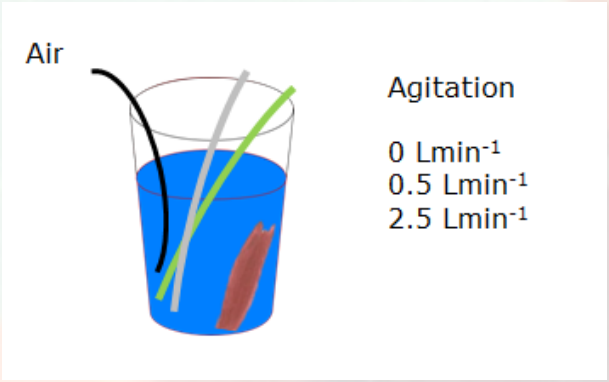
3. Result: Applying fertilization step



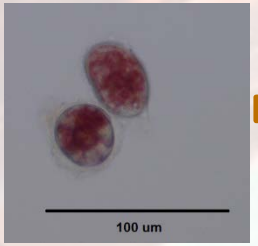


4. Spore settlement (SporeUse2)

- Preference study
- Assess effect of alginate coating and agitation



- Clean rope
- Coated rope
- Spores in excess
- N=3



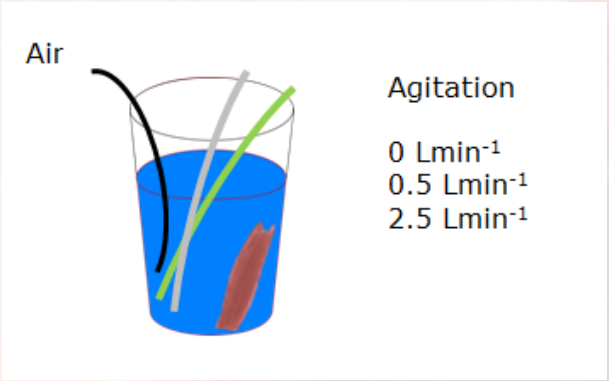
Prepare fertile tissue
12 h drying

Soaking 24h.
Alginate solution

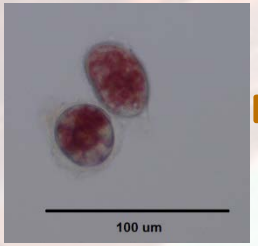
Day-1

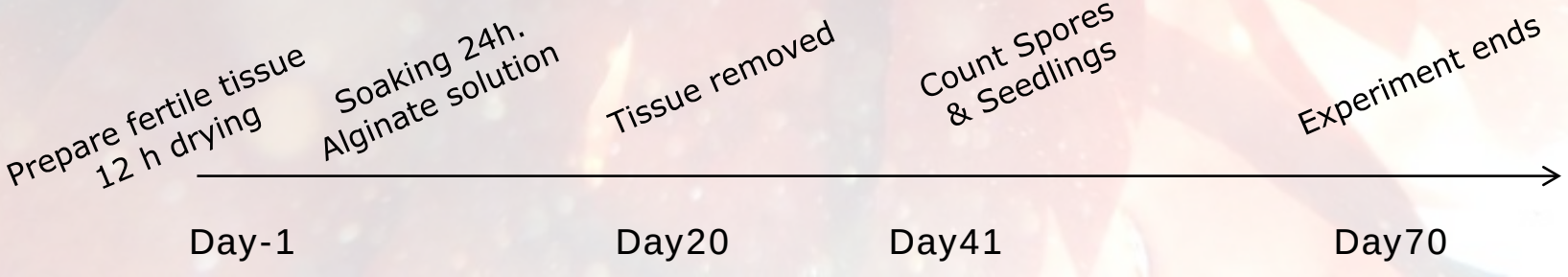
4. Spore settlement (SporeUse2)

- Preference study
- Assess effect of alginate coating and agitation

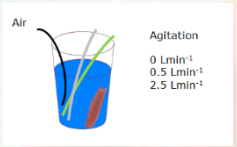


- Clean rope
- Coated rope
- Spores in excess
- N=3

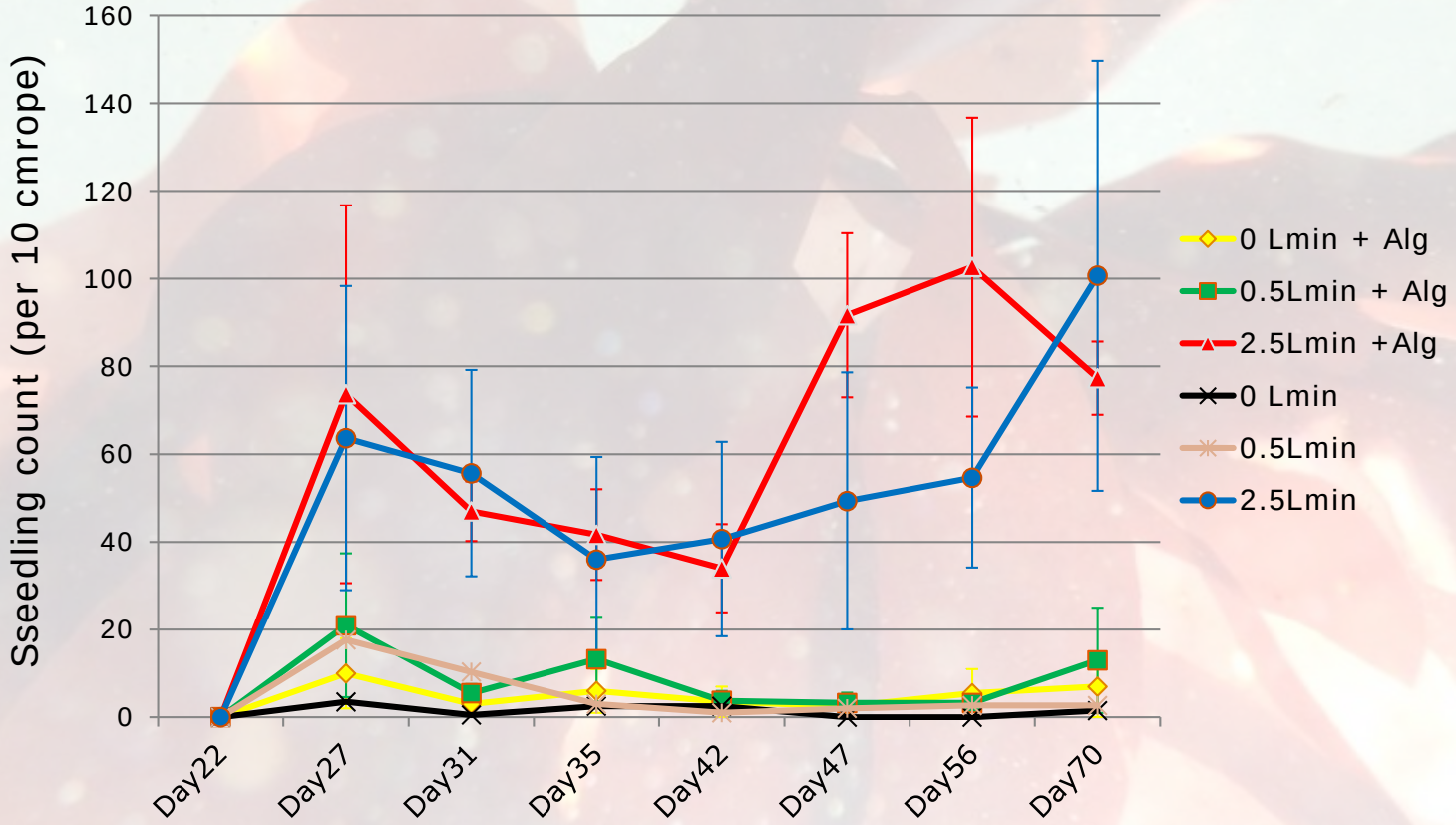




4. Results: Spore settlement
Alginate coating + agitation

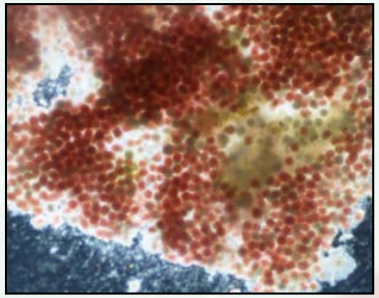


Number of seedlings dependent on agitation

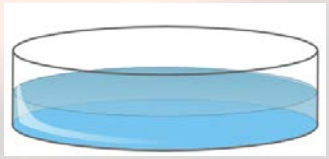


4. Spore settlement (SporeUse3)

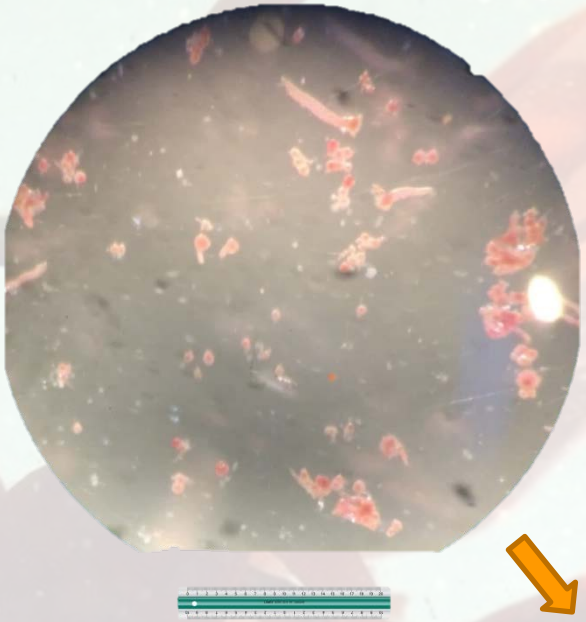
Can we store, dispurse and re-settle spores to substrate?



1 minute



30 days
Low light
5 ° C



15 mL

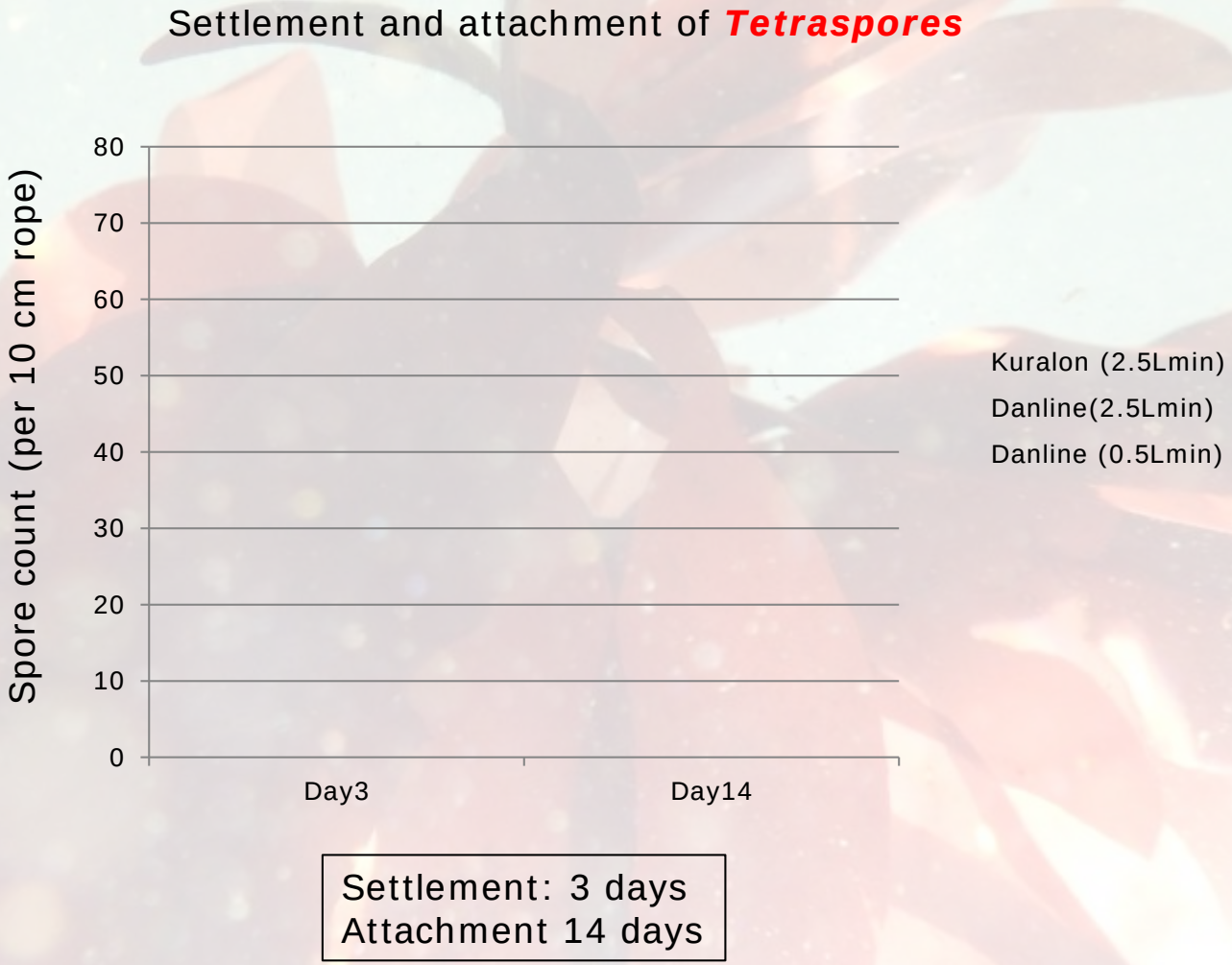
Air agitation
0.5 $Lmin^{-1}$
2.5 $Lmin^{-1}$

Ropes
5 mm Danline (PV)
2 mm Kuralon

4. Results: Spore settlement (SporeUse3)

Danline

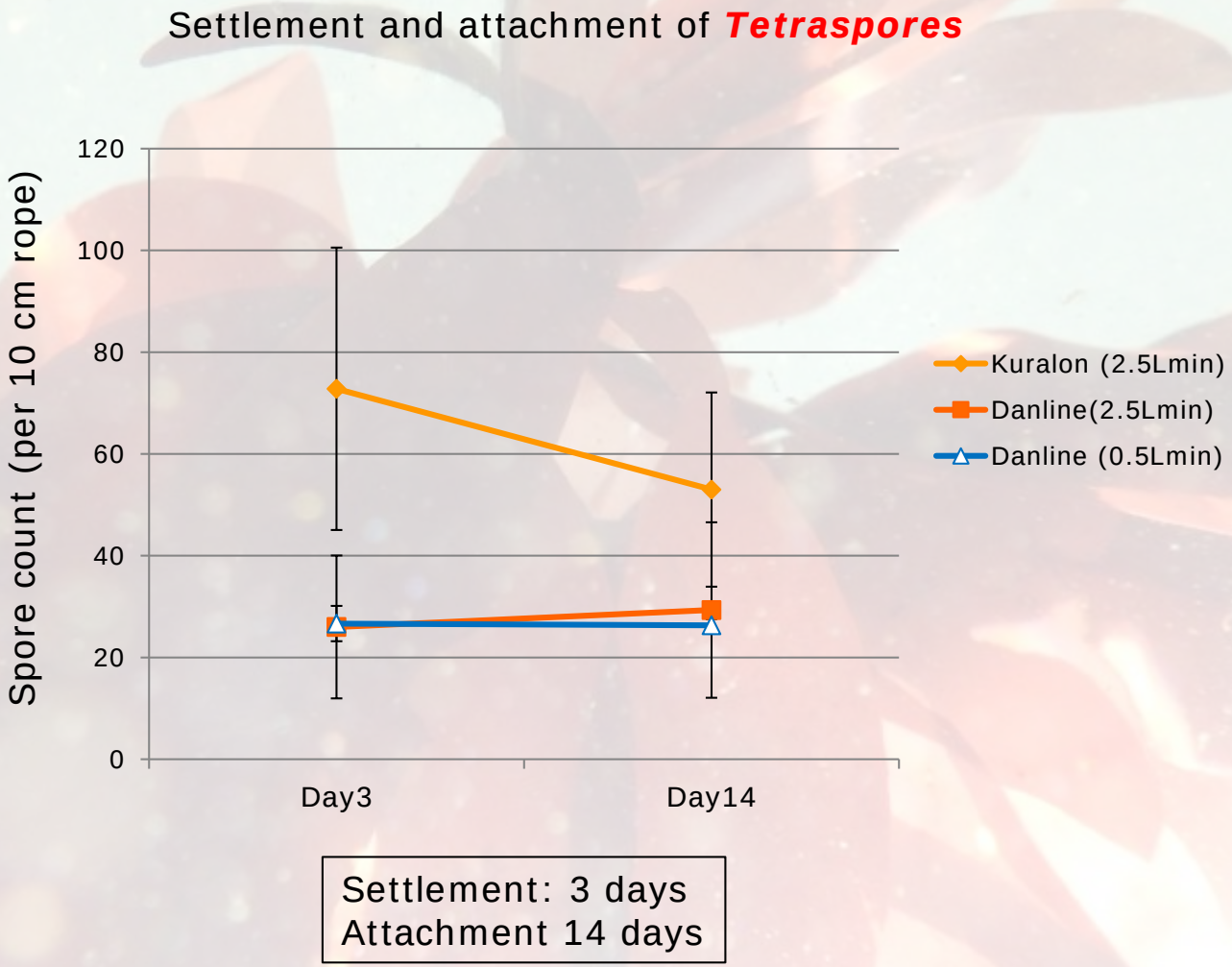
Kuralon



4. Results: Spore settlement (SporeUse3)

Danline

Kuralon

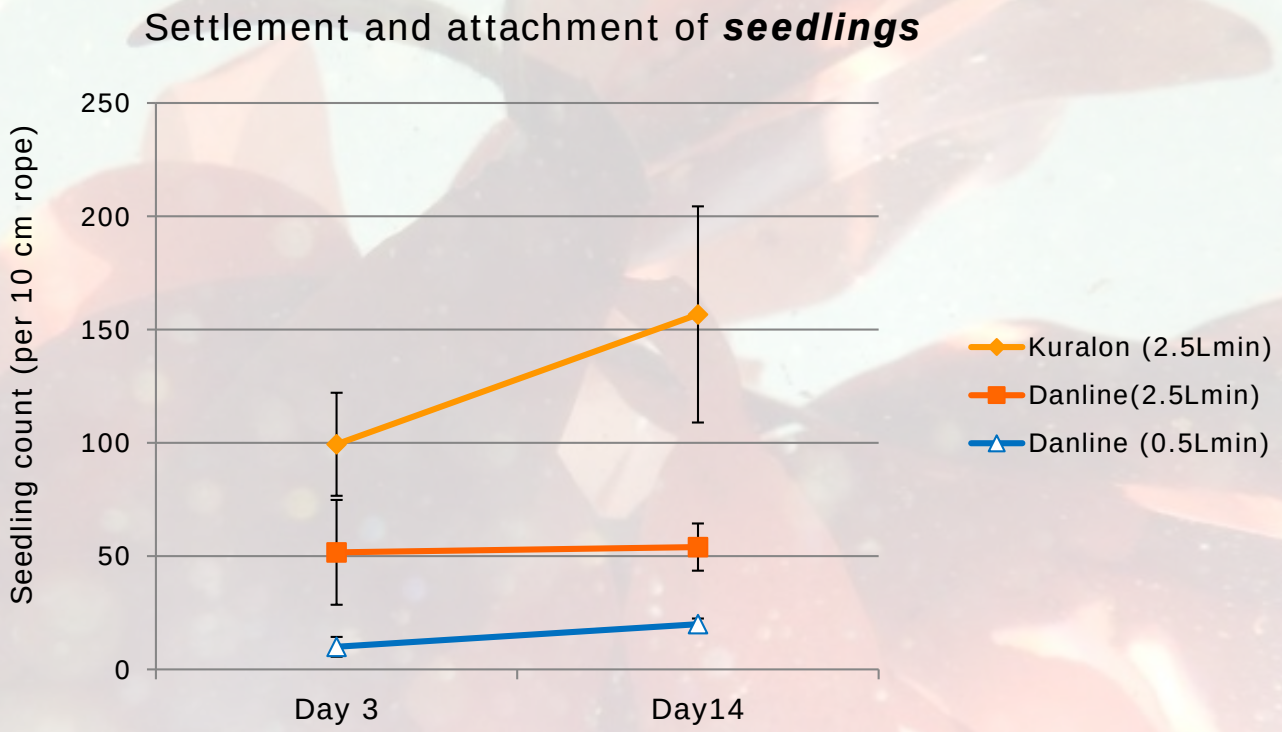


Settlement: 3 days
Attachment 14 days

4. Results: Spore settlement (SporeUse3)

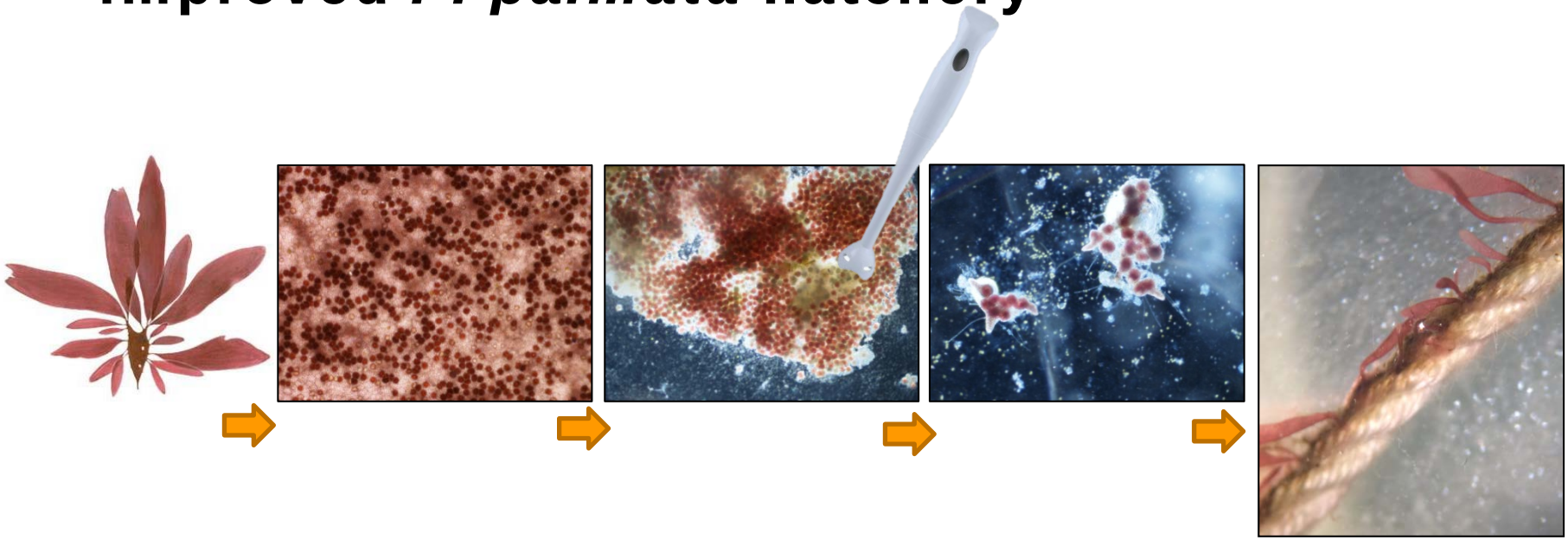
Danline

Kuralon



Settlement: 3 days
Attachment 14 days

Findings and suggestions for improved *P. palmata* hatchery



- Time duration and agitation increase spore release and agitation resulted in higher seedling density
- Blending the spore release provide dispersal of spores and seedlings Kuralon rope show higher density compared to Danline rope
- Fertilization step; adding male gamets increase numbers of seedlings
- No clear effect of Alginate pretreatment

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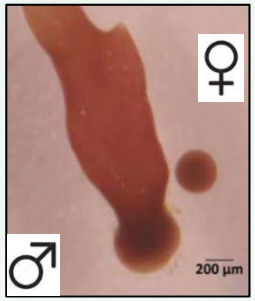
www.skaldyrcenter.aqua.dtu.dk/

$$M2_i = \frac{\sum_j \frac{dR}{dt} N_j \frac{\varphi_{ji}}{\varphi_j}}{N_i \omega_i}$$

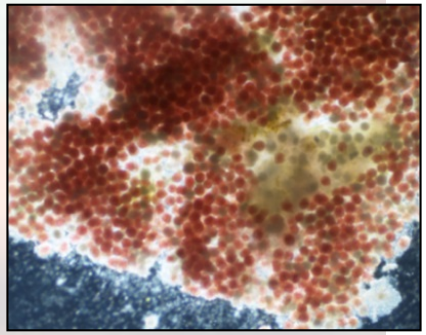
$$\Delta \int_a^b \varepsilon \Theta^{\sqrt{17}} + \Omega \int \delta e^{i\pi} = \infty \neq \{2.7182818284\} \chi^2 \gg \sum !$$

DTU Aqua
National Institute of Aquatic Resources

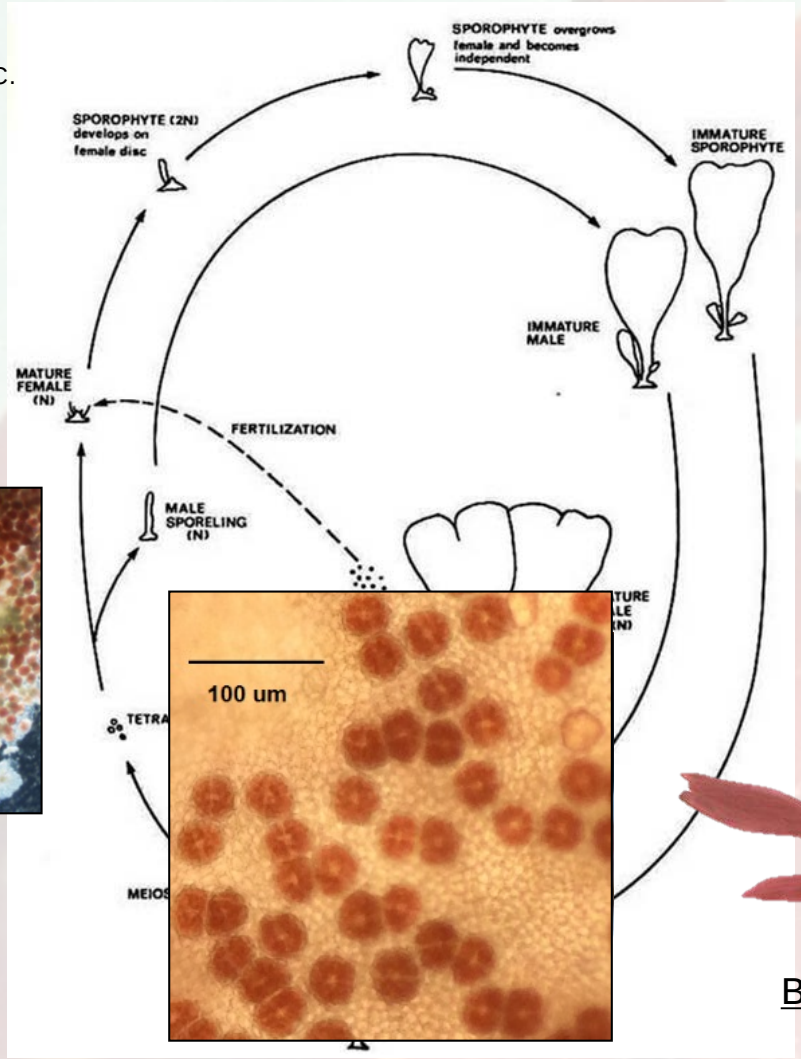
Photo: Constanza C.



Germination
1:1



Spore release

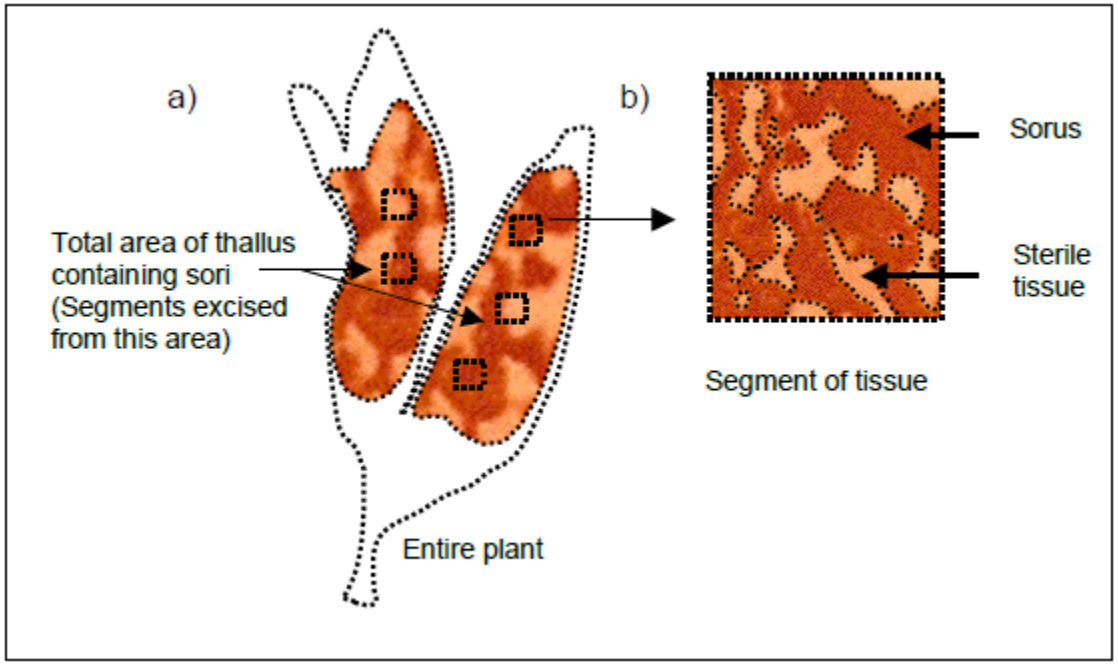


Fertility (Jan-April)

Seedlings

Biomass

1 season
0.3-0.9 kg/m


a)
b)
Total area of thallus containing sori (Segments excised from this area)
Entire plant
Sorus
Sterile tissue
Segment of tissue