1. PhD project

**Improve *Palmaria palmata* hatchery techniques**
- from tetraspores to seedling lines

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**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
Dissimination 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 not feasible with current cultivation techniques (Werner&Dring, 2011)
In Nature
Palmaria is often epi-phytic

DTU Aqua, Technical University of Denmark
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 \rightarrow \text{Fertile} \rightarrow \text{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!
My Objectives & Research Questions

- **Step 1. Prolong spore production.**
  Can we induce fertility during summer or pre-natural occurrence 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, disperse and get the spores to re-settle on substrate?
  Results on the effect of blending spores and rope type 50/50% -> 100?
1. Induction of spore formation

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

- 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

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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
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

- No agitation
  - ~ 195 spores/ml/g/h

- Higher release rate compared to 4 hours pre-treatment.
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
3. Applying a fertilization step (SporeUse1)

- 12 h drying
- Spore release & Inculcation
- 15 mL
- Air
- 500 mL

Day 0: Spore release & Inculation
Day 3: Addition
Day 7: Addition
Day 9: Addition
Day 11: 1. Data collection

+: addition of 10 mL
-: addition of 10 mL water
3. Result: Applying fertilization step

![Graph showing number of seedlings over time with fertilization](image)

- Count (# per 10 cm rope)
- Number of seedlings
- (n=6) ± SE

- Spore release & Incubation
- Day 0
- Day 3
- Day 7
- Day 9
- Day 11
- Data collection
4. Spore settlement (SporeUse2)

- Preference study
- Assess effect of alginate coating and agitation
  - Clean rope
  - Coated rope
- Spores in excess
- N=3

Day-1

Prepare fertile tissue
12 h drying

Soaking 24h.
Alginate solution
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

Seedling count (per 10 cm rope)
4. Spore settlement (SporeUse3)

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

- 30 days
- Low light
- 5 °C

1 minute

Air agitation
0.5 Lmin⁻¹
2.5 Lmin⁻¹

Ropes
5 mm Danline (PV)
2 mm Kuralon

15 mL
4. Results: Spore settlement (SporeUse3)

Settlement and attachment of *Tetraspores*

- **Danline**
- **Kuralon**

Spore count (per 10 cm rope)

- Day 3
- Day 14

- Kuralon (2.5L/min)
- Danline (2.5L/min)
- Danline (0.5L/min)

Settlement: 3 days
Attachment: 14 days
4. Results: Spore settlement (SporeUse3)

Settlement and attachment of *Tetraspores*

Settlement: 3 days
Attachment 14 days
4. Results: Spore settlement (SporeUse3)

Settlement and attachment of seedlings

Seedling count (per 10 cm rope)

- **Kuralon (2.5L/min)**
- **Danline (2.5L/min)**
- **Danline (0.5L/min)**

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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Germination
1:1

Spore release

Fertility (Jan-April)

Seedlings
1 season
0.3-0.9 kg/m

Biomass

Photo: Constanza C.