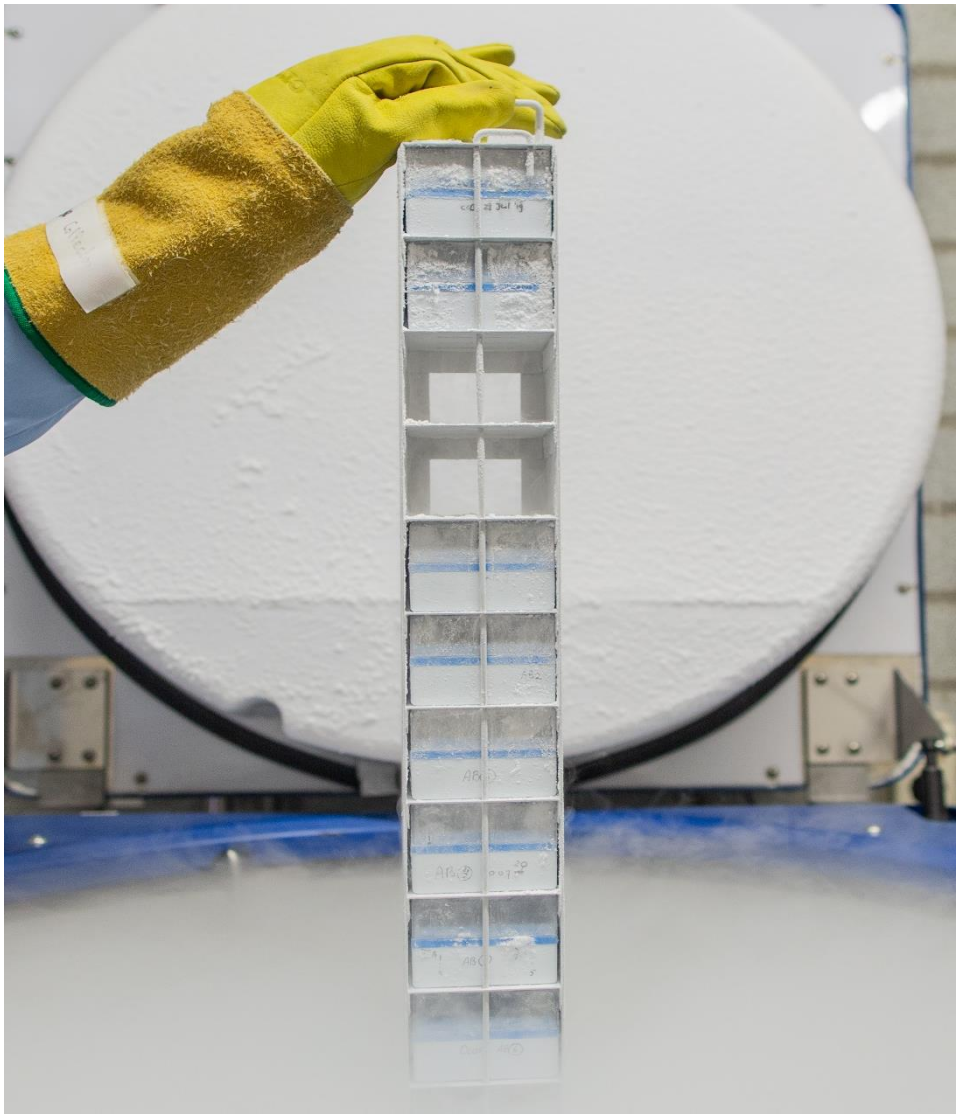

Cryopreservation of *Ulva* spp. and *Saccharina latissima*

Optimised protocols of cryopreservation, recovery and (long-term) regrowth of *Ulva* spp. and *Saccharina latissima* gametophytes and sporophytes

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UNIVERSITY & RESEARCH

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1 Wageningen University & Research

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Wageningen, November 2023

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Summary
In this report the optimised protocols for cryopreservation and long-term re-growth for *Ulva* spp. and *Saccharina latissima* are described.

Keywords: seaweed, biobanking

Report WPR-2023-1275

Photo cover: Loek Buter

Contents

Summary

In the seaweed field, both scientists and practitioners have a strong interest in standardized protocols for cryopreserving various seaweeds. Lee & Nam (2016) developed protocols for *Ulva prolifera* (Ulvales, Chlorophyta). Fort & Gachon (2019, not published) started with optimising the protocol of Lee & Nam (2016) for *Ulva* spp. in the GeniAlg-project and we continued optimising the *Ulva*-protocol during the GeniAlg-project (2017-2021). In 2023 our Aquatic Production Systems group of the Wageningen University and Research optimised a protocol of cryopreservation and regrowth for *Saccharina latissima*. The protocol is largely adapted from Visch et al. (2019). In this report both optimised protocols for cryopreservation and (long-term) regrowth are described. Experiments have shown that the protocols are workable to cryo-store the seaweeds for a long time, in a robust and solid way and after recovery the seaweeds are able to regrow (long-term).

1 Introduction

Kelp forests absorb significant amounts of nutrients, and therefore kelp forests may be utilized as bioremediators, especially along the coastal areas with excessive amounts of land-based nutrient input (Umanzor & Stephens 2022). Alternatively, when cultivated commercially, these "forests" may contribute to the circular economy via a re-use of the land-based derived nutrients and its chemical end-products. In this way, kelp forests may prevent eutrophication near coastal areas thus preserve ecosystem biodiversity, and additionally the seaweed biomass produced may again be re-used in the form of biostimulants. E.g., the last decades, biostimulants derived from seaweeds have shown to increase productivity of arable crops under both stressed (e.g. salinity and drought stress) and non-stressed conditions (Ayodeji et al. 2022). Besides being an efficient bioremediator, kelp forests may also enhance the biodiversity via provision of additional habitats, it can be a positive stimulus for nature-inclusive cultivation methods. The windmill farms for the Dutch coast create new opportunities for the development of aquaculture (the Dutch government aims for 300 km² multi-use facilities in the North Sea by 2030, amongst others seaweed farming). In the past, arable farming has focused too much on monoculture with its negative impact on biodiversity. Nowadays there is an increased understanding of the importance of maintaining the genetic variation of crops for sustainable resilient food systems and of maintaining biodiversity of the ecosystem for an optimal balance in growth of crops including plant disease prevention/protection.

Reliable and reproducible research on seaseeds and its commercial use depends on genetically stable materials. Genetic drift (accumulation of random mutations) has been allowed to run its course in the green seaweed labs for decades. Many other model systems, including the moss system, are kept frozen and after a number of generations the frozen culture is used again (Christianson 1998). This ensures that the same material is used for decades of research and limits genetic drift. Transformants (mutants/fluorescently labelled lines) are also stored in this way so that they are safely conserved for future research/commercial use with low maintenance costs. Seaweed growers have a similar demand for the cryopreservation of seaweed starting material so that biobanks become superfluous, thus minimizing space and maintenance costs. In addition, cryopreservation safeguards the valuable collection of genetic material, which is an important source for breeding seaweed crops with desirable characteristics in the future. In the seaweed field, both scientists and practitioners have a strong interest in standardized protocols for cryopreserving various seaweeds. For several seaweeds, including green algae *Ulva* spp. and kelps *Saccharina* sp., cryopreservation protocols are available in scientific literature (Charrier et al. 2018, Lee & Nam 2016, Visch et al. 2019), but their applicability and reproducibility remains uncertain until now. E.g. no scientific information is available on the robustness of the procedure and long-term re-growth after cryopreservation.

For cryopreservation, rapid cooling to the vitrification point is necessary for a high level of cell survival. Vitrification is a process in which water is solidified to a non-crystalline state through cooling, avoiding mechanical damage to the cells. At a temperature below vitrification point, water takes on an amorphous state. When the temperature rises above devitrification point water in the vitrified sample will spontaneously re-crystalizes. Rapid warming inhibits this re-crystallisation, allowing water to continue in an amorphous state which ensures a high level of cell survival. The use of cryoprotectant additives (CPAs) enhances the vitrification and devitrification process by elevating the vitrification and devitrification points. CPAs slow down the rate of crystal formation, partially dehydrate cells and raises the viscosity of the cell contents. By scientists a number of vitrification-protocols were developed in which these mechanisms are optimized in a one or two-step process followed by rapid cooling to -196 °C in liquid nitrogen (LN2) and a rapid heating/diluting process for recovery of the organism (Heesch et al. 2012).

Lee & Nam (2016) developed protocols for *Ulva prolifera* (Ulvales, Chlorophyta). In the GeniAlg-project Fort & Gachon (2019, not published) largely adapted the cryopreservation and recovery protocol for *Ulva* spp. (sea lettuce) from Lee & Nam (2016). We have made some minor adjustments to optimise the protocol of Fort & Gachon (2019) during the GeniAlg-project (2017-2021)(Fort et al. 2019, 2020, 2020,

2022, Jansen et al. 2022). Secondly a protocol of cryopreservation and regrowth for *Saccharina latissima* (sugar kelp) is optimised. The protocol is largely adapted from Visch et al. (2019) and optimised within the IF-project Aquatic Production Systems 2023 of Wageningen University and Research, Business Unit Agrosystems research. In this report the optimised protocols for cryopreservation and long-term re-growth for *Ulva* spp. and *Saccharina latissima* are described.

2 Cryopreservation of *Ulva* spp.

2.1 Protocol of cryopreservation of *Ulva* spp.

Fort & Gachon (2019, not published) largely adapted the cryopreservation and recovery protocol from Lee & Nam (2016). We have made some minor adjustments to optimise the protocol of Fort & Gachon (2019, not published) see fig 2.1.1 for the life cycle.

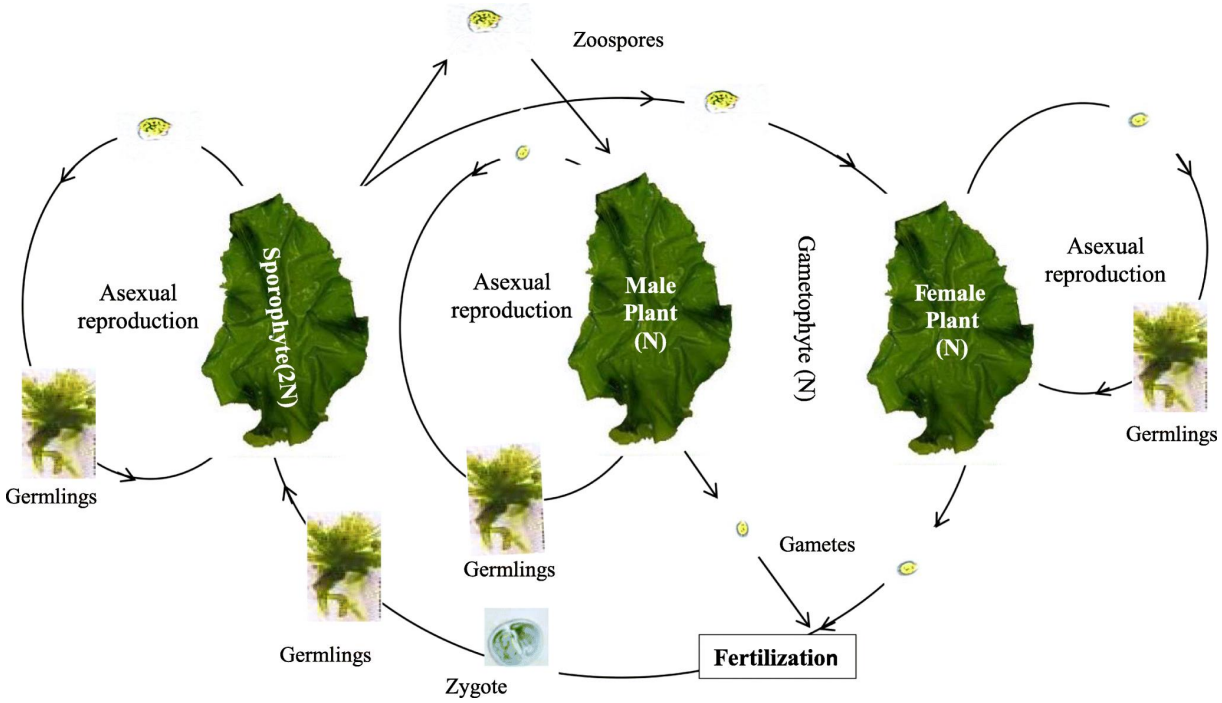

Figure 2.1.1 *Figure adapted from Mantri et al. 2020. Cited "Typical life cycle in Ulva"*

Our protocol for cryopreservation of thallus of Ulva spp. is as follows: The solutions and materials needed are: Fresh Ulva-thallus from the wild (Oosterschelde or North Sea) or kept at 6°C in long-term-storage climate chamber (up to 4 months or longer), with low light conditions (0,97 to 5,8 µmol $m^{-2}s^{-1}$ irradiance), 7 h light/17 h dark cycle, ~40 cm distance to RGBW led light source with spectrum showed in fig 2.1.2 Artificial seawater (ASW) 37.1 gram/L salt (Redseafish) made into demineralized water in 0.01M HEPES, pH 8. 40% glycerol stock solution in artificial seawater (ASW) made in 0.01M HEPES, pH 8. Cryovials® 2 ml in Simport plus boxes with lid (7.5 cm x 7.5 cm, 25 holes). Freeze Control CL-3300 Cryo Genesis with Program PROTO2 (Van Peer et al. (2005) not published) and Liquid Nitrogen barrel.

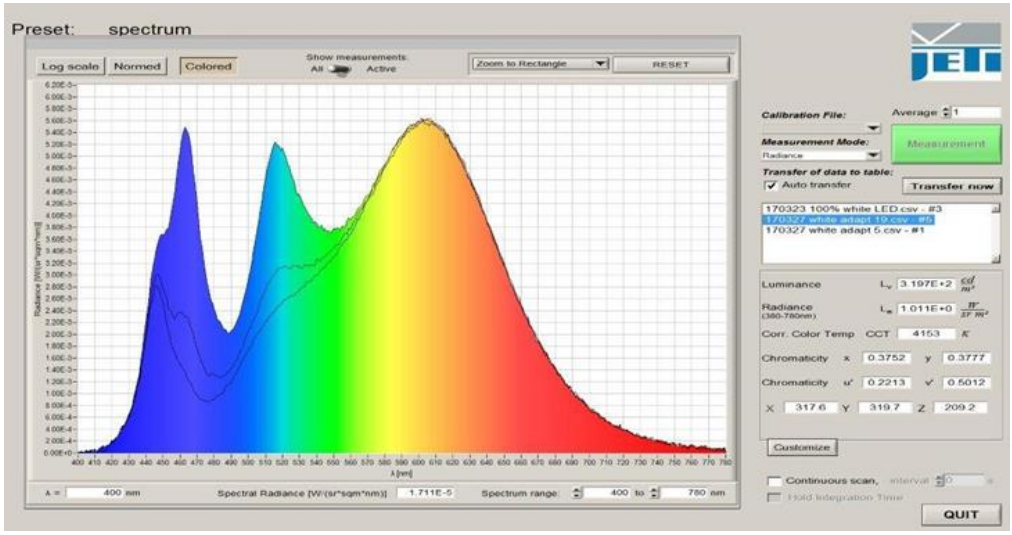

Figure 2.1.2 *Spectrum RGBW led light source in climate chambers (w1 and w2).*

To prepare thallus for cryopreservation punch with a drill *Ulva* discs of 1 cm diameter (fig 2.1.3) and place five discs per 2 ml cryovial® in 750 µl ASW, 0.01M HEPES, pH8. Add slowly 750 µl of 40% glycerol stock solution (75µl each time (in 10 steps) up to 750 µl, shaking gently between additions, typically over 15 minutes) for a final concentration of 20% glycerol. Incubate at room temperature for ~45 minutes. Place the cryovials® in the Freeze Control (fig. 2.1.4) and start the Program PROTO2: In two hours and 15 minutes, in 6 steps, the programme is run as follows: 1. Start at 20°C, 2. Cooling down to 10°C at 2°C/min, 3. Bell & Prompt, 4. Ramp to -28°C at 0.5°C/min, 5. Ramp to -80°C at -1°C/min, 6. End final state -80°C. Place cryovials® in Simport plus boxes with lid in liquid nitrogen and keep in nitrogen container (-196°C) till recovery.

Figure 2.1.3 *Punch with a drill Ulva discs of 1 cm diameter (Foto Luuk Buter).*

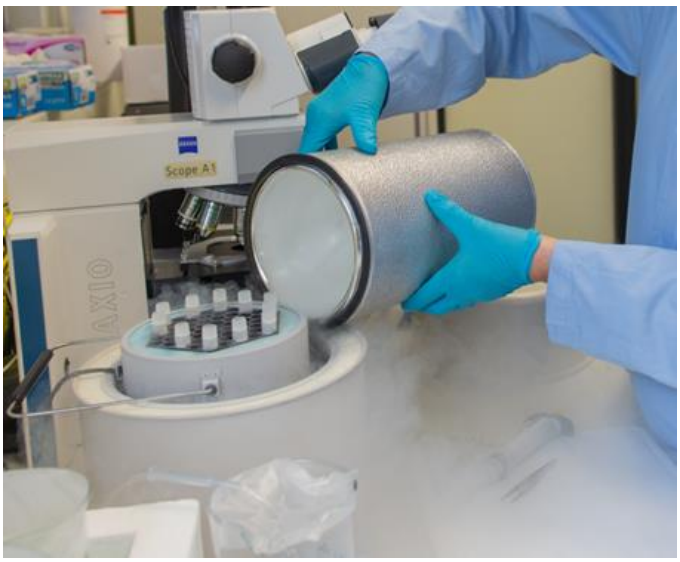

Figure 2.1.4 *Freeze Control CL-3300 Cryo Genesis (Foto Luuk Buter).*

2.2 Protocol of recovery and long-term regrowth of *Ulva* spp.

Our protocol for recovery and long-term regrowth of cryopreserved thallus of *Ulva* spp. is as follow: Materials needed for recovery: Nutrients CellHIF2P (Varicon Aqua Solutions Ltd.), stock solution 100g/L in demineralized water, water bath or thermoshaker and air pump (1 L air per minute), Artificial seawater (ASW) 37.1 gram/L salt (Redseafish) made into demineralized water in 0.01M HEPES, pH 8 and Filtered seawater (FSW) from the Oosterschelde in the Netherlands.

Recovery of thallus

Quickly thaw samples at 40 °C (water bath or thermoshaker) for ~1.5 minute until ice is gone. Incubate tissue in 50 ml beaker containing in 30 ml pre-cooled ASW 0.01M HEPES, pH8 solution on ice for 30 minutes. Place in 100 ml beaker containing 50 ml ASW in 0.01M HEPES ph8+0.05 ml CellHIF2P (from stock solution 100g/l) and place for one week, (no air bubbles) in a climate chamber at 6°C, low light conditions (0.97 to 5.83 µmol $m^{-2}s^{-1}$ irradiance), 7 h light/17 h dark cycle, ~40 cm distance to RGBW led light source with spectrum showed in fig. 2.1. On day 7 not renew the medium (no air bubbles) and place beaker of 100 ml to a climate chamber at 14°C, 12h light/12h dark cycle, 80 µmol $m^{-2}s^{-1}$ irradiance, 30 cm distance to RGBW led light source for 4 days. Dead cells will be visible after ~1-3 days, with patches of live green tissue and spores or gametes will be formed. Do not remove the dead tissue when replacing the media. On day 11 renew the medium with 50 ml ASW in HEPES +0.05 ml CellHIF2P (from stock solution 100g/l) (no air bubbles) at 14 °C, 12 h light/12 h dark cycle, 80 µmol $m^{-2}s^{-1}$ irradiance in climate chamber, distance 30 cm to light-source for 3 days.

Regrowth of thallus

After 14 days of recovery, replace media with normal FSW 50 ml (no need for buffering) plus nutrients 0.05 ml CellHIF2P (from stock solution 100g/L), add bubbling (1 L air per minute) and place under higher light ~150-200 µmol $m^{-2}s^{-1}$ irradiance 30 cm distance to RGBW led light source (Fig. 1) for 4 days. On day 18 renew the medium with 200 ml FSW plus 0.2 ml CellHIF2P (from stock solution 100g/L) in beaker of 800 ml at 14 °C, 12 h light/12 h dark cycle, ~150-200 µmol $m^{-2}s^{-1}$ irradiance, distance 30 cm to RGBW led light-source, for 3 days, perform air-bubbles ((1 L air per minute).

Maintain in that stage for 46 days, replacing media every 3-4 days with 200 ml FSW plus 0.2 ml CellHIF2P (from stock solution 100g/L) in beaker of 800 ml at 14 °C, 12 h light/12 h dark cycle, ~150-200 µmol $m^{-2}s^{-1}$ irradiance, distance 30 cm to RGBW led light-source, perform air-bubbles (1 L air per minute). After ~25 days spores or gametes will grow in small 'spider' thallus on dead tissue or 'spider' thallus will

grow free floating in the media. Monitor re-growth of thallus and the growth of 'spider' thallus every 3-4 days with camera by making photos of the thallus above mm-paper. After 46 days of post-recovery, weight the tissue (after gently blotting in tissue paper to remove excess moisture).

L**ong-term re-growth**

For an extra 21 days take a small amount of thallus of day 46 and weight the tissue (after gently blotting in tissue paper to remove excess moisture). Replacing media every 3-4 days with 200 ml FSW plus 0.2 ml CellHIF2P (from stock solution 100g/L) in beaker of 800 ml at 14 °C, 12 h light/12 h dark cycle, ~150-200 µmol $m^{-2}s^{-1}$ irradiance, distance 30 cm to RGBW led light-source, perform air-bubbles (1 litre air per minute). Monitor re-growth of thallus and the growth of 'spider' thallus every 3-4 days with camera. After the extra 21 days of long-term re-growth, weight the tissue (after gently blotting in tissue paper to remove excess moisture).

Use the programme ImageJ to measure the area of *Ulva*-thallus (photos) for monitoring the growth in time.

The protocol is based on seven experiments performed in 2018 till 2022. See § 2.3 for the experimental data and set up.

2.3 Experiments cryopreservation *Ulva* spp.

Seven experiments were done with thallus of *Ulva* spp. populations collected in the Oosterschelde of the Netherlands (Fig. 2.3.1) and in France, to achieve an optimised cryopreservation protocol and to test this protocol for *Ulva* spp. populations. Briefly, the experiments are described with their outcomes.

Figure 2.3.1 *Locations wild-collected thallus of Ulva spp. in the Oosterschelde of the Netherlands.*

Experiment 1 Cryopreserved three *Ulva* spp. populations for 15 days, 2020-01-20.

Results of regrowth of cryopreserved Ulva spp. populations (B1-UsZEE191014, C1-UsHEE_STRAND191014, D1-UsWIM190429(Coll.SAM): The Ulva-populations survived the cryopreservation in liquid nitrogen (-196 °C) after thawing for at least 14 days. Two populations (B1, D1) did grow vegetative and sporulated with grown into spider-shaped thallus for 23 and 34 days (see fig. 2.3.1, 2.3.2, 2.3.3, 2.3.4 and tables 2.3.1, 2.3.2 and 2.3.4). One population (C1) only sporulated without growth and showed no vegetative growth.

Figure 2.3.2 *Growth of spores (100 x magnification, photo made 28 days after recovery (thawing 2020-02-04, photo 2020-03-03). Medium with spores kept in climate camber 2 at 20°C (17 h l, 7h d), population D1, see Table 2.3.4 (exp. 1).*

Table 2.3.1 *Relative growing rates of Ulva growth experiment 1 population B1-UsZEE191014.*

RGR B1	y=cx²+bx+a		2nd order polynomiaal	
Exp 1.	RGR=b+2cx			
day	**ln**	**b**	**c**	**RGR**
20	-0.955	1.564	-0.029	0.404
23	-0.051	1.564	-0.029	0.230
30	0.00	1.564	-0.029	-0.176

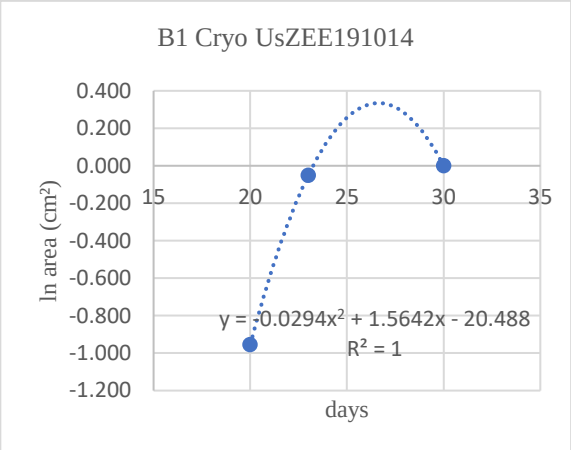

Figure 2.3.3 *2nd order polynomiaal of Ulva growth experiment 1 population B1-UsZEE191014.*

Table 2.3.2 *Relative growing rates of Ulva growth experiment1 population D1-UsWIM190429(Coll.SAM).*

RGR D1	y=cx²+bx+a		2nd order polynomiaal	
Exp. 1	RGR=b+2cx			
day	**ln**	**b**	**c**	**RGR**
20	1.07	0.9831	-0.0165	0.3231
23	2.00	0.9831	-0.0165	0.2241
28	2.58	0.9831	-0.0165	0.0591
34	2.40	0.9831	-0.0165	-0.1389

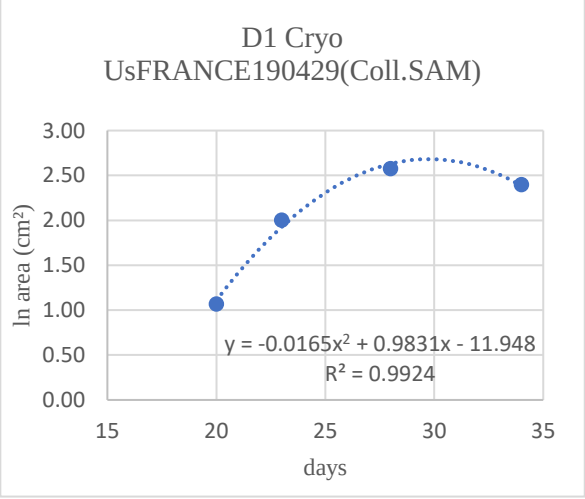

Figure 2.3.4 *2nd order polynomiaal of Ulva growth experiment 1 population D1-UsWIM190429(Coll.SAM).*

Experiment 2 Cryopreserved five *Ulva* spp. populations for 25 days, 2020-08-20.

Results of regrowth of cryopreserved Ulva spp. populations (UsNEE2-200727, UsYER-haven, UsHEE-H-200727, UsHEE-S-200727,UsKAMP-pontje-200727): The Ulva-populations survived the cryopreservation in liquid nitrogen (-196 °C) after thawing for at least 7 days. The thallus was placed on day 7 (2020-09-21) in outside growing tanks in nets in Yerseke (Wageningen Marine Research, Jansen et al. 2022). After three days the thallus was eaten by lobsters.

Experiment 3 Cryopreserved five *Ulva* spp. populations for 94 or 101 days, 2020-06-22 & 2020-06-29.

Results of regrowth of cryopreserved Ulva spp. populations E1- UsNEE-2-200605, G1- UsZUI200207[1], H1- Us FRANCE190429(Coll.SAM)[2], K1- UsYER200605, L1- UsKAM200605: all the populations survived the cryopreservation and sporulated or formed gametes, which grown into thallus (measured for 46 days)(fig. 2.3.5, table 2.3.4). We do not know if the thallus of the populations are gametophytes (N) or sporophytes (2N), because the spores or gametes were not noticed. For three spider-thallus of population K1 a growth experiment was done for three extra weeks. The three small 'spider' thallus grew to larger normal shaped thallus (fig. 2.3.6, table 2.3.4).

Figure 2.3.5 *Regrowth of spores or gametes of population K1 after 46 days of recovery of the cryopreserved population (exp. 3).*

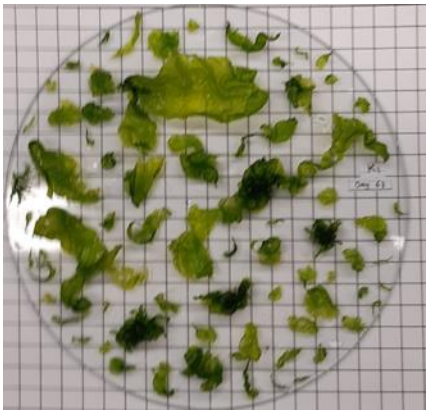

Figure 2.3.6 *Regrowth of three small 'spider' thallus of population K1.Growth into larger normal shaped thallus after 67 days of recovery of the cryopreserved population (exp. 3).*

Experiment 4 Cryopreserved five *Ulva* spp. populations for 154 or 161 days (duplo of exp. 3) 2020-06-22 & 2020-06-29.

Results of regrowth of cryopreserved Ulva spp. populations E2- UsNEE-2-200605, G2- UsZUI200207[1], H2- Us FRANCE190429(Coll.SAM)[2], K2- UsYER200605, L2- UsKAM200605: all the populations survived the cryopreservation and sporulated or formed gametes, which grew into thallus (measured for 46 days)(table 2.3). Population E2 grew vegetative and has also sporulated or gametes were formed, but this was not noticed.

The spores or gametes did grow into thallus, not known if the thallus of population E2 was a sporophyte (2N) or gametophyte (N). Population G2 sporulated on day 14 and meiospores (N) were measured with stigma (red colour, light receptor). The meiospores (N) grew into "spiders". In a follow up experiment the "ultra small spiders" grew larger for an extra 21 days in a larger 'spider' thallus (table 2.3.4). Therefor population G2 is a sporophyte (2N). Population H2 formed gametes (N) on day 10, 11 and 14 after recovery. The gametes grew to become normal thallus. Therefor population H2 is a gametophyte (N). Population K2 formed gametes on day 10, 11 and 14 after recovery. On day 14 gamete with 2 flagella is noticed. The gametes (N) grew to become normal thallus. Therefore population K2 is a gametophyte (N). In a follow up experiment for three weeks one small 'spider' thallus grew to larger normal shaped thallus (fig 2.3.7). Population L2 formed gametes (N) on day 11 and 14 after recovery (fig. 2.3.8). The gametes (N) grew to normal thallus, therefor population L2 is a gametophyte (N).

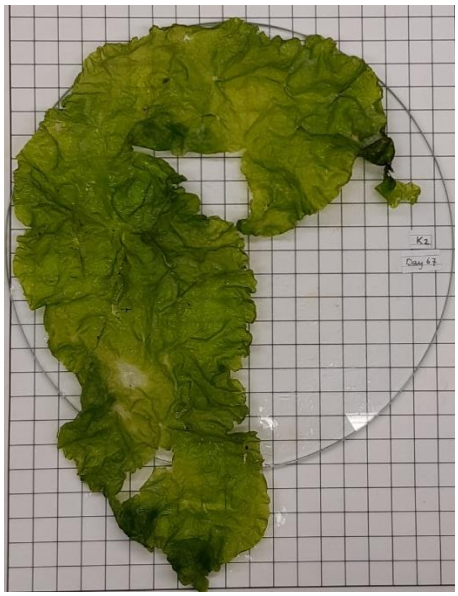

Figure 2.3.7 *Growth of one 'spider' thallus of population of K2 after 67 days of recovery, fresh weight (FW) 7.441 gram, start 0.510 gram FW (day 46) RGR[13] is (ln 7441 – ln 510)/21=(8.91-6.23)/21=0.13 (exp 4).*

[13]*Relative Growth Rate*

$$RGR = \frac{\ln W_t - \ln W_0}{t}$$

[1]Zuidpier, IJmuiden, Netherlands, [2]Escalles, France

Figure 2.3.8 *Growth of gametes (N) of population L2 after 14 days of recovery, 600x Nikon 80i (exp. 4).*

Experiment 5 Cryopreserved six *Ulva* spp. populations for 1.5 up to 14 months (Duplo's or same location of exp. 1, 2, 3 and 4), 2020 & 2021.

Results of regrowth of cryopreserved Ulva spp. populations B2- UsZEE191014, I1- UsHEE_STRAND200605, R1- UsNEE-2-200605, V2- UsYER-haven-200727, Y2- UsKAMP-pontje_200727, FA1- UsZUI200207: All the populations survived the cryopreservation and sporulated or formed gametes, which grew into thallus. In a follow up experiment six semi-big, semi-medium and semi-small 'spider' thallus of population B2 and five thallus-strings from V2 were grown for three extra weeks. One small 'spider' thallus did not grow, but the other five 'spider' thallus of B2 and five thallus-strings of V2 did grow well (see fig. 2.3.9 and table 2.3.4).

Figure 2.3.9 *Growth of thallus-string V2 67 days after recovery (rep 1) with growth of new formed gametes (N) on part of the thallus (exp.5).*

Experiment 6 Cryo Growth experiment with two cryo-*Ulva*-populations from experiment 5 for three weeks, 2020 & 2021 (see table 2.3.3 set up).

Results of growth of cryopreserved *Ulva* spp. populations FA1- UsZUI200207 and V2- UsYER-haven-200727 after recovery grown for 46 days (exp. 5) and stored for 168 days at 6 °C in long-term-storage climate chamber w1 (up to 4 months or longer), with low light conditions (0,97 to 5,8 µmol $m^{-2}s^{-1}$ irradiance), 7 h light/17 h dark cycle, ~40 cm distance to RGBW led light source with spectrum (fig 2.1.1): both populations grew well in climate chamber w2 but did grew less well in the light-chamber lk (see fig. 2.1.1 and 2.3.10, table 2.3.2). A cause in different growth could be the difference in light spectrums between the chambers.

Table 2.3.3 *Set up growth experiment for three weeks of Ulva-thallus of two cryo-stored populations derived from experiment 5, grown for 46 days after recovery and stored for 168 days at 6 °C.*

Pop[1]	Replicate (n=)	conc. 100 g/l Stock CellHIF2P (ml)	Location[2]	Light	Temp (°C)
FA1	4	0.2 ml	w2	12h light 12h dark	15
FA1	4	0.4 ml*	w2	12h light 12h dark	15
V2	4	0.2 ml	w2	12h light 12h dark	15
V2	4	0.4 ml*	w2	12h light 12h dark	15
FA1	4	0.2 ml	lk	12h light 12h dark	15
FA1	4	0.4 ml*	lk	12h light 12h dark	15
V2	4	0.2 ml	lk	12h light 12h dark	15
V2	4	0.4 ml*	lk	12h light 12h dark	15

[1]FA1 = UsZUI200207, V2 = UsYER-haven-200727

[2]w2=climate chamber 2 (fig. 2.1.1), lk=light-chamber (fig. 2.3.10) have different light spectrums.

*The concentration of CellHIF2P (stock solution 100g/L) was 0.4 ml in 200 ml Fresh Seawater (FSW)(400 mg CellHIF2P) instead of the protocol 0.2 ml in 200 ml FSW (200 mg CellHIF2P).

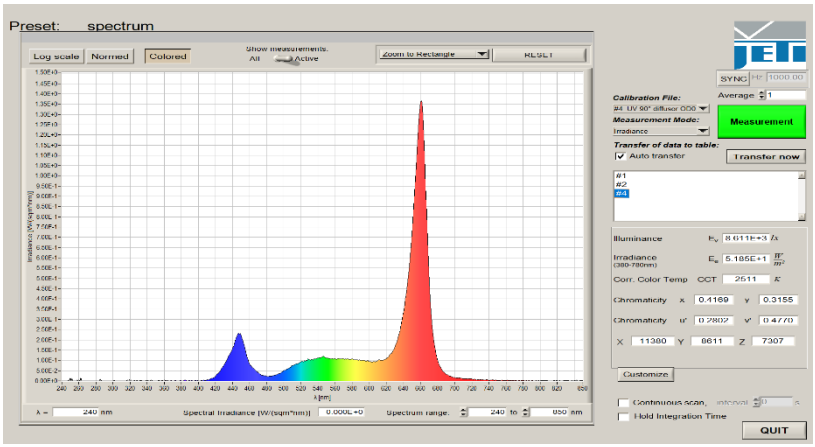

Figure 2.3.10 *Spectrum RGBW led in light-chamber (lk) with RGBW-led-lights.*

Experiment 7 Cryo Growth experiment with three cryo-*Ulva*-populations from experiment Sam Bras (internship 10-2021) for three weeks, start after 46 days of recovery (7 replicates).
Results of growth of cryopreserved *Ulva* spp. populations of AB6(18-24)-Us-AM211011[1], AB6(1-7)-Us-M-Slender211001[2], AB6(10-16)-Us-Zeebrug211014 after 66 days of recovery: all populations grew well (see fig. 2.3.11 and table 2.3.4).

[1]Schelphoek (51°41'49.9"N 3°48'38.5"E), [2]*Ulva mutabilis* Slender type (easy propagation and uniformity (Lovlie, 1964; Nordby & Hoxmark, 1972)

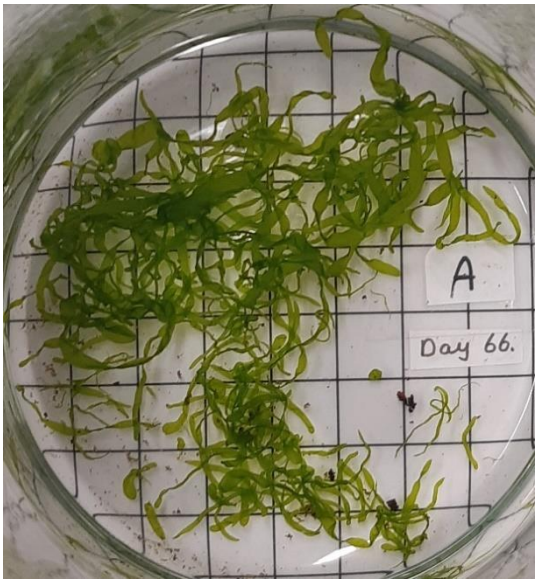

Figure 2.3.11 *Growth of Ulva mutabilis² Slender type rep. 1 for three weeks, start 45 days after recovery (area 0.006 cm², Fresh Weight 0.004 gram). On day 66 the area was 17.440 cm² and 0.581 gram Fresh Weight. Area was measured with the programme ImageJ (exp.7, table 2.3.4).*

Table 2.3.4 *Regrowth of cryopreserved Ulva spp., Area and Fresh Weights (FW) of seven experiments, used protocol §2.1 and §2.2.*

Cryo-exp	Date start growing day0	Ulva-pop (date collected in the wild)	Code population	Date-measurement	Days (growing)after recovery[1]	Area cm2	FW-g	Replicate	Location[2]	Photos made on days after recovery for Area-measurement or following the regrowth after cryopreservation
1	20-1-2020	UsZEE191014	B1	24-2-2020	20	0.385	NA	1	w2	20, 23, 28
1	20-1-2020	UsZEE191014	B1	27-2-2020	23	0.950	NA	1	w2	20, 23, 28
1	20-1-2020	UsZEE191014	B1	5-3-2020	30	0.000	NA	1	w2	20, 23, 28
1	20-1-2020	UsFRANCE190429(Coll.SAM)	D1	24-2-2020	20	2.915	NA	1	w2	20, 23, 28,34
1	20-1-2020	UsFRANCE190429(Coll.SAM)	D1	27-2-2020	23	7.410	NA	1	w2	20, 23, 28,34
1	20-1-2020	UsFRANCE190429(Coll.SAM)	D1	3-3-2020	28	13.160	NA	1	w2	20, 23, 28,34
1	20-1-2020	UsFRANCE190429(Coll.SAM)	D1	9-3-2020	34	11.000	NA	1	w2	20, 23, 28,34
3	1-10-2020	UsNEE-2-200605	E1	16-11-2020	46	48.810	1.879	1	w2	11, 14, 18, 25, 28, 32, 35, 39, 42, 46
3	1-10-2020	UsZUI200207	G1	16-11-2020	46	47.040	2.052	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
3	1-10-2020	Us FRANCE190429(Coll.SAM)	H1	16-11-2020	46	51.230	2.182	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
3	1-10-2020	UsYER200605	K1	16-11-2020	46	18.970	1.518	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
3	1-10-2020	UsYER200605	K1-1	16-11-2020	46	no photo	0.297	1	w2	only 3 spider-thallus start growth-exp-for 3 extra weeks
3	1-10-2020	UsYER200605	K1-1	7-12-2020	67	101.800	4.201	1	w2	49, 53, 56, 60, 63, 67
3	1-10-2020	UsKAM200605	L1	12-10-2020	11	2.514	NA	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
3	1-10-2020	UsKAM200605	L1	5-11-2020	35	99.650	NA	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
3	1-10-2020	UsKAM200605	L1	9-11-2020	39	250.600	NA	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
3	1-10-2020	UsKAM200605	L1	16-11-2020	46	367.600	9.136	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
4	30-11-2020	UsNEE-2-200605	E2	15-1-2021	46	31.220	1.759	1	w2	8,9,11,14,46
4	30-11-2020	UsZUI200207	G2	15-1-2021	46	33.540	1.572	1	w2	8,9,11,14,46,67
4	30-11-2020	UsZUI200207	G2-1	15-1-2021	46	no photo	0.069	1	w2	ultra-small 'spider'thallus of population G2 60 Us ZUI200207 for 3 extra weeks
4	30-11-2020	UsZUI200207	G2-1	5-2-2021	67	44.880	2.635	1	w2	8,9,11,14,46,67
4	30-11-2020	Us FRANCE190429(Coll.SAM)	H2	15-1-2021	46	46.425	2.981	1	w2	8,9,11,14,46
4	30-11-2020	UsYER200605	K2	15-1-2021	46	24.800	1.089	1	w2	8,9,11,14,46
4	30-11-2020	UsYER200605	K2-1	15-1-2021	46	no photo	0.51	1	w2	one small 'spider'thallus of population K2 64 UsYER200605 for 3 extra weeks
4	30-11-2020	UsYER200605	K2-1	5-2-2021	67	216.100	7.441	1	w2	8,9,11,14,46,67
4	30-11-2020	UsKAM200605	L2	15-1-2021	46	44.390	6.001	1	w2	8,9,11,14,46
5	1-4-2021	UsZEE191014	B2	17-5-2021	46	53.920	3.043	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
5	1-4-2021	UsZEE191014	B2-1	17-5-2021	46	no photo	2.9	1	w2	original of many 'spider'-thallus for 3 extra weeks
5	1-4-2021	UsZEE191014	B2-1	7-6-2021	67	103.600	7.55	1	w2	46,50,54,57,60,63,67
5	1-4-2021	UsZEE191014	B2-2A	17-5-2021	46	13.725	0.056	1	w2	one big 'spider'-thallus for 3 extra weeks
5	1-4-2021	UsZEE191014	B2-2A	7-6-2021	67	63.820	0.38	1	w2	46,50,54,57,60,63,67
5	1-4-2021	UsZEE191014	B2-2B	17-5-2021	46	1.228	0.056	2	w2	one big 'spider'-thallus for 3 extra weeks
5	1-4-2021	UsZEE191014	B2-2B	7-6-2021	67	10.780	0.59	2	w2	46,50,54,57,60,63,67
5	1-4-2021	UsZEE191014	B2-3A	17-5-2021	46	0.324	0.011	1	w2	one medium 'spider'-thallus for 3 extra weeks
5	1-4-2021	UsZEE191014	B2-3A	7-6-2021	67	10.360	0.42	1	w2	46,50,54,57,60,63,67
5	1-4-2021	UsZEE191014	B2-3B	17-5-2021	46	0.337	0.011	2	w2	one medium 'spider'-thallus for 3 extra weeks
5	1-4-2021	UsZEE191014	B2-3B	7-6-2021	67	4.361	0.33	2	w2	46,50,54,57,60,63,67
5	1-4-2021	UsZEE191014	B2-4A	17-5-2021	46	0.062	0.002	1	w2	one small 'spider'-thallus for 3 extra weeks
5	1-4-2021	UsZEE191014	B2-4A	7-6-2021	67	7.457	0.41	1	w2	46,50,54,57,60,63,67
5	1-4-2021	UsZEE191014	B2-4B	17-5-2021	46	0.032	0.002	2	w2	one small 'spider'-thallus for 3 extra weeks
5	1-4-2021	UsZEE191014	B2-4B	7-6-2021	67	0.000	0	2	w2	46,50,54,57,60,63,67
5	1-4-2021	UsHEE_STRAND200605	I1	17-5-2021	46	25.060	3.396	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
5	1-4-2021	UsNEE-2-200605	R1	17-5-2021	46	41.570	3.676	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
5	1-4-2021	UsYER-haven-200727	V2	17-5-2021	46	38.790	4.543	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
5	1-4-2021	UsYER-haven-200727	V2-1	17-5-2021	46	1.596	0.037	1	w2	outgrow of gametes for 3 extra weeks
5	1-4-2021	UsYER-haven-200727	V2-1	7-6-2021	67	115.900	6.05	1	w2	46,50,54,57,60,63,67
5	1-4-2021	UsYER-haven-200727	V2-2	17-5-2021	46	1.696	0.048	2	w2	outgrow of gametes for 3 extra weeks
5	1-4-2021	UsYER-haven-200727	V2-2	7-6-2021	67	54.870	2.64	2	w2	46,50,54,57,60,63,67
5	1-4-2021	UsYER-haven-200727	V2-3	17-5-2021	46	1.995	0.087	3	w2	outgrow of gametes for 3 extra weeks
5	1-4-2021	UsYER-haven-200727	V2-3	7-6-2021	67	76.030	5.04	3	w2	46,50,54,57,60,63,67
5	1-4-2021	UsYER-haven-200727	V2-4	17-5-2021	46	2.494	0.072	4	w2	outgrow of gametes for 3 extra weeks
5	1-4-2021	UsYER-haven-200727	V2-4	7-6-2021	67	33.590	2.77	4	w2	46,50,54,57,60,63,67
5	1-4-2021	UsYER-haven-200727	V2-5	17-5-2021	46	2.195	0.025	5	w2	outgrow of gametes for 3 extra weeks
5	1-4-2021	UsYER-haven-200727	V2-5	7-6-2021	67	23.470	1.05	5	w2	46,50,54,57,60,63,67
5	1-4-2021	UsKAMP-pontje_200727	Y2	17-5-2021	46	40.900	8.905	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
5	1-4-2021	UsZUI200207	FA1	17-5-2021	46	27.960	5.193	1	w2	11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46
6	1-11-2021	UsYER-haven-200727	V2-1	1-11-2021	0	3.635	0.532	1	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-1	22-11-2021	21	16.488	0.519	1	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-2	1-11-2021	0	3.046	0.351	2	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-2	22-11-2021	21	10.886	0.600	2	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-3	1-11-2021	0	1.948	0.222	3	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-3	22-11-2021	21	5.582	0.483	3	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-4	1-11-2021	0	0.840	0.063	4	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-4	22-11-2021	21	3.534	0.204	4	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-1*	1-11-2021	0	1.736	0.286	1	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-1*	22-11-2021	21	6.162	0.270	1	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-2*	1-11-2021	0	2.893	0.586	2	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-2*	22-11-2021	21	9.933	0.544	2	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-3*	1-11-2021	0	1.036	0.102	3	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-3*	22-11-2021	21	2.641	0.290	3	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-4*	1-11-2021	0	1.558	0.148	4	lk	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-4*	22-11-2021	21	4.666	0.215	4	lk	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-1	1-11-2021	0	2.426	0.755	1	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-1	22-11-2021	21	30.803	1.881	1	w2	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-2	1-11-2021	0	2.046	0.141	2	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-2	22-11-2021	21	30.443	1.979	2	w2	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-3	1-11-2021	0	0.899	0.107	3	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-3	22-11-2021	21	24.512	1.604	3	w2	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-4	1-11-2021	0	1.586	0.114	4	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-4	22-11-2021	21	27.360	1.057	4	w2	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-1*	1-11-2021	0	2.575	0.281	1	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-1*	22-11-2021	21	46.555	2.653	1	w2	0,3,7,10,14,17,21

6	1-11-2021	UsYER-haven-200727	V2-2*	1-11-2021	0	2.170	0.325	2	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-2*	22-11-2021	21	22.939	1.175	2	w2	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-3*	1-11-2021	0	1.444	0.189	3	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-3*	22-11-2021	21	20.482	1.389	3	w2	0,3,7,10,14,17,21
6	1-11-2021	UsYER-haven-200727	V2-4*	1-11-2021	0	0.713	0.070	4	w2	outgrow of gamete for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsYER-haven-200727	V2-4*	22-11-2021	21	12.742	0.449	4	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-1	1-11-2021	0	2.967	0.151	1	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-1	22-11-2021	21	3.468	0.262	1	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-2	1-11-2021	0	2.217	0.540	2	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-2	22-11-2021	21	3.279	0.570	2	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-3	1-11-2021	0	1.693	0.126	3	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-3	22-11-2021	21	3.425	0.324	3	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-4	1-11-2021	0	1.971	0.421	4	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-4	22-11-2021	21	4.679	0.539	4	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-1*	1-11-2021	0	3.092	0.150	1	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-1*	22-11-2021	21	4.634	0.218	1	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-2*	1-11-2021	0	3.466	0.724	2	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-2*	22-11-2021	21	7.029	0.641	2	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-3*	1-11-2021	0	2.161	0.203	3	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-3*	22-11-2021	21	4.019	0.262	3	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-4*	1-11-2021	0	1.567	0.223	4	lk	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-4*	22-11-2021	21	6.024	0.607	4	lk	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-1	1-11-2021	0	2.650	0.280	1	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-1	22-11-2021	21	6.978	0.467	1	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-2	1-11-2021	0	2.629	0.452	2	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-2	22-11-2021	21	10.211	0.766	2	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-3	1-11-2021	0	1.948	0.174	3	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-3	22-11-2021	21	9.233	0.465	3	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-4	1-11-2021	0	1.784	0.306	4	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-4	22-11-2021	21	16.241	1.424	4	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-1*	1-11-2021	0	3.177	0.340	1	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-1*	22-11-2021	21	13.304	0.968	1	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-2*	1-11-2021	0	2.618	0.361	2	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-2*	22-11-2021	21	9.063	0.510	2	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-3*	1-11-2021	0	2.097	0.163	3	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-3*	22-11-2021	21	12.889	0.676	3	w2	0,3,7,10,14,17,21
6	1-11-2021	UsZUI200207	FA1-4*	1-11-2021	0	1.409	0.162	4	w2	outgrow of gamete/spores for 3 extra weeks after biobanking 6°C
6	1-11-2021	UsZUI200207	FA1-4*	22-11-2021	21	12.025	1.310	4	w2	0,3,7,10,14,17,21
7	29-11-2021	Us-AM211011	AB6-18	13-1-2022	45	1.274	0.023	1	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-AM211011	AB6-18	3-2-2022	66	10.390	0.692	1	w2	45,52,59,66
7	29-11-2021	Us-AM211011	AB6-19	13-1-2022	45	10.090	0.416	2	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-AM211011	AB6-19	3-2-2022	66	28.380	1.609	2	w2	45,52,59,66
7	29-11-2021	Us-AM211011	AB6-20	13-1-2022	45	4.077	0.170	3	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-AM211011	AB6-20	3-2-2022	66	11.740	0.498	3	w2	45,52,59,66
7	29-11-2021	Us-AM211011	AB6-21	13-1-2022	45	2.495	0.181	4	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-AM211011	AB6-21	3-2-2022	66	7.776	0.363	4	w2	45,52,59,66
7	29-11-2021	Us-AM211011	AB6-22	13-1-2022	45	0.961	0.085	5	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-AM211011	AB6-22	3-2-2022	66	5.721	0.201	5	w2	45,52,59,66
7	29-11-2021	Us-AM211011	AB6-23	13-1-2022	45	3.815	0.112	6	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-AM211011	AB6-23	3-2-2022	66	15.990	0.535	6	w2	45,52,59,66
7	29-11-2021	Us-AM211011	AB6-24	13-1-2022	45	2.621	0.081	7	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-AM211011	AB6-24	3-2-2022	66	6.022	0.126	7	w2	45,52,59,66
7	29-11-2021	Us-M-Slender211001	AB6-1	13-1-2022	45	0.006	0.004	1	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-M-Slender211001	AB6-1	3-2-2022	66	17.440	0.581	1	w2	45,52,59,66
7	29-11-2021	Us-M-Slender211001	AB6-2	13-1-2022	45	2.877	0.092	2	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-M-Slender211001	AB6-2	3-2-2022	66	30.150	1.433	2	w2	45,52,59,66
7	29-11-2021	Us-M-Slender211001	AB6-3	13-1-2022	45	0.046	0.095	3	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-M-Slender211001	AB6-3	3-2-2022	66	18.870	0.710	3	w2	45,52,59,66
7	29-11-2021	Us-M-Slender211001	AB6-4	13-1-2022	45	3.894	0.146	4	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-M-Slender211001	AB6-4	3-2-2022	66	12.960	0.240	4	w2	45,52,59,66
7	29-11-2021	Us-M-Slender211001	AB6-5	13-1-2022	45	2.095	0.054	5	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-M-Slender211001	AB6-5	3-2-2022	66	4.224	0.104	5	w2	45,52,59,66
7	29-11-2021	Us-M-Slender211001	AB6-6	13-1-2022	45	3.204	0.067	6	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-M-Slender211001	AB6-6	3-2-2022	66	31.410	1.249	6	w2	45,52,59,66
7	29-11-2021	Us-M-Slender211001	AB6-7	13-1-2022	45	6.529	0.220	7	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-M-Slender211001	AB6-7	3-2-2022	66	39.430	1.836	7	w2	45,52,59,66
7	29-11-2021	Us-Zeebrug211014	AB6-10	13-1-2022	45	17.950	0.592	1	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-Zeebrug211014	AB6-10	3-2-2022	66	32.810	1.519	1	w2	45,52,59,66
7	29-11-2021	Us-Zeebrug211014	AB6-11	13-1-2022	45	8.944	0.553	2	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-Zeebrug211014	AB6-11	3-2-2022	66	33.090	1.709	2	w2	45,52,59,66
7	29-11-2021	Us-Zeebrug211014	AB6-12	13-1-2022	45	12.630	0.506	3	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-Zeebrug211014	AB6-12	3-2-2022	66	40.800	3.917	3	w2	45,52,59,66
7	29-11-2021	Us-Zeebrug211014	AB6-13	13-1-2022	45	3.643	0.153	4	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-Zeebrug211014	AB6-13	3-2-2022	66	27.810	0.793	4	w2	45,52,59,66
7	29-11-2021	Us-Zeebrug211014	AB6-14	13-1-2022	45	2.842	0.021	5	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-Zeebrug211014	AB6-14	3-2-2022	66	33.450	1.273	5	w2	45,52,59,66
7	29-11-2021	Us-Zeebrug211014	AB6-15	13-1-2022	45	3.302	0.262	6	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-Zeebrug211014	AB6-15	3-2-2022	66	26.080	0.485	6	w2	45,52,59,66
7	29-11-2021	Us-Zeebrug211014	AB6-16	13-1-2022	45	3.131	0.160	7	w2	outgrow of gamete/spores for 3 extra weeks
7	29-11-2021	Us-Zeebrug211014	AB6-16	3-2-2022	66	18.040	0.557	7	w2	45,52,59,66

*The concentration of CellHIF2P (stock solution 100g/L) was 0.4 ml in 200 ml Fresh Seawater (FSW)(40 mg CellHIF2P/200 ml) instead of the protocol 0.2 ml in 200 ml FSW (20 mg CellHIF2P/200 ml).

[1]Except experiment 6 the growth experiment started at day 0 which was after 46 days of recovery and stored for 168 days at 6 °C in long-term-storage climate chamber w1 with low light conditions (0,97 to 5,8 µmol $m^{-2}s^{-1}$ irradiance), 7 h light/17 h dark cycle.

[2]w2=climate chamber 2 (fig. 2.1.1), lk=light-chamber (fig. 2.3.10) have different light spectrums.

Relation of growth parameters area and (fresh)weights of thallus (experiment 1 to 7)

A method to measure the growth could be to measure the area over a time period. Unfortunately, sea lettuce can be triggered by environmental changes to sporulate or to form gametes. By measuring the area with camara images the seaweed will almost not been disturbed and the chance to sporulate or to form gametes has been reduced. The question is, what is the difference in growth if measured the areas instead of measured the fresh weights? What is the relation of areas and fresh weights of the new formed thallus to measure the growth over a time period.

Therefor the data is analysed with linear regression. The analysis of the areas and fresh weights measured in the experiments (see fig 2.3.9) showed that the area parameter is useable for growth experiments (R^2=0.6247) instead of the fresh weights. However, to improve accuracy measuring fresh weights at the start and finishing of the experiments will be a good addition, because sometimes the thallus 'leaves' overlap and the area measurement will be under estimated.

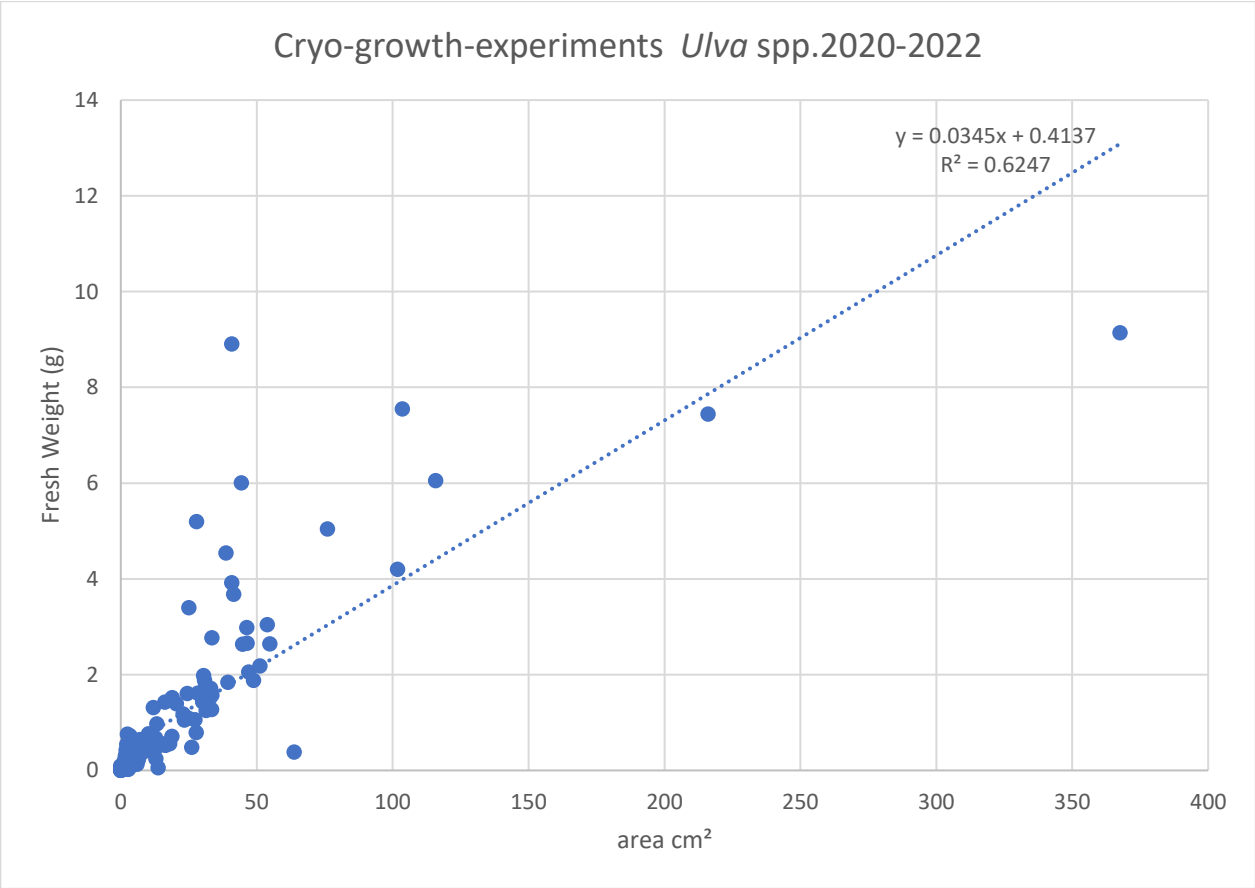

Figure 2.3.9 *Linear Regression analyse of the parameters area and fresh weights of thallus regrowth of Ulva spp. populations after cryopreservation (experiments 1 to 7).*

Discussion & Conclusion

Of the 24 cryopreserved *Ulva*-populations (experiment 2 excluded), 23 populations recovered and grew, after sporulation or gamete formation, in normal shaped thallus.

In conclusion, 96% of the cryopreserved *Ulva*-populations recovered well and regrowth after cryopreservation for a storage time period of at least 15 days up to 14 months.

Our experiments showed that our optimised protocol to cryopreserve sea lettuce (*Ulva* spp.) works well for longer term storage in a robust and workable way and the cryopreserved gametophytes (N) and sporophytes (2N) are able to regrowth (after sporulation (meiospores (N)) or gamete forming (N)) in normal gametophytes (N). Whether the newly formed gametophytes were males or females was not determined.

3 Cryopreservation of *Saccharina latissimia*

3.1 Protocol of cryopreservation of *Saccharina latissima*

The protocol is largely adapted from Visch et al. (2019) and optimised within the IF-project Aquatic Production Systems 2023 Seaweed Cultivation of Wageningen University and Research (see fig. 3.1.1 for the life cycle).

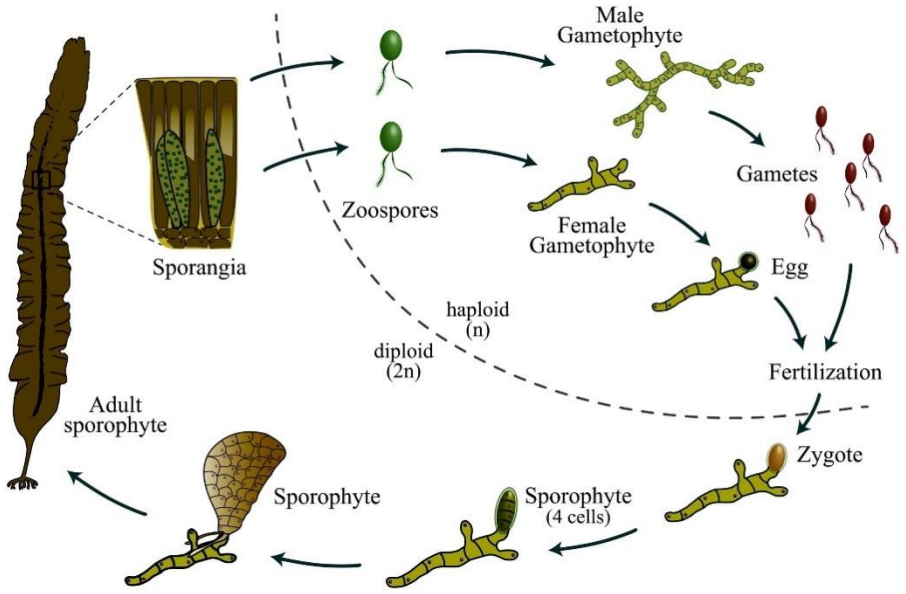

Figure 3.1.1 *Figure adapted from Visch et al. 2019. Cited "Life cycle of kelp (e.g., Saccharina latissima). During meiosis, zoospores (n) are formed in sporangia by a large multicellular sporophyte (2n). The spores settle onto the seafloor and develop into male and female gametophytes (n). Sterile gametophytes can be clonally propagated, and used as seed stock for further breeding and cultivation. Male and female gametophyte form antheridia that produce sperm and oogonia that produce eggs, respectively. The sperm fertilizes the egg, and a zygote is formed that develops into a sporophyte (2n)."*

Our protocol for cryopreservation of male and female gametophytes of *Saccharina latissima* is as follows: The solutions and materials needed are: Gametophytes males, gametophytes females kept at 11 °C in climate chamber with low light conditions (10 µmol $m^{-2}s^{-1}$ irradiance), 14 h light/10 h dark cycle, 4 cm distance to red light source (RGBW LED panel, with red light spectrum showed in fig 3.1.2 F/2 complete[1] and F/2 complete[1] in 0.01M HEPES, pH 8 and F/2 complete[1] made in 0.01M HEPES, pH 8 with DMSO (20%) and D-sorbitol (18%). Cryovials® 2 ml in Simport plus boxes with lid (7.5 cm x 7.5 cm, 25 holes). Freeze Control CL-3300 Cryo Genesis with own program and Nitrogen Liquid barrel.

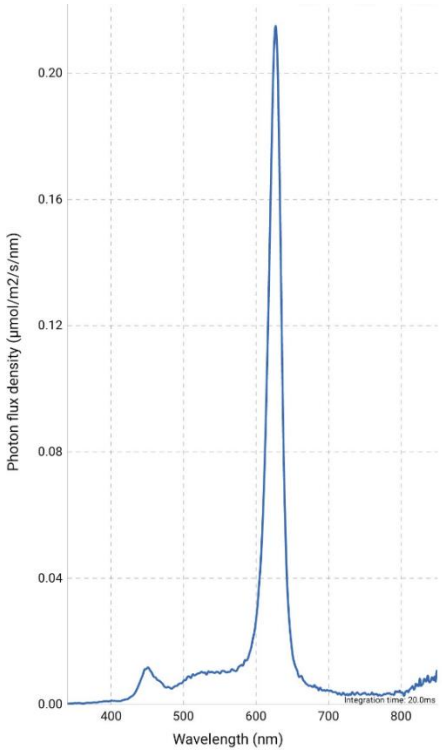

Figure 3.1.2 *Spectrum of red light incubator.*

¹ F/2 Complete is the addition of the three stocks listed below to demineralised water containing 34 gram/l Instant Ocean Sea Salt dissolved after which it is filter sterilised with 0.22 µm filter.
F/2 stock solution 1: Component: NaNO3 75 $g.L^{-1}$, NaH2PO4 5 $g.L^{-1}$. Concentration in final volume: 1mL/L
F/2 stock solution 2: Component: FeCL3 3.15 $g.L^{-1}$, EDTA 4,36 $g.L^{-1}$, CoCL2 10 $mg.L^{-1}$, MnCL2 180 $mg.L^{-1}$, ZnSO4 22 $mg.L^{-1}$, Na2MoO4 6.3$mg.L^{-1}$, CuSO4 9.8 $mg.L^{-1}$. Concentration in final volume: 1mL/L.
F/2 stock solution3. Component: B12 1$mg.L^{-1}$, Biotin 1$mg.L^{-1}$, Thiamine HCL 20 $mg.L^{-1}$. Concentration in final volume: 0.5 mL/L

To prepare the gametophytes for cryopreservation cut the male and female gametophytes in small pieces and keep separate. Pipet 750 µl on ice chilled F/2 complete in 0.01M HEPES in 2 ml cryovial® and add the gametophyte pieces. Add slowly 750 µl of DMSO (20%) plus D-sorbitol (18%) stock solution (75µl each time (in 10 steps) up to 750 µl, shaking gently between additions, typically over 15 minutes) for a final concentration of 10% DMSO and 9% D-sorbitol. Incubate on ice chilled for ~45 minutes. Place the cryovials® in the Freeze Control and start the own program: In 2 hours and 20 minutes, in 6 steps, the programme run as follows: 1. Start at 10°C, 2. Cooling down to -40°C at 0.5°C/min, 3. Bell & Prompt, 4. Hold for 15 min at -40°C, 5. Ramp to -80°C at -1°C/min, 6. End final state -80°C. Place cryovials® in Simport plus boxes with lid in liquid nitrogen and keep in nitrogen container (-196°C) till recovery.

3.2 Protocol of recovery and regrowth of *Saccharina latissimia*

Our protocol for recovery and regrowth of cryopreserved male and female gametophytes of *Saccharina latissimia*: Materials needed for recovery: water bath or thermoshaker, F/2 complete¹ plus F/2 complete¹ in 0.01M HEPES, pH 8, 6 and 24 well plates. Climate chambers 11 °C with low light conditions (10 µmol $m^{-2}s^{-1}$ irradiance), 14 h light/10 h dark cycle and red light, spectrum showed in fig 3.1 and white

light, spectrum showed in fig 3.2, tweezer, needles, plastic beakers (Ø 6.4 cm, height 6.5 cm) with lid (with 6 small holes) and plastic beakers (Ø 9 cm, height 14 cm) with lid (with air filter). Long-term-storage climate chamber (up to 4 months or longer), 6°C with low light conditions (0,97 to 5,8 µmol $m^{-2}s^{-1}$ irradiance), 7 h light/17 h dark cycle, ~40 cm distance to RGBW led light source with spectrum showed in fig 2.1.

Recovery and regrowth of gametophytes

Quickly thaw samples containing pieces of gametophytes at 40 °C (water bath or thermoshaker) for ~1.5 minute until ice is gone. Incubate tissue in 6 well plate (on ice) in 12 ml pre cooled F/2 complete in 0.01M Hepes, pH 8 for 30 minutes. Than place tissue in another 6 well plate (on ice) in 12 ml pre cooled F/2 complete in 0.01M Hepes, pH 8. Place the 6 well plate for one day in the dark in a climate chamber at 8 °C. On day 2 place the 6 well plate in climate chamber at 11 °C in low light conditions (10 µmol $m^{-2}s^{-1}$ irradiance), 14 h light/10 h dark cycle, 4 cm distance to red light source (fig 3.1). On day 7 renew the medium with F/2 complete (without Hepes). On day 10 check the gametophytes for viability and contamination, and place the viable gametophytes in 12 ml new F/2 complete (without Hepes) in 6 well plates at the same conditions. Renew the medium of the gametophytes on day 24 in 12 ml F/2 complete (without Hepes) in 6 well plates and check for viability and contamination. Place at the same conditions. In general renew the medium every month with F/2 complete (without Hepes). On day 36 check for viability and contamination and place at the same conditions. On day 50 check for viability and contamination and renew the medium. Create small fragments of the gametophytes with two needles by pulling them apart, before placing them in the new medium.

Induction of male and female reproductive organs

Put 78 days after recovery the regrowth viable male and female gametophytes from the cryopreservation and male and female gametophytes not cryopreserved in separate 24 well plates in 2 ml F/2 complete (without Hepes) for crossings to develop sporophytes. Place the 24 well plates in a climate chamber with white light (fig. 3.2.1) at 11 °C. Check for antheridia (gametes) and oogonia (eggs) 10 days after induction in white light (day 88).

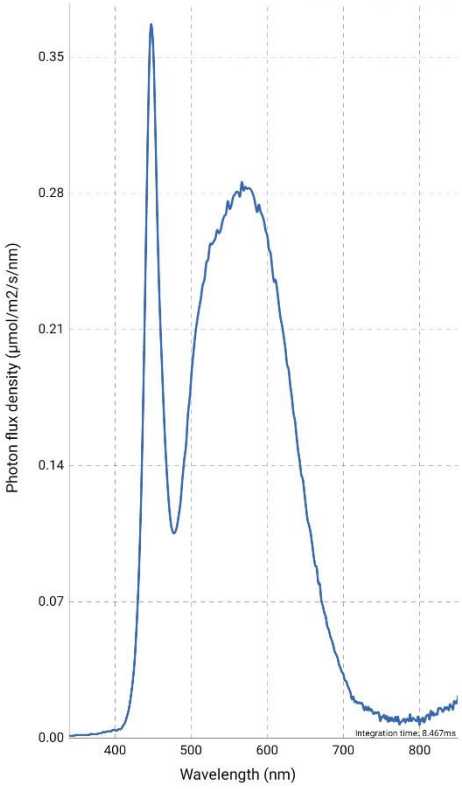

Figure 3.2.1 *Spectrum of white light incubator.*

Crossings of non en cryopreserved gametophytes

Make four different crossings after 10 days of induction into white light as follow: 1) no cryo female x no cryo male, 2) no cryo female x cryo male, 3) cryo female x no cryo male, 4) cryo female x cryo male by combine the male and female gametophytes in the generative phase together with a tweezer or needle in 2 ml F/2 complete in the 24 wells plate of day 78. Do not renew the medium.

Sporophyte development of non en cryopreserved gametophytes

After 11 days check the crossings (day 99) for development on sporophytes. Place the growth sporophytes 14 days after crossing (day 102) in 200 ml plastic beakers with lid (with 6 small holes) in 100 ml F/2 complete (without Hepes) in a climate chamber with white light (fig. 3.1) at 11 °C. Renew the medium 20 days after crossing (day 108) with F/2 complete (without Hepes) eand check for viability and contamination.

Monitoring growth of sporophytes

Check for growth 27 days after crossing (day 115) by making photos of the sporophytes above mm-paper and renew the medium with 100 ml F/2 complete (without Hepes) per beaker. Make 32 days after crossing photos of the same sporophytes (day 120) above mm-paper and add 25 ml F/2 complete (without Hepes) per beaker. Make photos again 38 days after crossing (day 126) and stop the growth-experiment. To store the sporophytes: place them at 6°C in a long-term storage climate chamber with low light conditions (0,97 to 5,8 µmol $m^{-2}s^{-1}$ irradiance), 7 h light/17 h dark cycle, ~40 cm distance to RGBW led light source (fig 2.1.2).

Use the programme ImageJ to measure the area of the sporophytes (photos) for monitoring the growth in time.

The protocol is based on an experiment performed in 2022 and 2023 (see §3.3).

3.3 Experiment *Saccharina latissima*

Experiment 1

On the 12th of December 2022, five gametophytes[1] males (SaDKaar F/2-FE OL red light) and five gametophytes[1] females (SaNoLek, F/2 Ol red light) were cryopreserved.

After 35 days of cryopreservation (protocol §3.1). the gametophytes were thawed (16th of January 2023) The total period for recovery, crossing and regrowth was 126 days (till 22th of May 2023, see protocol §3.2).

Recovery and regrowth of gametophytes

24 days after recovery the female and male gametophytes were still vital, but very small (see fig. 3.3.1 & 3.3.2)

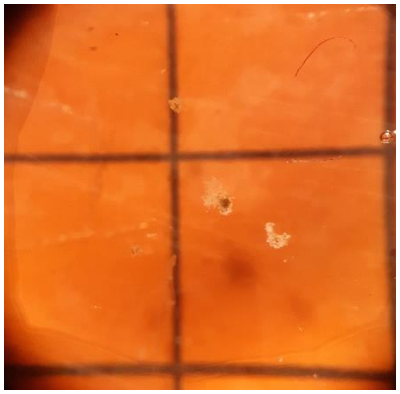

Figure 3.3.1 *Photo of regrowth of cryopreserved female gametophytes, 24 days after recovery (9th of February 2023, box is 1 square centimetre).*

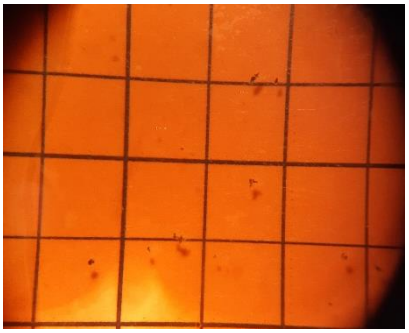

Figure 3.3.2 *Photo of regrowth of cryopreserved male gametophytes, 24 days after recovery (9th of February 2023, box is 1 square centimetre).*

Induction of male and female reproductive organs

The reproduction organs of female gametophytes were seen after ten days of induction in white light (14th of April 2023) (fig. 3.3.3)

[1]The gametophytes were derived from Laboratory of Cell and Developmental Biology at the Wageningen University & Research.

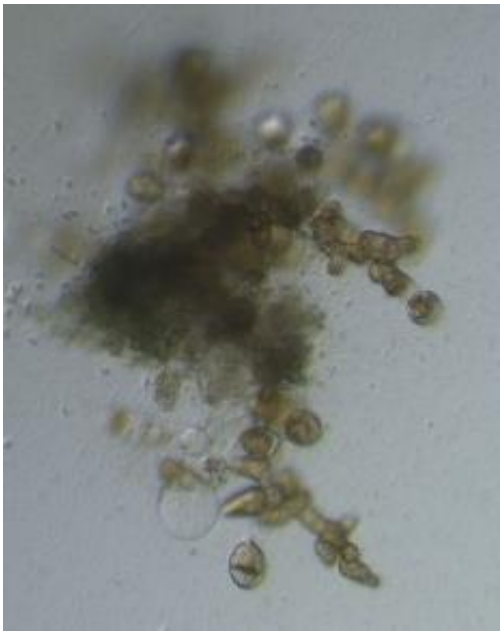

Figure 3.3.3 *Photo of developed vital oocytes of cryopreserved female gametophytes (sample code sl6fc) after ten days of induction in white light (100x).*

Crossing

After 14 days (24 days after induction in white light) the gametophytes crossings **1)** no cryo female x no cryo male, **2)** no cryo female x cryo male, **3)** cryo female x no cryo male and **4)** cryo female x cryo male developed in new sporophytes (28th of April 2023, see §3.2, fig. 3.3.3, 3.3.4, 3.3.5 & 3.3.6).

Figure 3.3.3 *Photo of the newly formed sporophyte after 14 days of crossing **1)** no cryo female x no cryo male gametophyte, 24 days after induction in white light (100x), §3.2 section Crossings of non en cryopreserved gametophytes.*

Figure 3.3.4 *Photo of the new formed sporophyte after 14 days of crossing 2. no cryo female x cryo male gametophyte, 24 days after induction in white light (100x) §3.2 section Crossings of non and cryopreserved gametophytes.*

Figure 3.3.5 *Photo of the new formed sporophyte after 14 days of crossing 3. cryo female x no cryo male gametophyte, 24 days after induction in white light (100x) §3.2 section Crossings of non and cryopreserved gametophytes.*

Figure 3.3.6 *Photo of the new formed sporophyte after 14 days of crossing 4. cryo female x cryo male gametophyte, 24 days after induction in white light (100x) §3.2 section Crossings of non and cryopreserved gametophytes.*

Growth of sporophytes

After 32 days (42 days after induction in white light) the sporophytes of crossings 1) no cryo female x no cryo male, 2) no cryo female x cryo male, and 4) cryo female x cryo male were grown to larger thallus. The sporophytes of crossing 3) cryo female x no cryo male did not grown well. (16th of May 2023, see §3.2, fig. 3.3.7, 3.3.8 & 3.3.9).

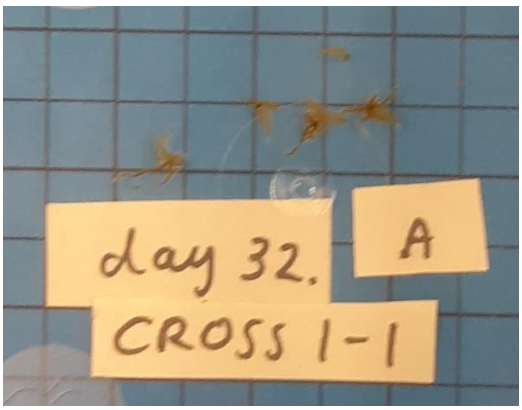

Figure 3.3.7 *Photo of growth of the new formed sporophytes after 32 days of crossing 1. no cryo female x no cryo male gametophyte, 42 days after induction in white light, §3.2 section Crossings of non and cryopreserved gametophytes (box is 1 square centimetre).*

Figure 3.3.8 *Photo of growth of the new formed sporophytes after 32 days of crossing 2. no cryo female x cryo male gametophyte, 42 days after induction in white light, §3.2 section Crossings of non and cryopreserved gametophytes (box is 1 square centimetre).*

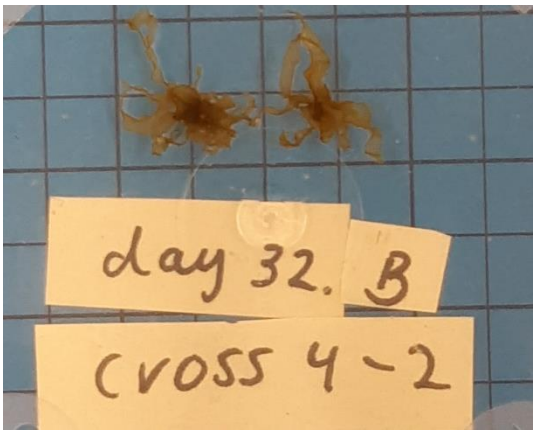

Figure 3.3.9 *Photo of growth of the new formed sporophytes after 32 days of crossing 4. cryo female x cryo male gametophyte, 42 days after induction in white light, §3.2 section Crossings of non and cryopreserved gametophytes (box is 1 square centimetre).*

Discussion & Conclusion

Cryopreservation of female and male gametophytes is possible. The recovered cryopreserved female and male gametophytes were able to develop into new sporophytes in all the four crossings 1) no cryo female x no cryo male, 2) no cryo female x cryo male, and 4) cryo female x cryo male. The longer term regrowth was successful for crossing 1, 2 and 4.

Our experiment showed that our optimised protocol works well to preserve *Saccharina latissima* gametophytes for the longer term in a robust and workable way and the recovered gametophytes are able to form new sporophytes.

4 Conclusion

In this report optimised protocols for cryopreservation of *Ulva* spp. and *Saccharina latissima* have been described and tested. It was shown that even after long-term storage, cryo-preserved biomass could successfully be re-grown for longer periods.

This report is not only relevant for commercial seaweed cultivation companies, but also for institutes worldwide that safeguard genetic diversity of seaweeds.

5 Acknowledgements

We would like to thank Thijs Ketelaar, Norbert de Ruijter, Otto van der Linden and Harald Holm of the Laboratory of Cell Biology of the Wageningen University and Research for helpful suggestions, use of the equipment and seaweed materials and we would like to thank Willem Visser of Business Unit Agro Systems Research of the Wageningen University and Research for technical support.

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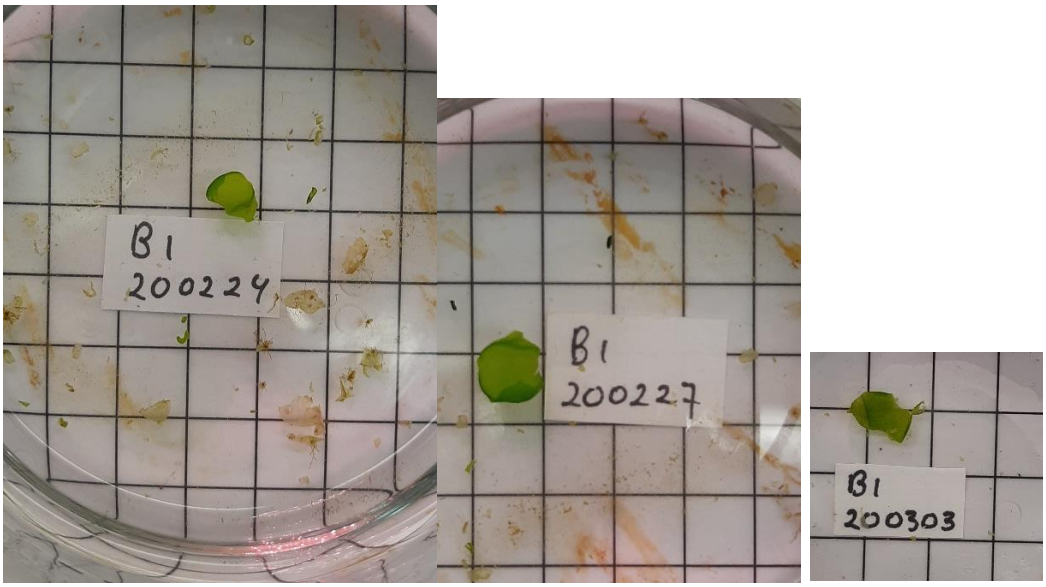
Annex 1 Data *Ulva* experiment 1

Data experiment 1

Table 1.1 4 Ulva spp. population in duplo frost in liquid Nitrogen -196 °C

Code	pop.	Code GeniAlg	from storage
A1	45	UsWim190909	6 °C
A2	45	UsWim190909	6 °C
B1	50	UsZEE191014	6 °C
B2	50	UsZEE191014	6 °C
C1	51	UsHEE_STRAND191014	6 °C
C2	51	UsHEE_STRAND191014	6 °C
D1	31	UsFRANCE190429(Coll.SAM)	6 °C
D2	31	UsFRANCE190429(Coll.SAM)	6 °C



B1 20-02-24 (day 20) ø 0.7 cm circle, area is 0.385 cm²

B1 20-02-27 (day 23) ø approx. 1.1 cm circle, surface area is 0.950 cm²

0.950 cm²- 0.385 cm²= 0.565 cm² increase in 3 days, the RGR is (ln 0.950 – ln 0.385)/3=0.301

$$RGR = \frac{\ln W_t - \ln W_0}{t}$$

B1 20-03-03 (day 28) ø approx. 1.0 x 0.5 cm, surface area is 0.5 cm², growth stagnates.

B1 20-03-05 (day 30) thallus tissue was disintegrated.

Table 1.2 RGR of Ulva population 50 B1 UsZEE191014 after recovery of cryopreservation -196 °C.

RGR B1	$y=cx^2+bx+a$		2nd order polynomial	
	$RGR=b+2cx$			
day	**ln**	**b**	**c**	**RGR**
20	-0.955	1.564	-0.029	0.404
23	-0.051	1.564	-0.029	0.230
30	0.00	1.564	-0.029	-0.176

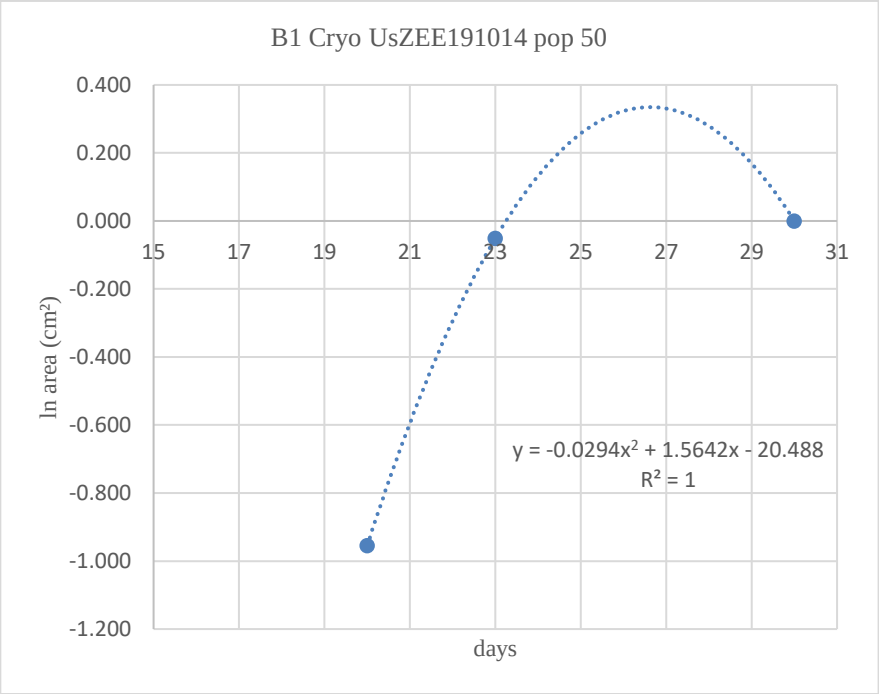
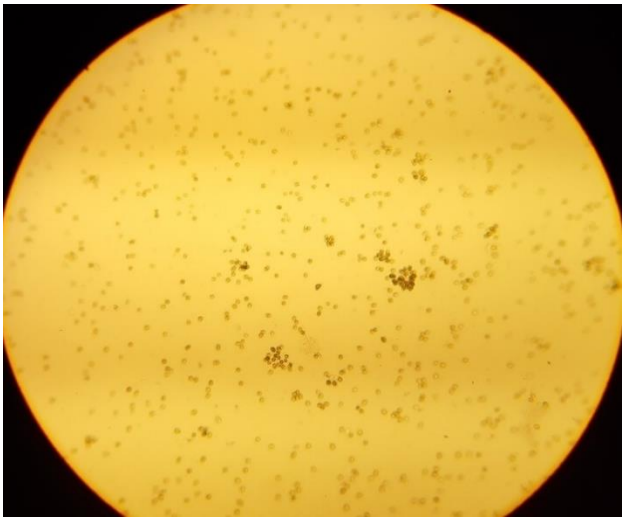
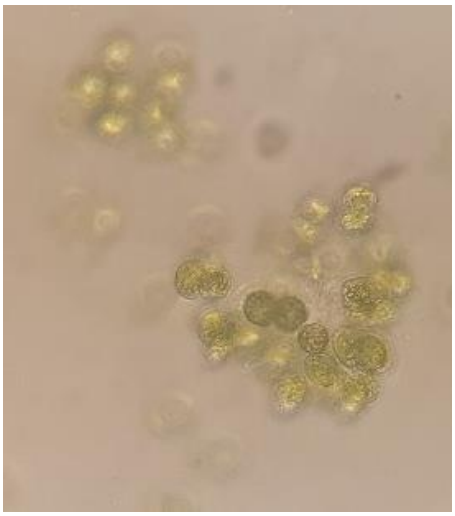


Fig 1.1 RGR of Ulva population 50 B1 UsZEE191014 after recovery of cryopreservation -196 °C.

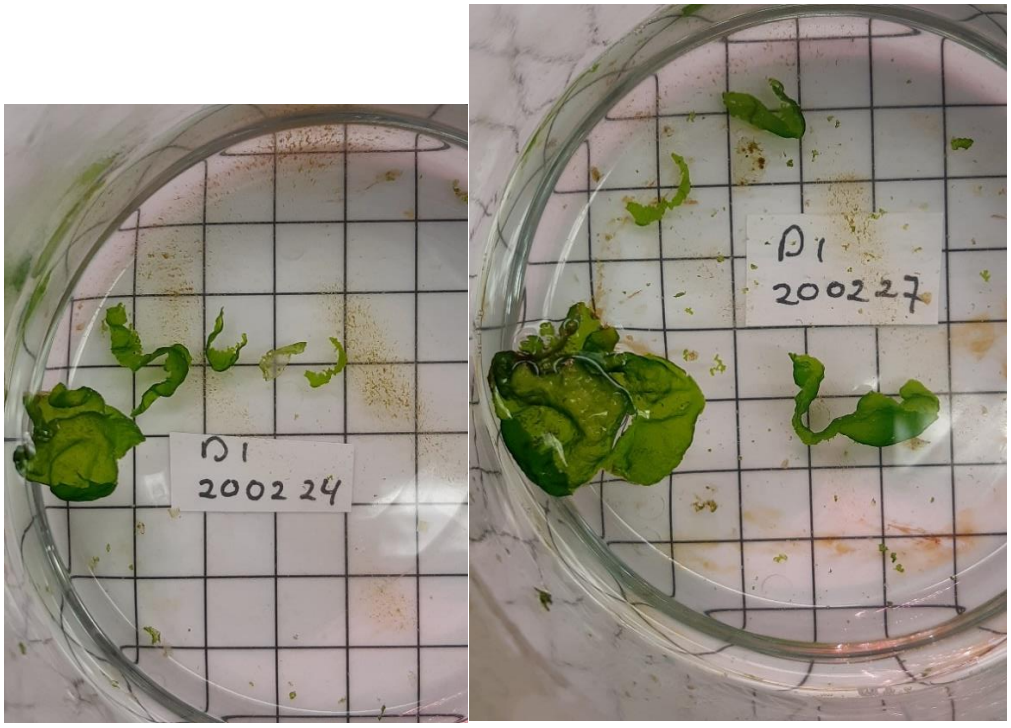
B1 Outgrow of spores, see photo above, renewed the medium 24th of February 2020. Picture made on 3th of March 2020 (day 28), 100 x magnification. Medium with spores kept in climate chamber 2 at 20°C (17 h l, 7h d).





C1 20-02-04 (day 0) to 20-02-14 (day 10), the thallus tissue was sporulated, see photo's above, spores were not growing, but still green. Pictures were made on 9th of March 2020, 100x and 400x magnification. The spores were kept in the medium; 50 ml ASW in 0.01M HEPES ph8+0.05 ml CellHIF2P. First at 6°C (7 h l, 17 h d) for one week, than at 15°C (12h l, 12 h d) for 2 weeks, than from 20-2-27(day 23) at 20°C (17 h l, 7h d) till 9th of March 2020 (day 34).

C1 20-02-24 (day 20) no growth, thallus tissue was sporulated, between day 0 and day 10.



D1 20-02-24 (day 20) ø approx. 1.6 cm circle, area is 2.01 cm² (largest thallus piece)

Approx. 2 x 0.4 cm, area is 0.8 cm² (2nd large thallus piece)

Approx. 0.7 x 0.15 cm, area is 0.105 cm² (3th large thallus piece)

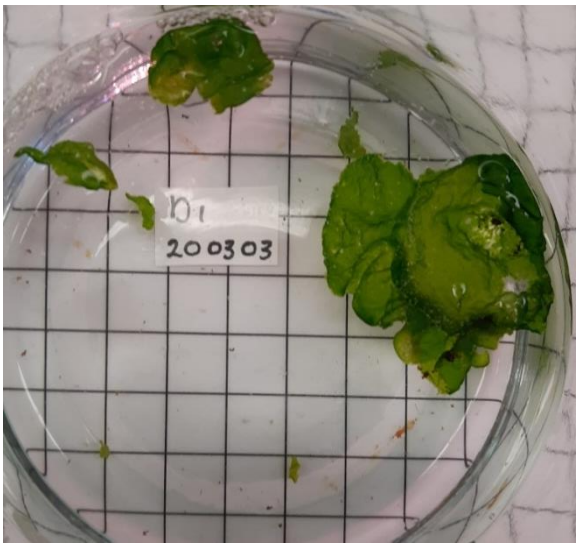
D1 20-02-27 (day 23) ø approx. 2.7 cm circle, area is 5.72 cm² (largest thallus piece)

Approx. (1.5 x 0.5)+(1.5 x 0.3 cm), area is 1.2 cm² (2nd large thallus piece)

Approx. 1.4 x 0.35 cm, area is 0.49 cm² (3th large thallus piece)

7.41 cm²-2.915 cm² = 4.495 cm² increase in 3 days, the RGR is (ln 7.41 – ln 2.915)/3=0.311

$$RGR = \frac{\ln W_t - \ln W_0}{t}$$

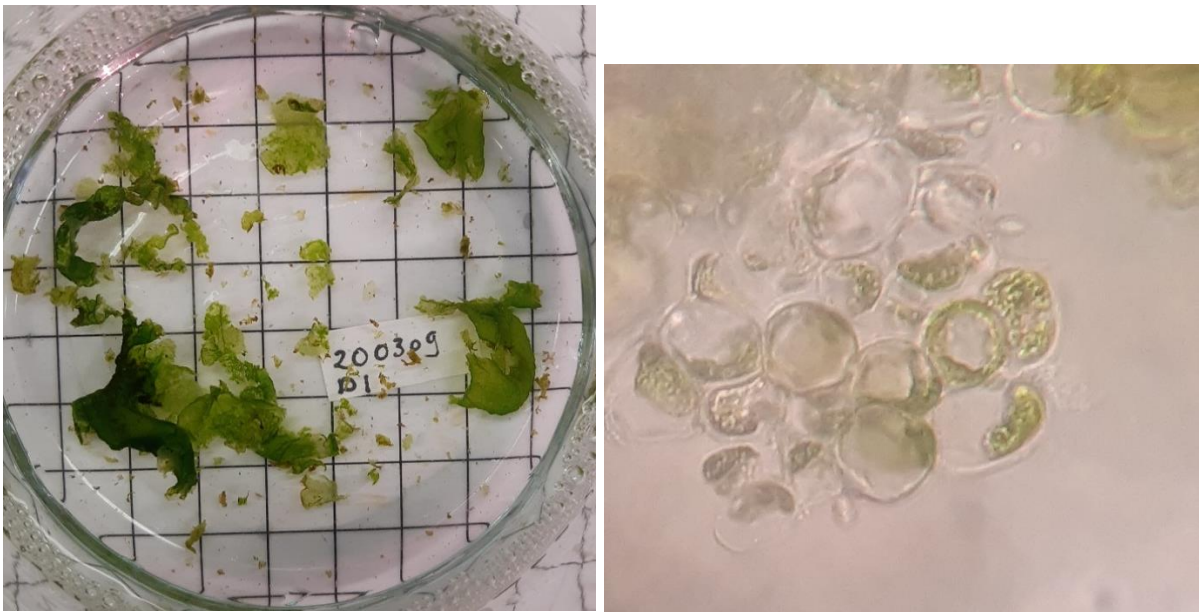


D1 20-03-03 (day 28) ø approx. 3.5 cm circle, area is 9.62 cm² (largest thallus piece)

Approx. 2.1 x 1.4 cm, area is 2.94 cm² (2nd large thallus piece)

Approx. 1.2 x 0.5 cm, area is 0.6 cm² (3th large thallus piece)

13.16 cm² - 7.41 cm² = 5,75 cm² increase in 5 days, the RGR is (ln 13.16 – ln 7.41)/4= 0.115



D1 20-03-09 (day 34) Thallus disintegrated and sporulated (400 x), approx. 11 cm².

Table 1.3 RGR of Ulva population 31 UsFRANCE190429(Coll.SAM) after recovery of cryopreservation -196 °C.

RGR D1	$y=cx^2+bx+a$ RGR=b+2cx		2nd order polynomiaal	
day	**ln**	**b**	**c**	**RGR**
20	1.07	0.9831	-0.0165	0.3231
23	2.00	0.9831	-0.0165	0.2241
28	2.58	0.9831	-0.0165	0.0591
34	2.40	0.9831	-0.0165	-0.1389

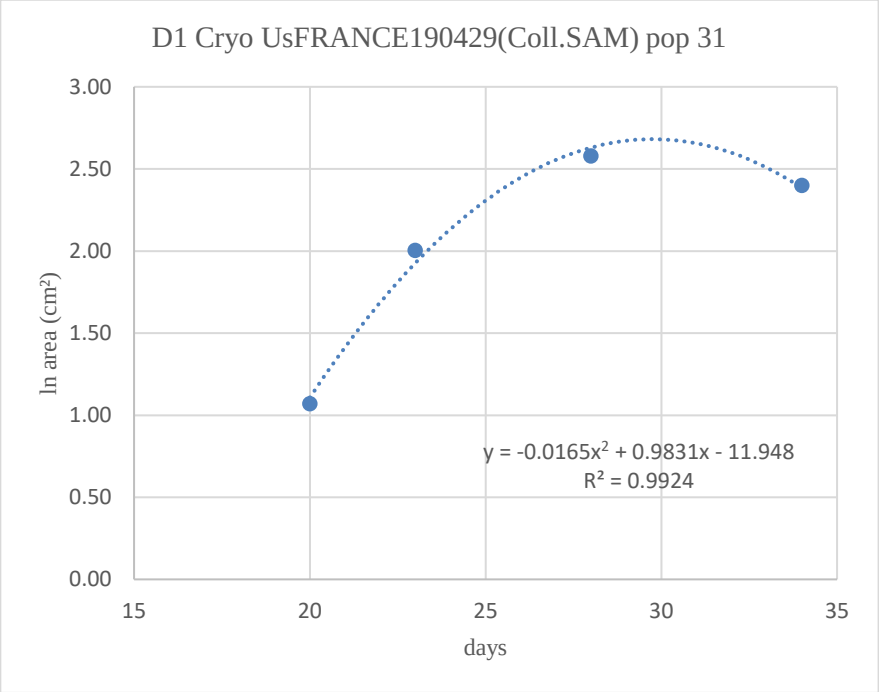


Fig 1.2 RGR of Ulva population 31 UsFRANCE190429(Coll.SAM) after recovery of cryopreservation -196 °C.

D1 Outgrow of spores, see photo's above, renewed medium on 21th of February 2020, 100 x, 25 x magnification. Pictures made on 3th of March 2020 (day 28). Medium with spores kept in climate camber 2 at 20°C (17 h l, 7h d).

Annex 2 Data *Ulva* experiment 3

Data experiment 3

Table 3.1 5 Ulva spp. population in duplo frost in liquid Nitrogen -196 °C

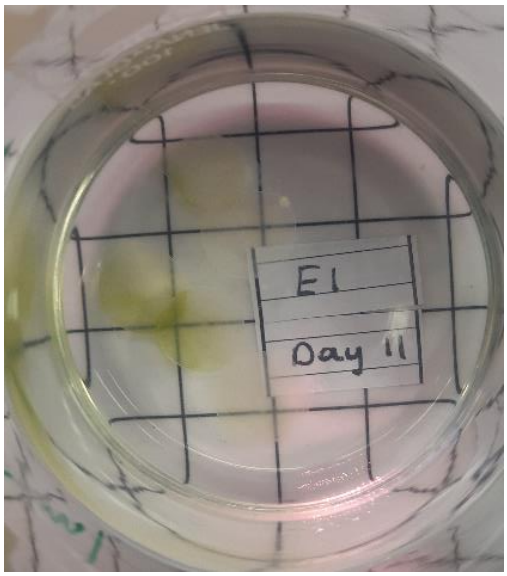
Code	pop.	Code GeniAlg	from storage
E1	62	UsNEE-2-200605	6 °C
E2	62	Us NEE-2-200605	6 °C
G1	60	UsZUI200207	6 °C
G2	60	Us ZUI200207	6 °C
H1	31	Us FRANCE190429(Coll.SAM)	6 °C
H2	31	Us FRANCE190429(Coll.SAM)	6 °C
K1	64	UsYER200605	6 °C
K2	64	Us YER200605	6 °C
L1	63	UsKAM200605	6 °C
L2	63	Us KAM200605	6 °C

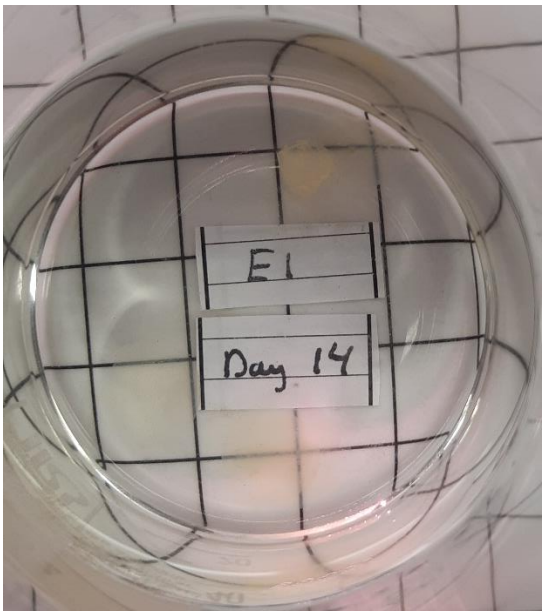
Table 3.2 5 Ulva spp. population 5 discs per vial, in duplo frost in liquid Nitrogen -196 °C, from one or more thallus blades.

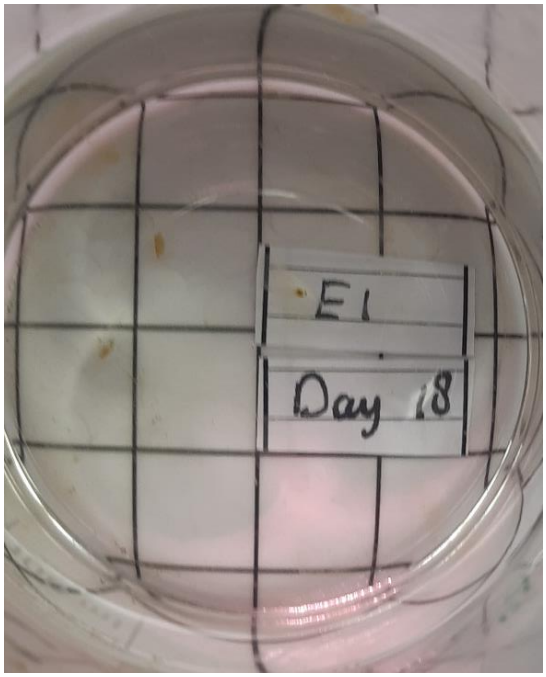
Box	Code	pop.	from storage	Date in -196 °C N	Thallus	from one thallesblade	Cryopres_FreezeControl
AB2	E1	62	6 °C	200622	5 cut discs per vial, Ø 1 cm no. 6	yes	PROTO2
AB2	G1	60	6 °C	200622	5 cut discs per vial, Ø 1 cm no. 6	NA	PROTO2
AB2	H1	31	6 °C	200622	5 cut discs per vial, Ø 1 cm no. 6	no	PROTO2
AB3	K1	64	6 °C	200629	5 cut discs per vial, Ø 1 cm no. 6	yes	PROTO2
AB3	L1	63	6 °C	200629	5 cut discs per vial, Ø 1 cm no. 6	yes	PROTO2

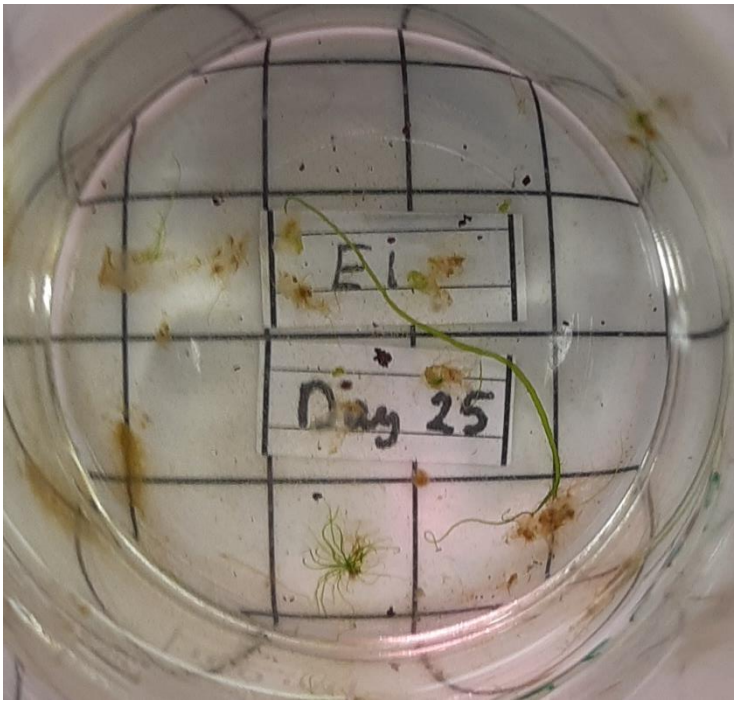
Table 3.3 Fresh Weight (FW) and Dry Weight (DW, 60 °C 24 h) of 5 Ulva spp cryopreserved, after 46 days of regrowth.

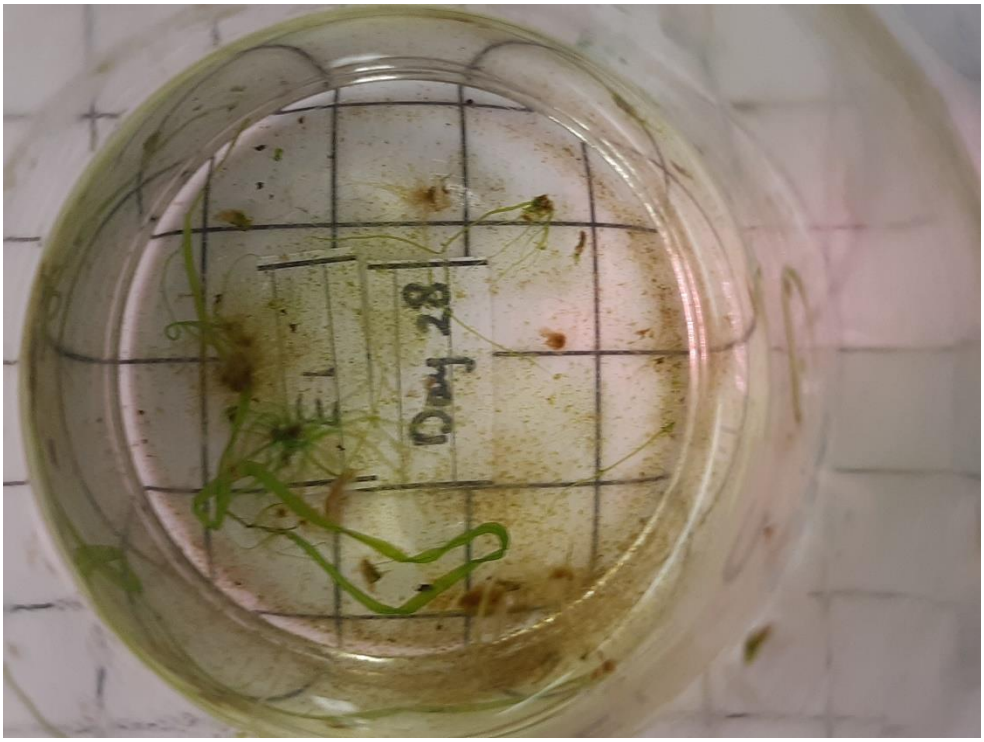
Code	pop.	FW (g) day46	DW (g) day46
E1	62	1.879	0.25
G1	60	2.052	0.22
H1	31	2.182	0.29
K1	64	1.518	0.22
L1	63	9.136	1.12

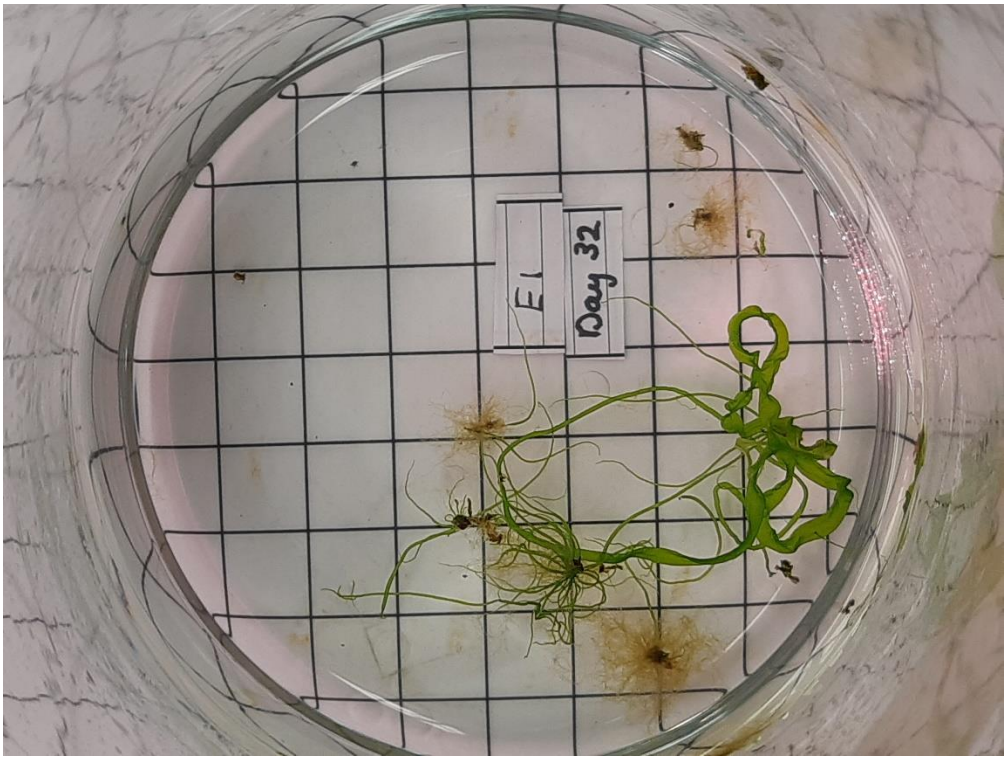


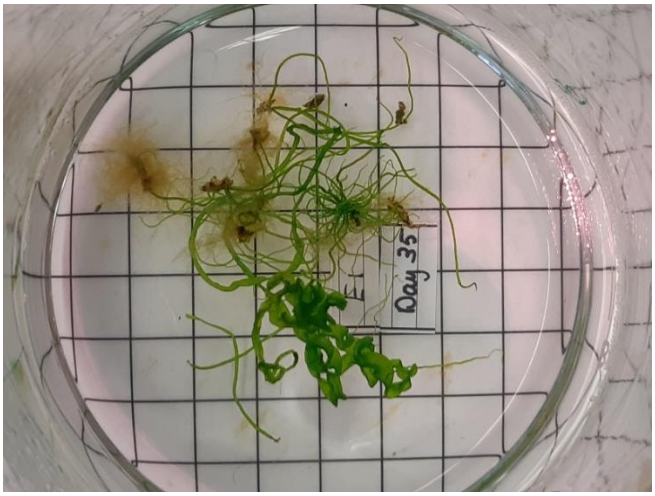


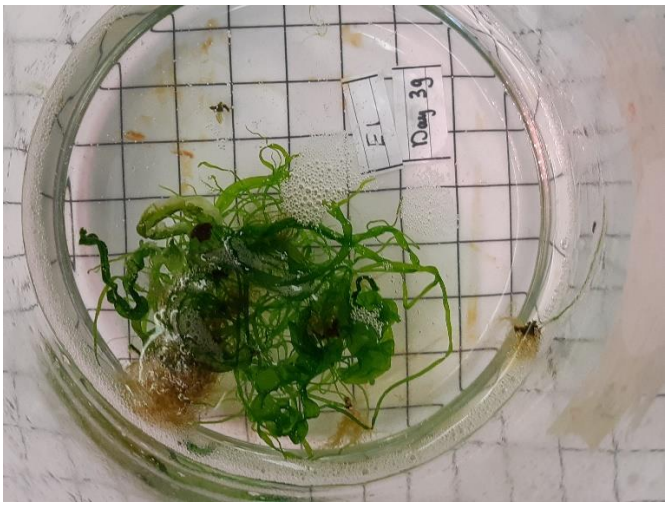

E1
Day 18

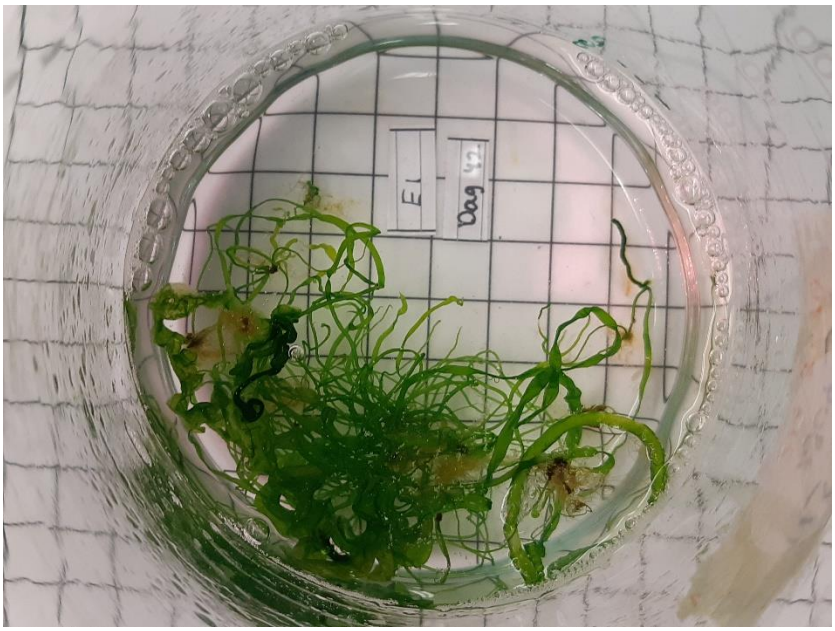

E1
Day 25

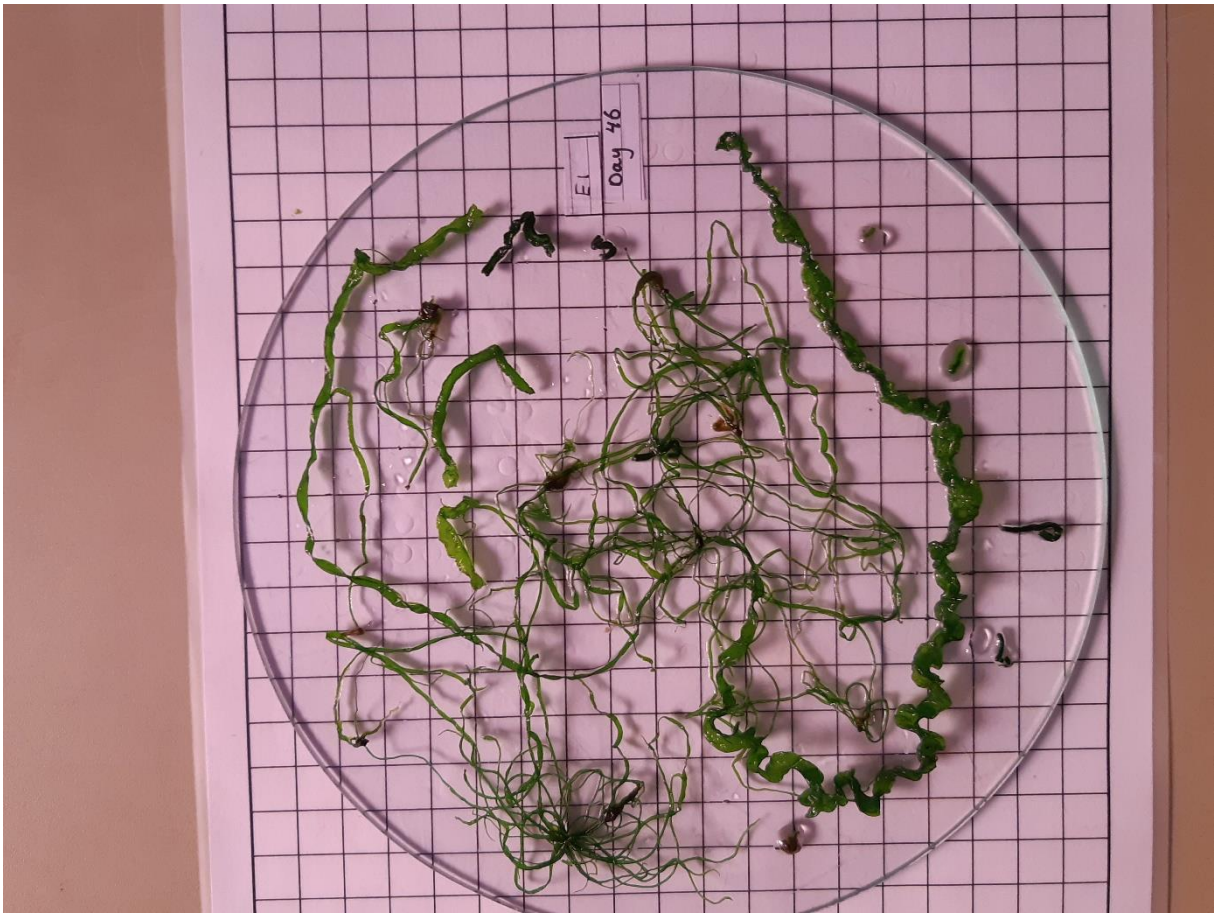

Day 28


E1
Day 32


E1
Day 35


E1
Day 39


E1
Day 42



E1 20-10-12 (day 11).

E1 20-10-15 (day 14).

E1 20-10-19 (day 18). Not renewed medium.

E1 20-10-22 (day 21). Not renewed medium, no photo.

E1 20-10-26 (day 25).

E1 20-10-29 (day 28).

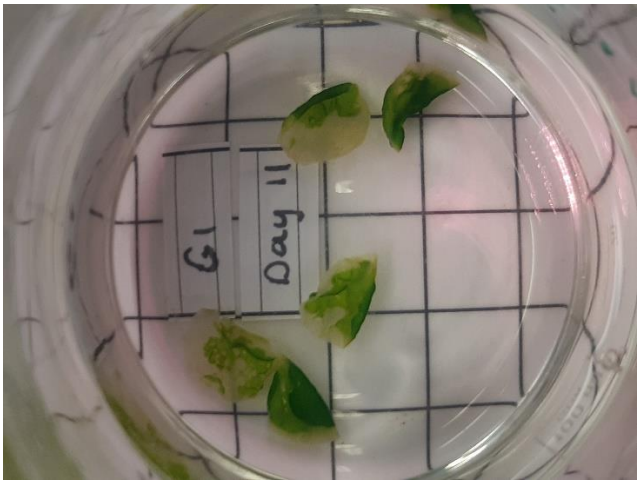
E1 20-11-02 (day 32).

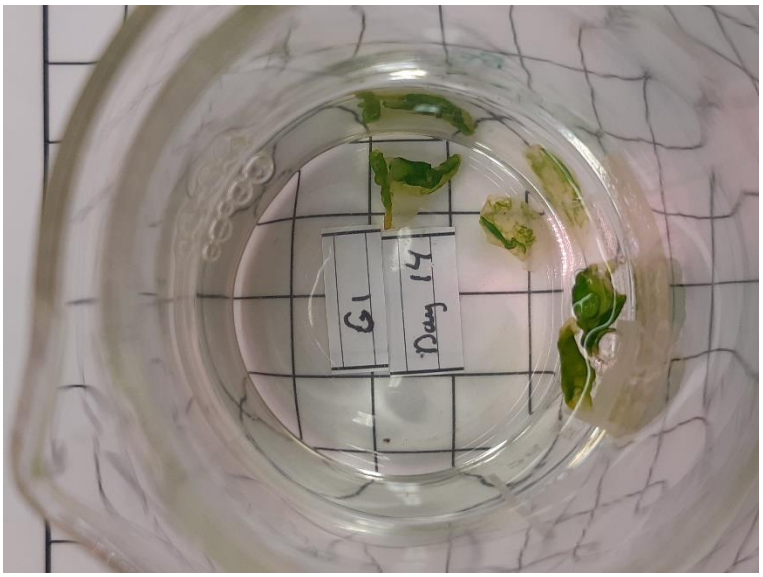
E1 20-11-05 (day 35).

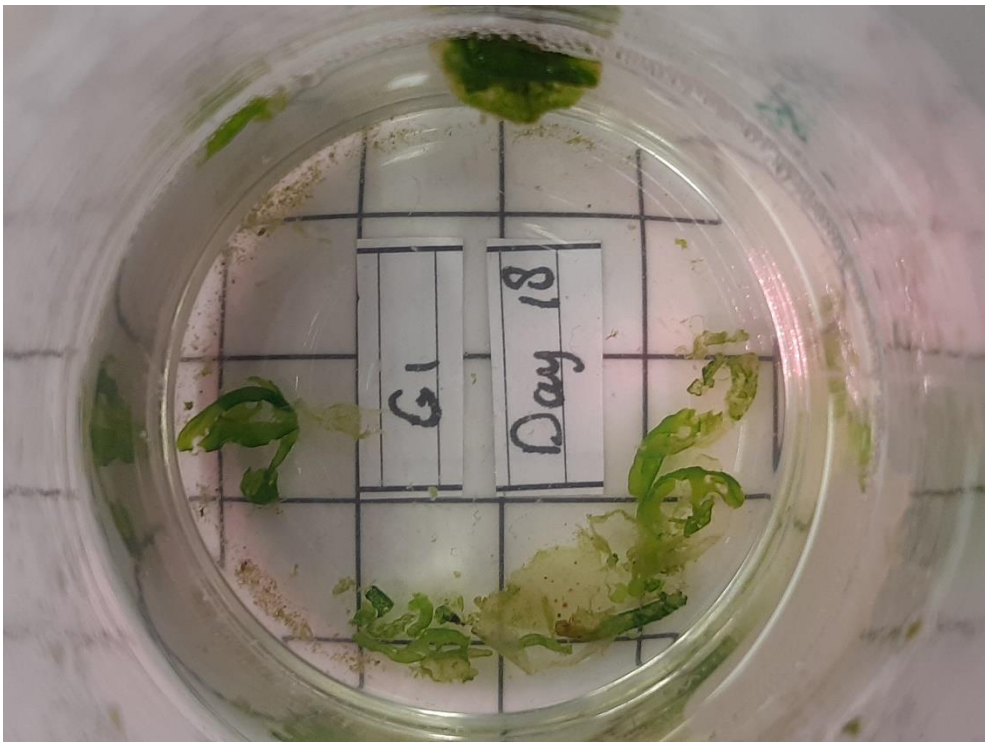
E1 20-11-09 (day 39).

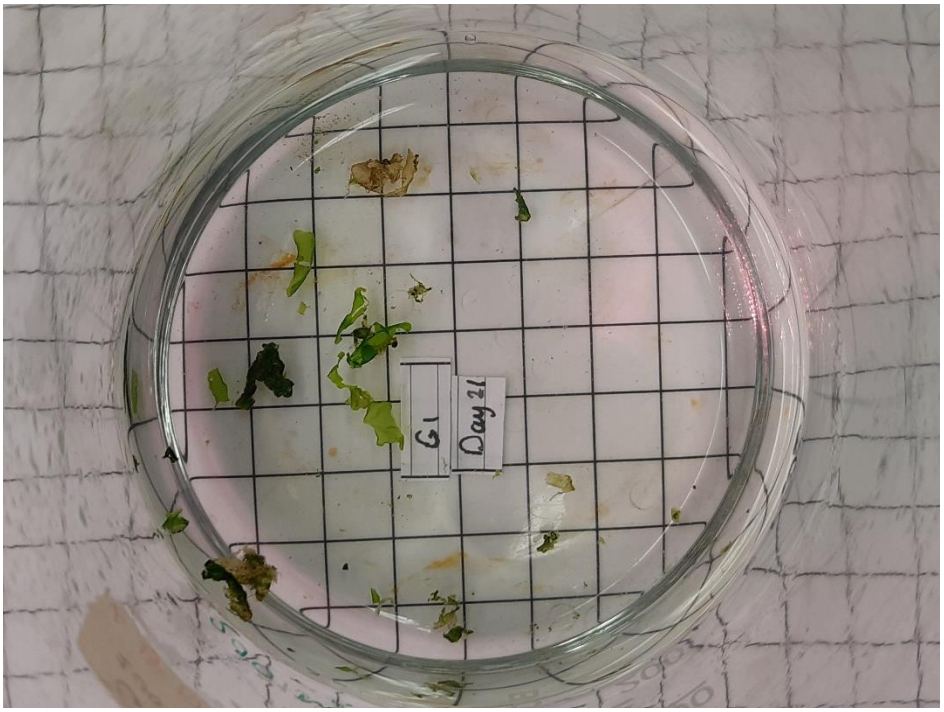
E1 20-11-12 (day 42).

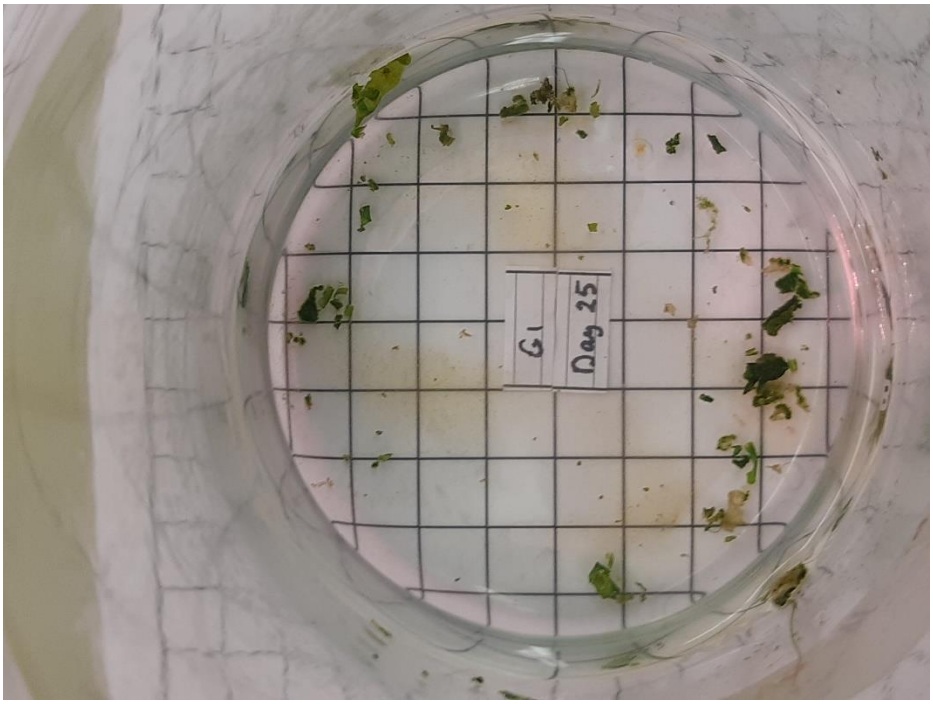
E1 20-11-16 (day 46).

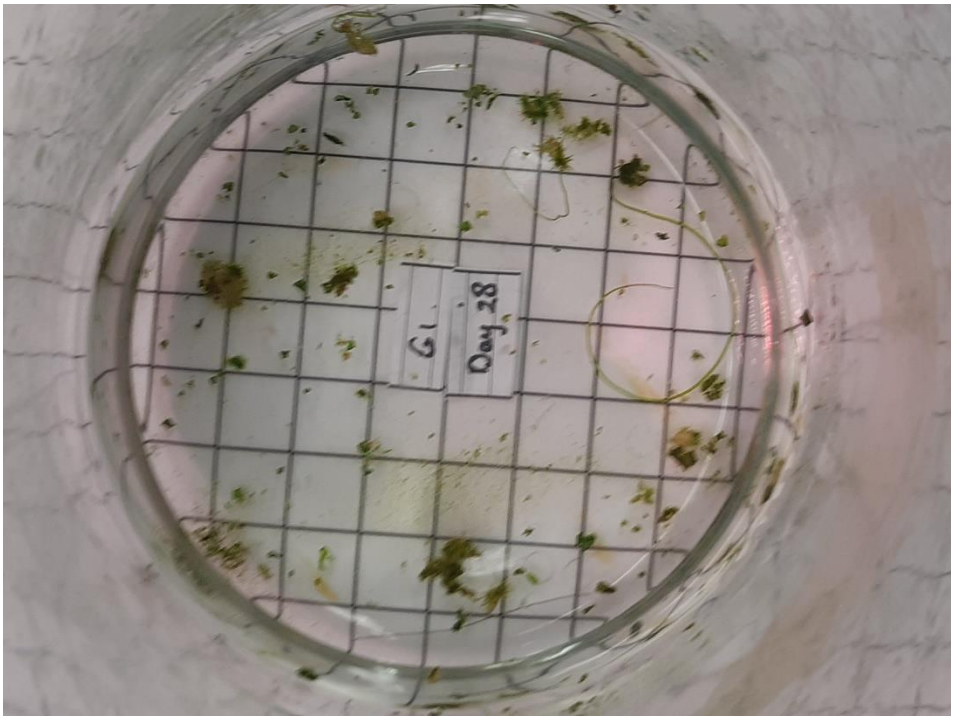

G1
Day 11

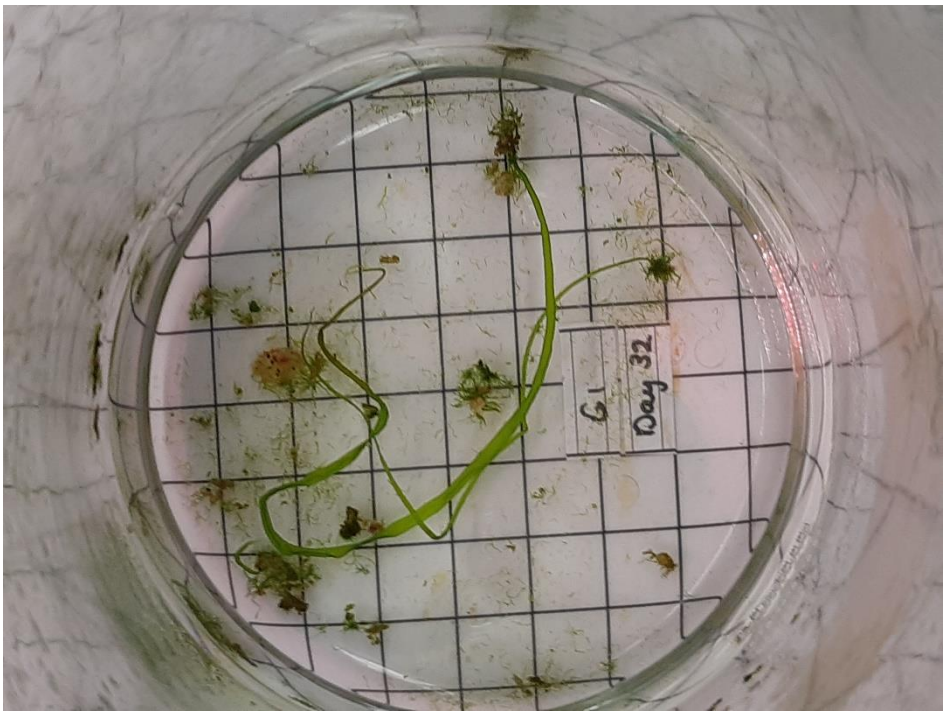

G1
Day 14


G1
Day 18


G1
Day 21


G1
Day 25


G1
Day 28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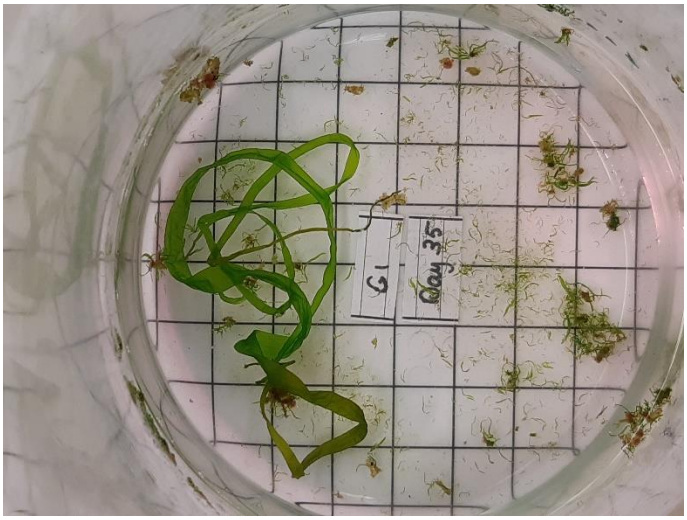
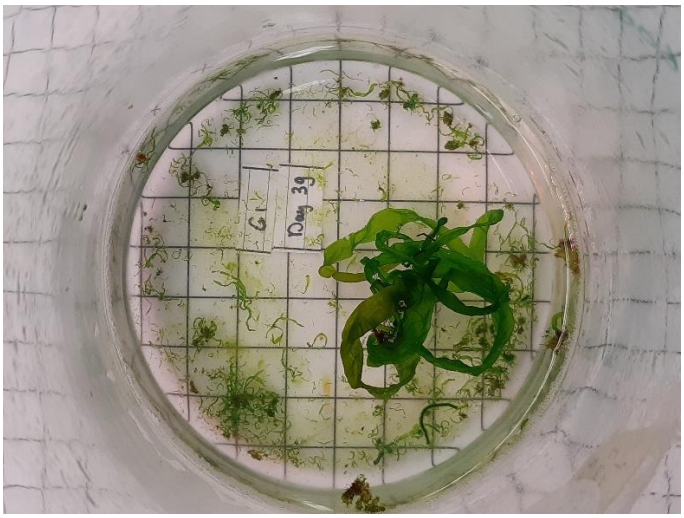
Day 32


G1
Day 35


G1
Day 39


G1
Day 42

G1 20-10-12 (day 11).

G1 20-10-15 (day 14).

G1 20-10-19 (day 18).

G1 20-10-22 (day 21).

G1 20-10-26 (day 25).

G1 20-10-29 (day 28).

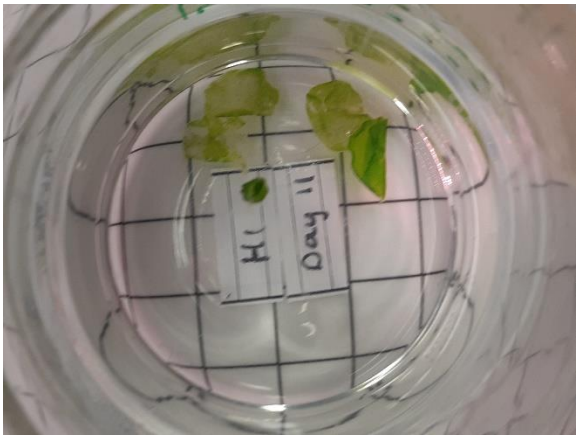
G1 20-11-02 (day 32).

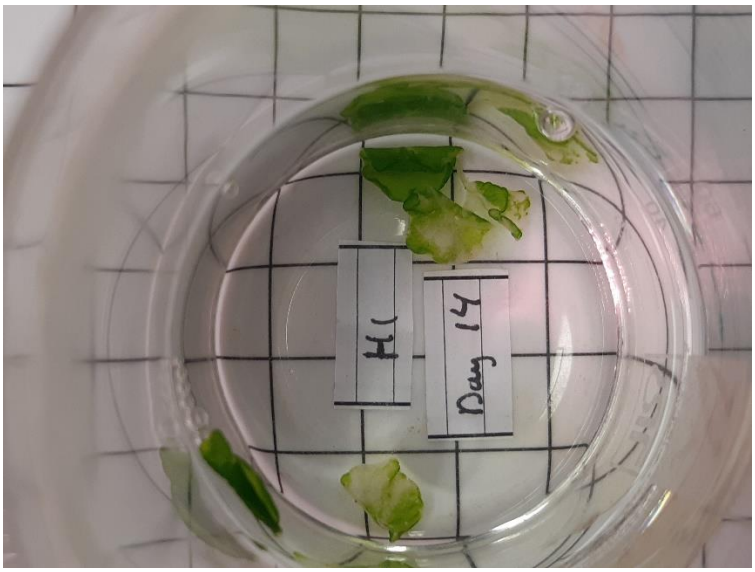
G1 20-11-05 (day 35).

G1 20-11-09 (day 39).

G1 20-11-12 (day 42).

G1 20-11-16 (day 46).


H1
Day 11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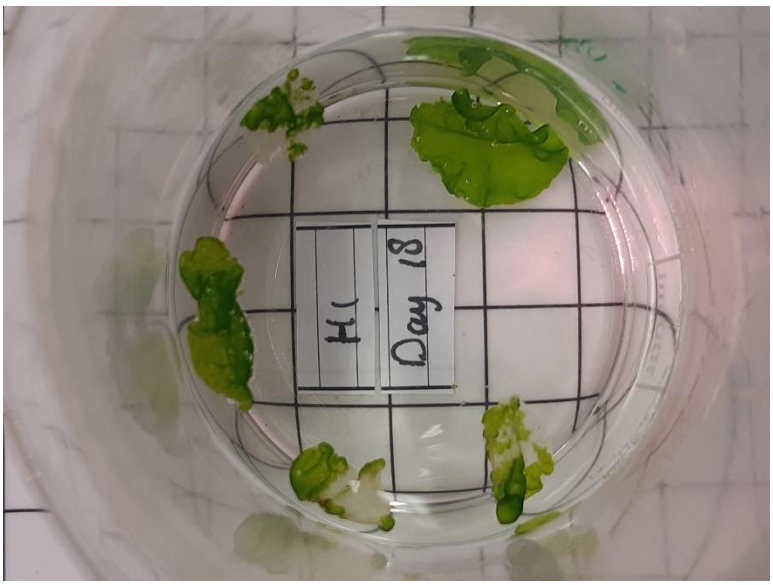
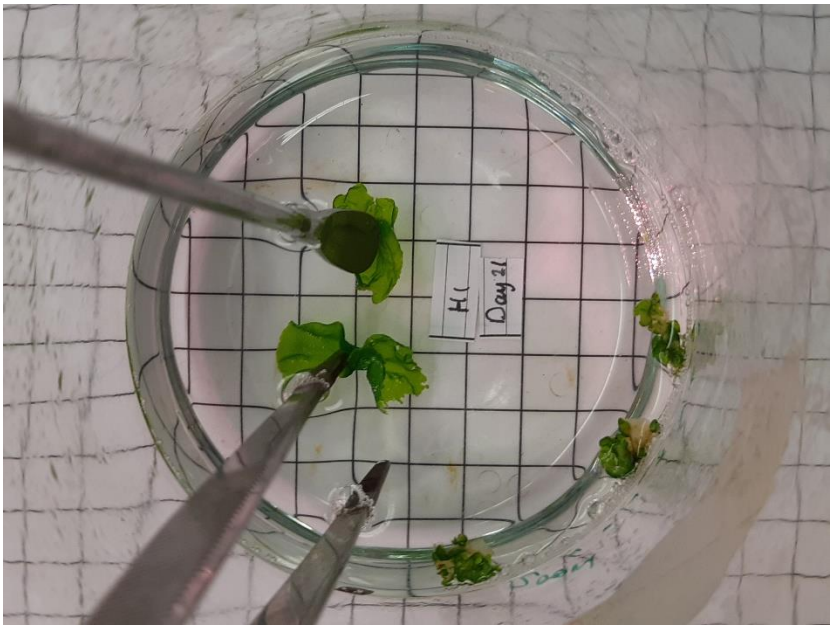
Day 14


H1
Day 18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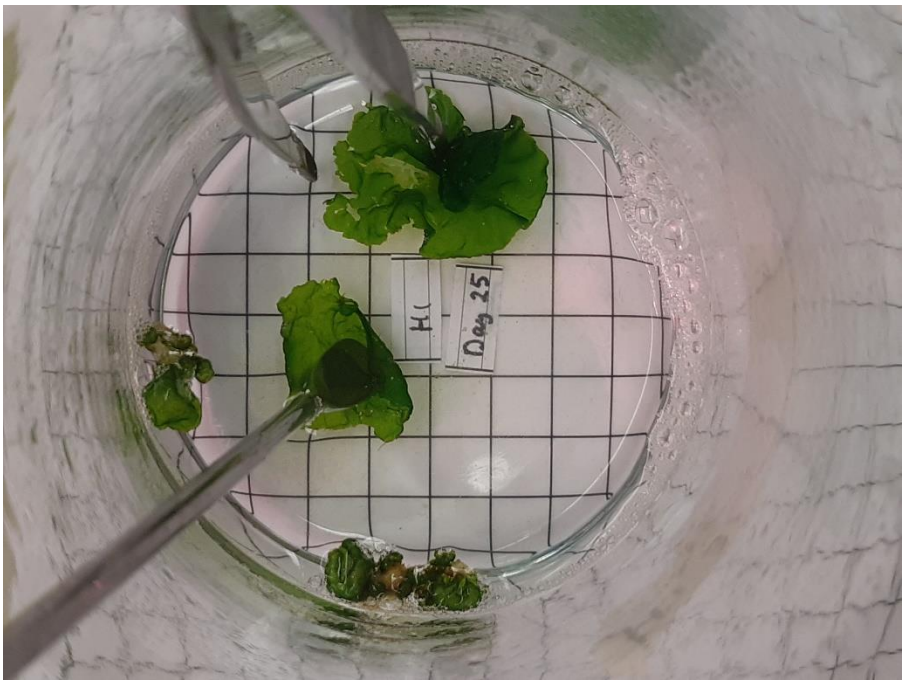
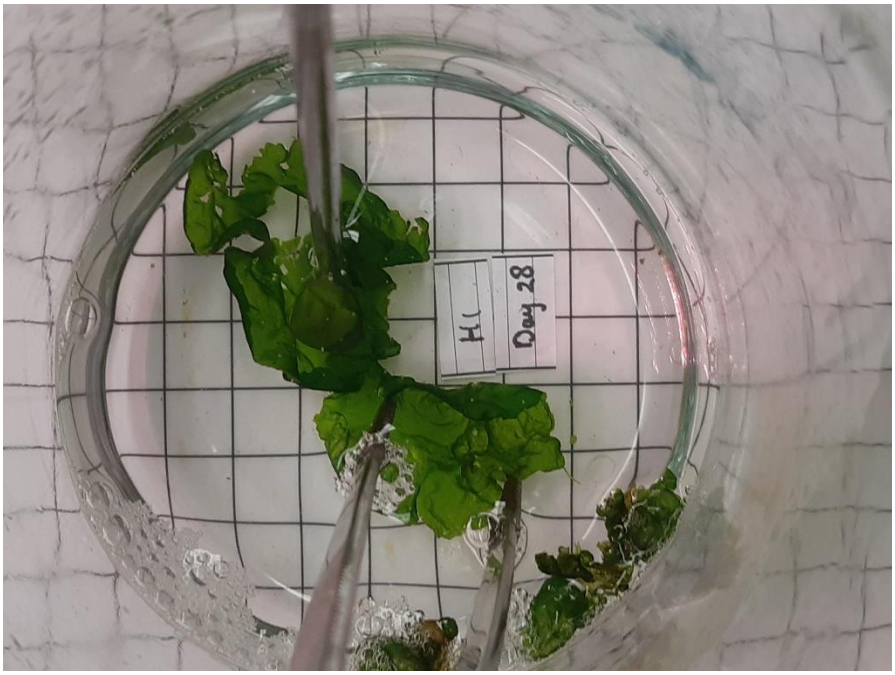
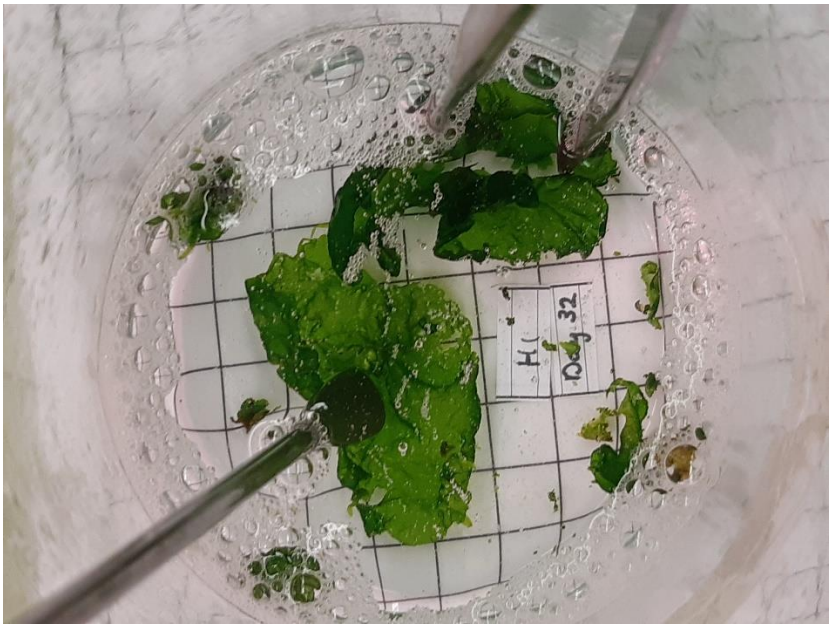
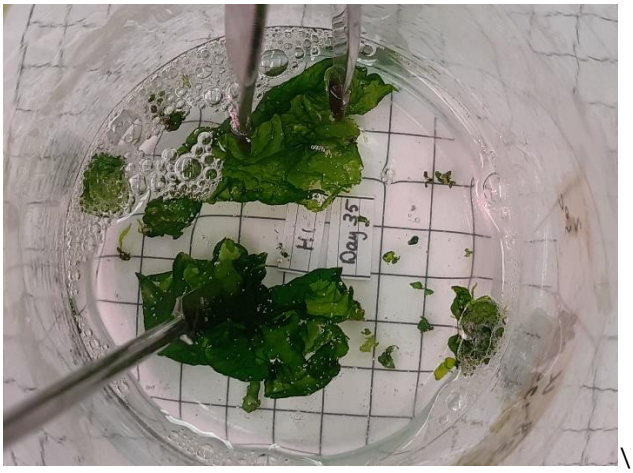
Day 21

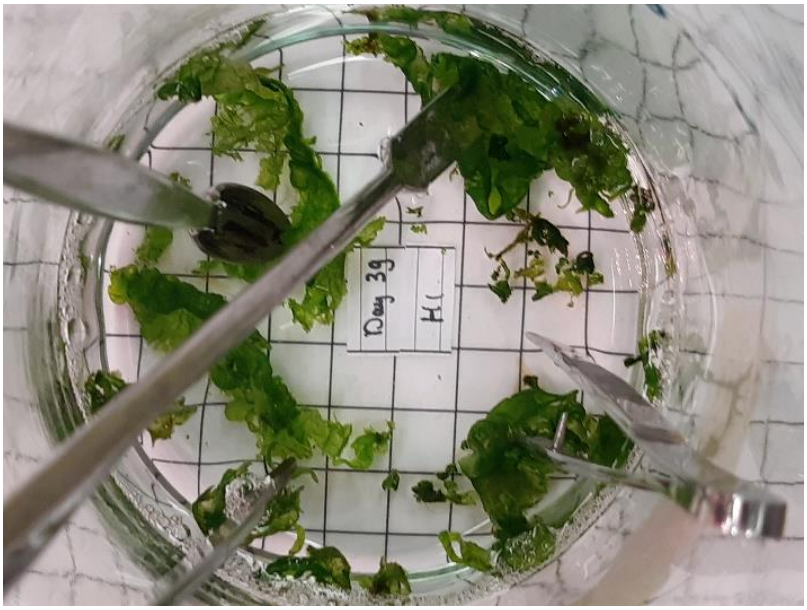

H1
Day 25

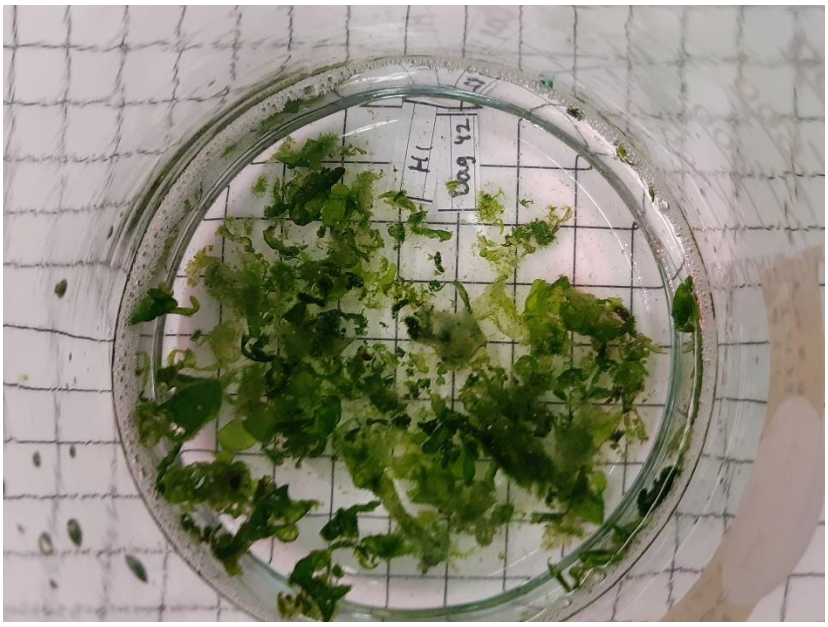

H(
Day 28

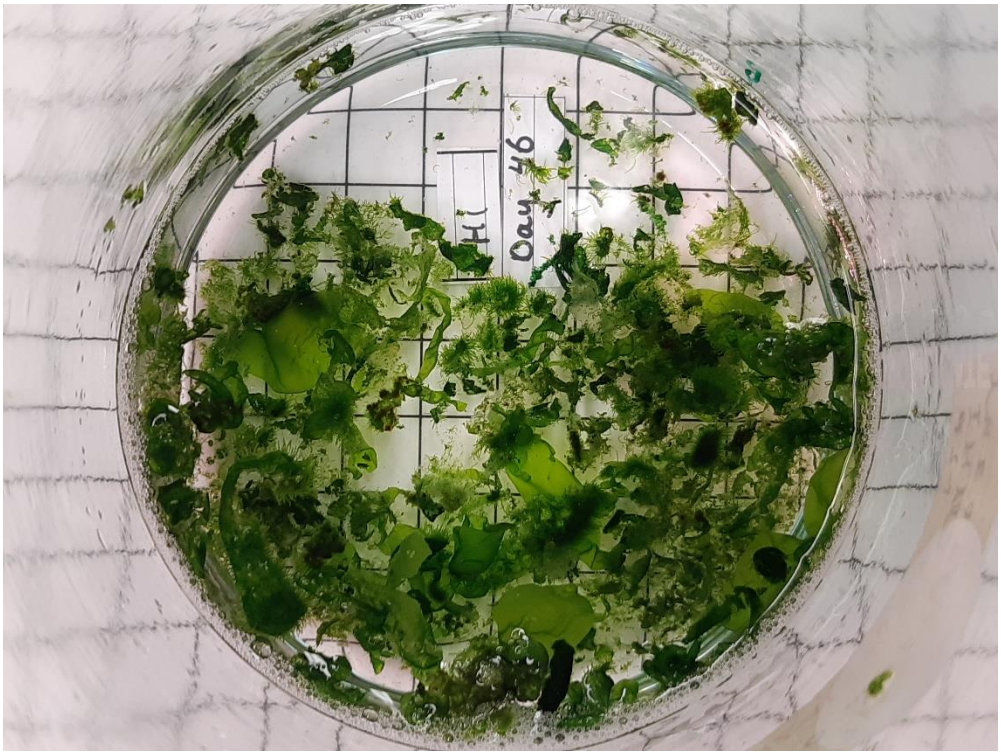

H(
Day 32


H(
Day 35

\


Day 39


Day 42



H1 20-10-12 (day 11).

H1 20-10-15 (day 14).

H1 20-10-19 (day 18).

H1 20-10-22 (day 21).

H1 20-10-26 (day 25).

H1 20-10-29 (day 28).

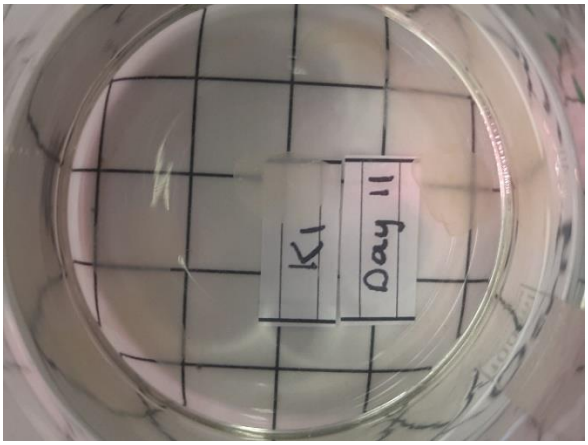
H1 20-11-02 (day 32).

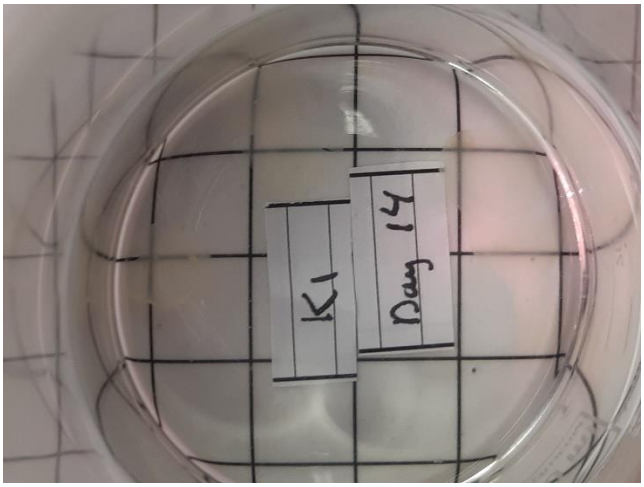
H1 20-11-05 (day 35).

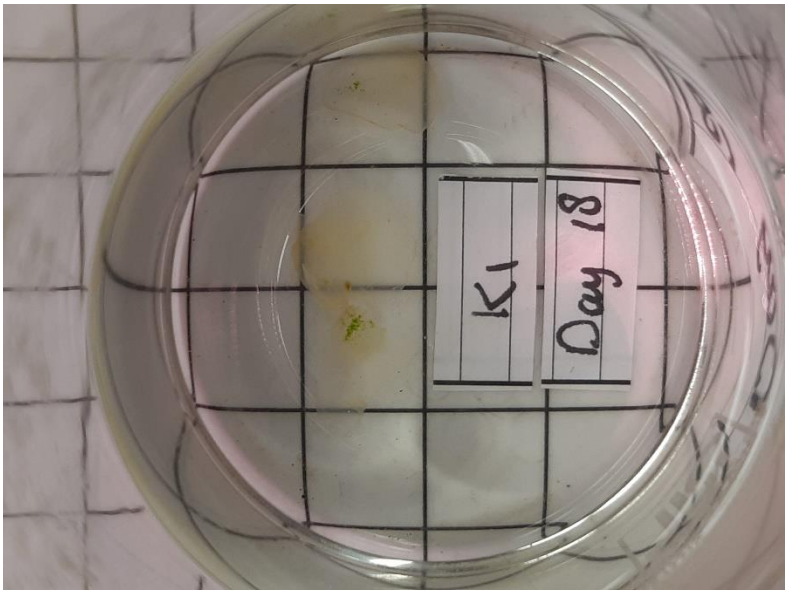
H1 20-11-09 (day 39).

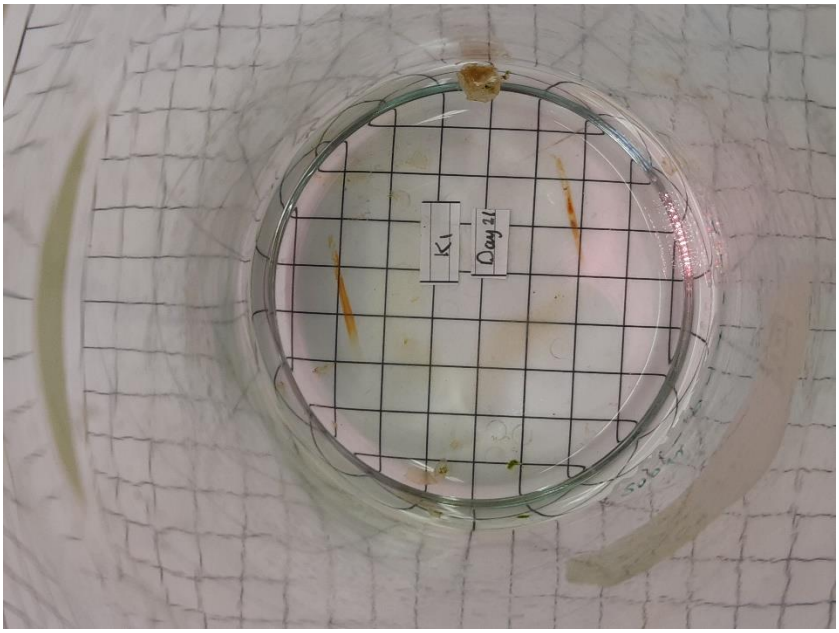
H1 20-11-12 (day 42).

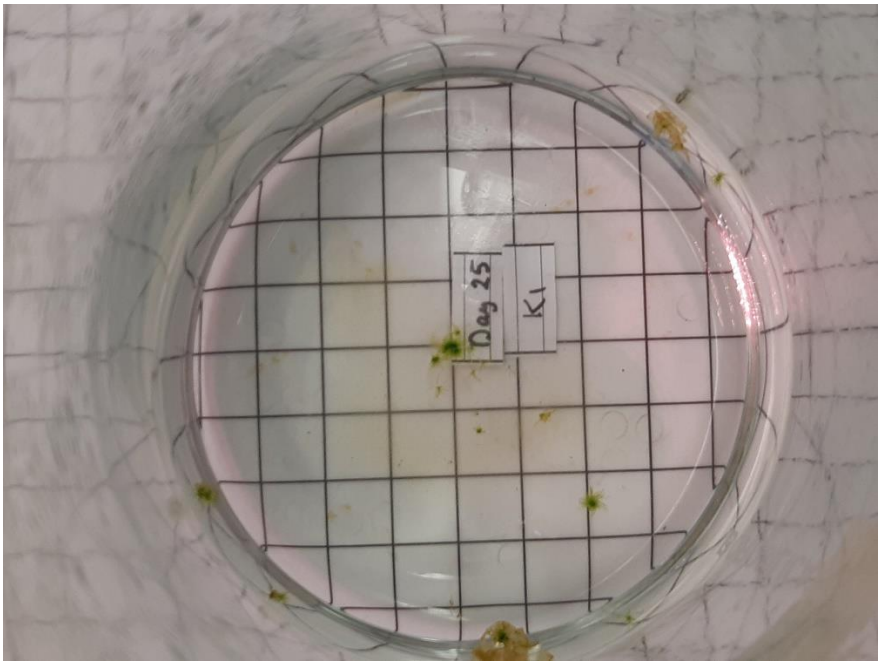
H1 20-11-16 (day 46).

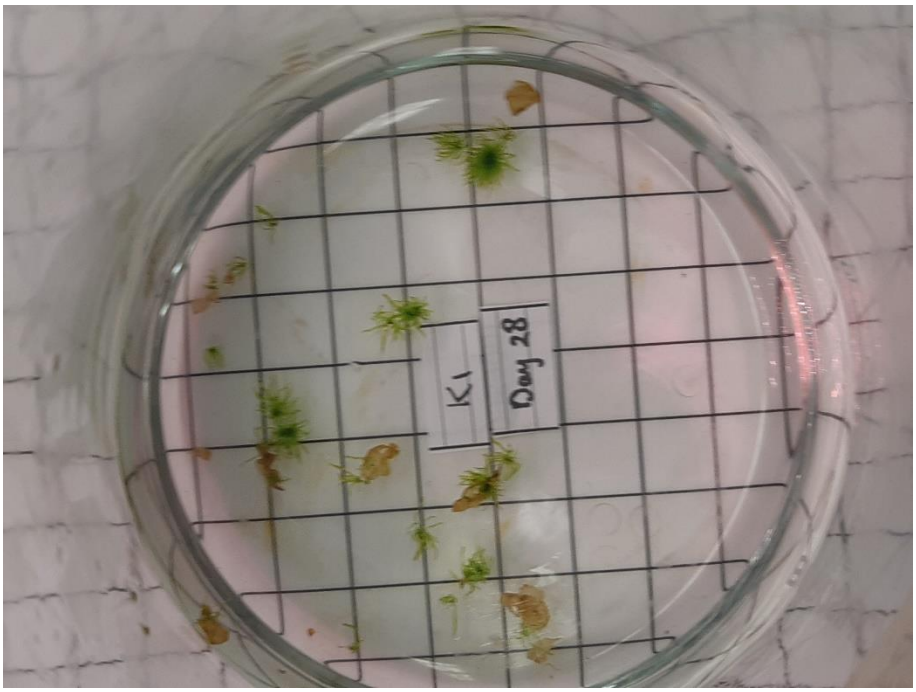


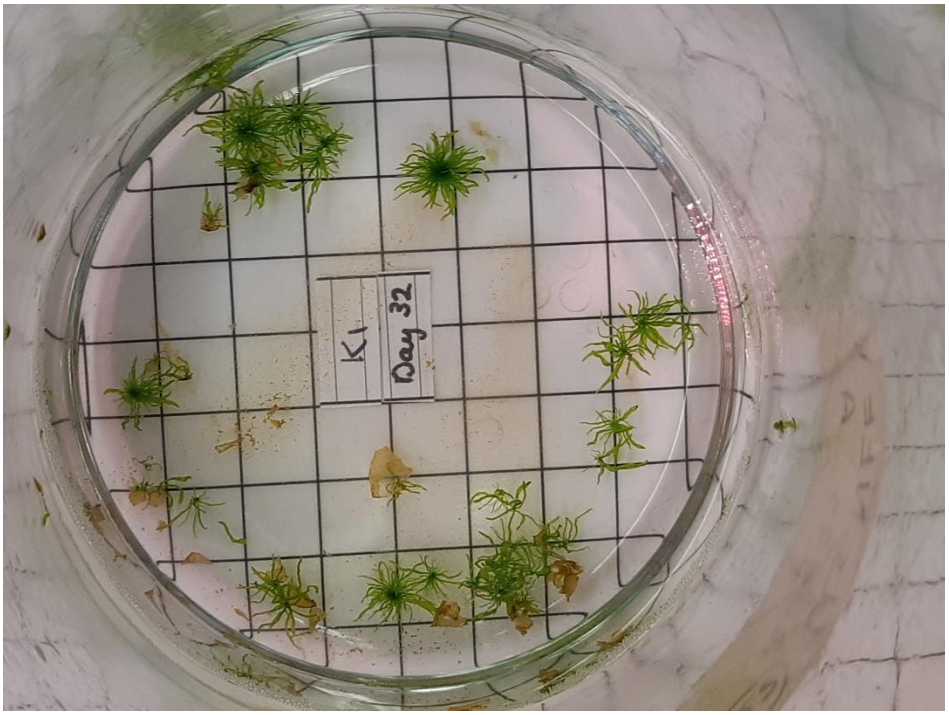

K1
Day 14

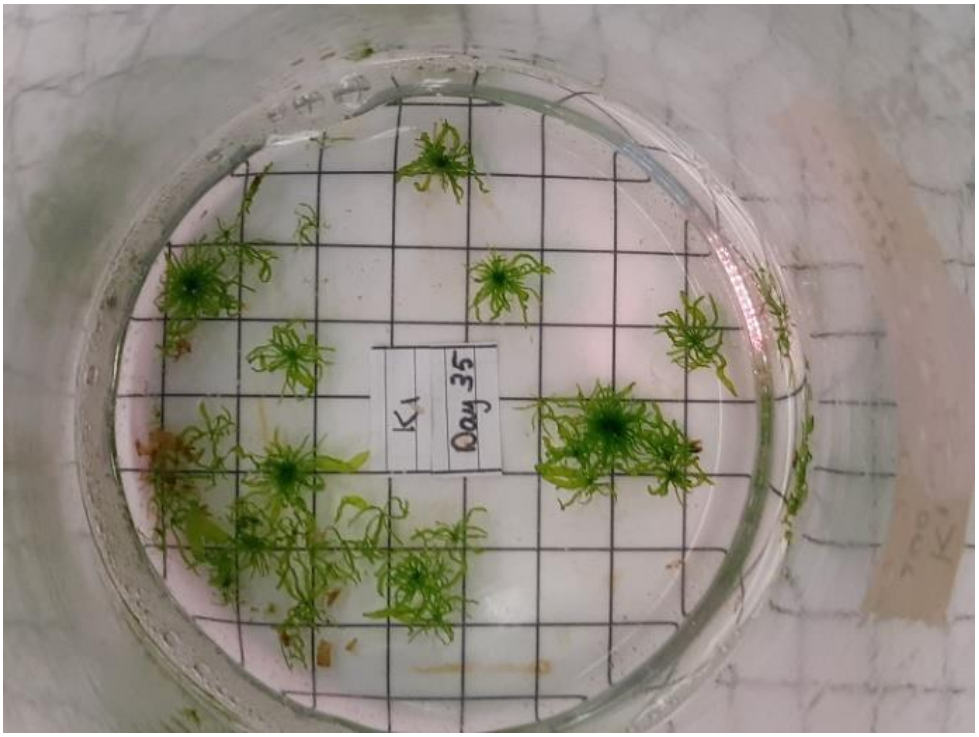

K1
Day 18

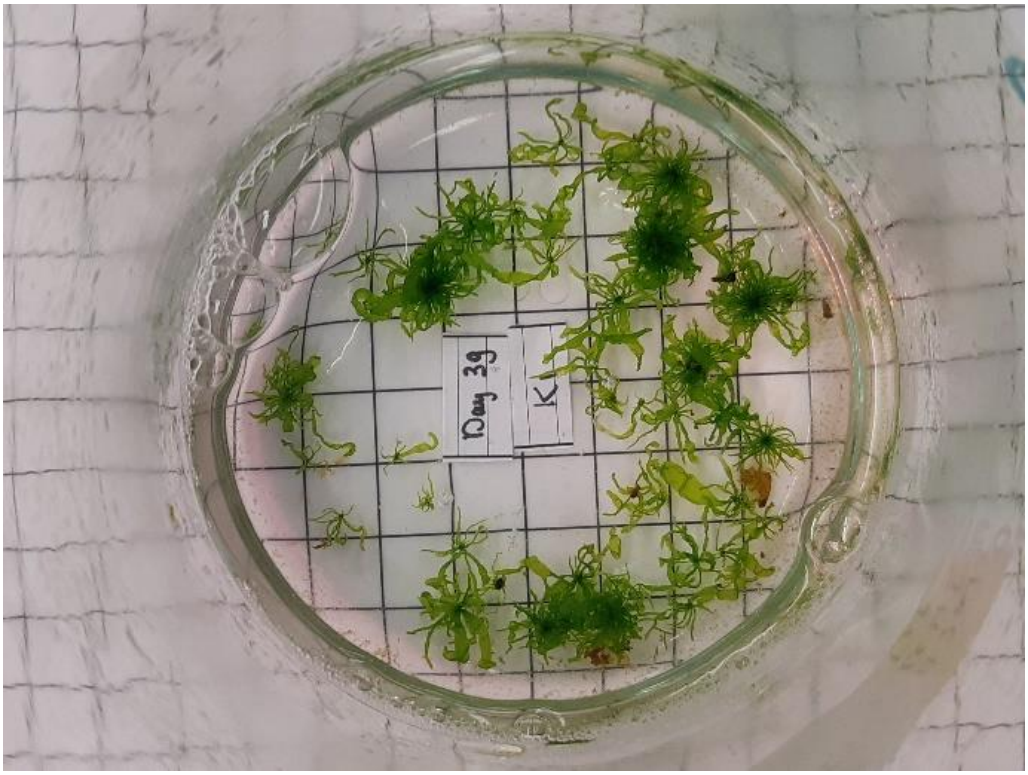

K1
Day 21

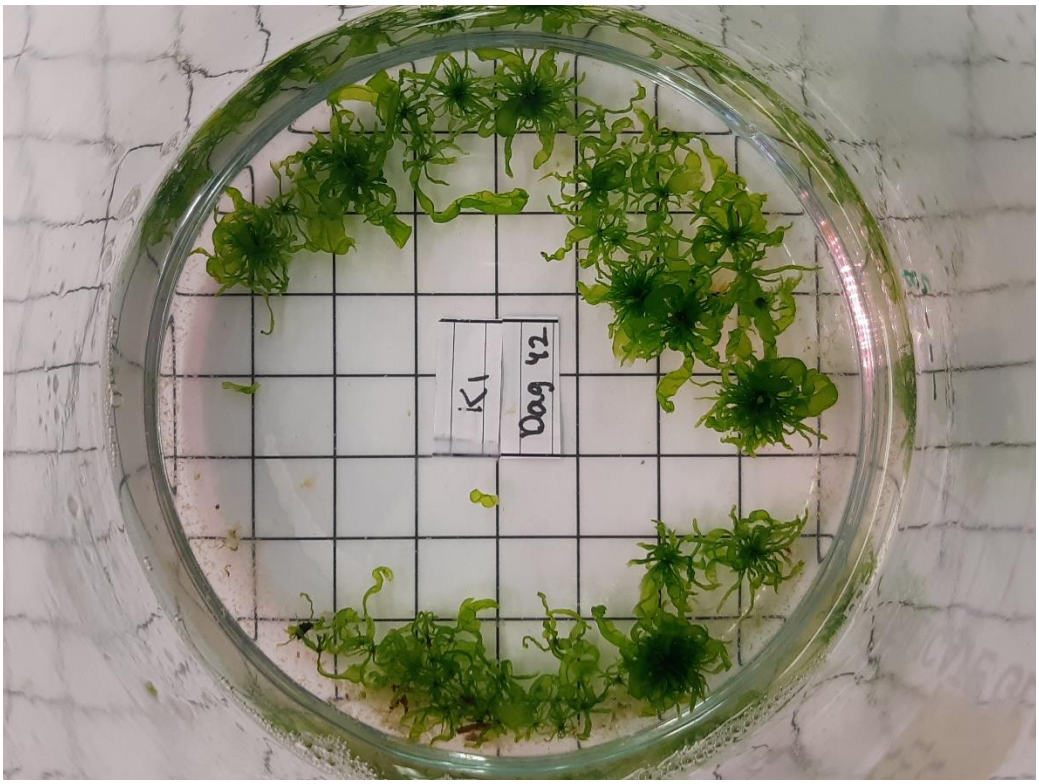

Day 25
K1

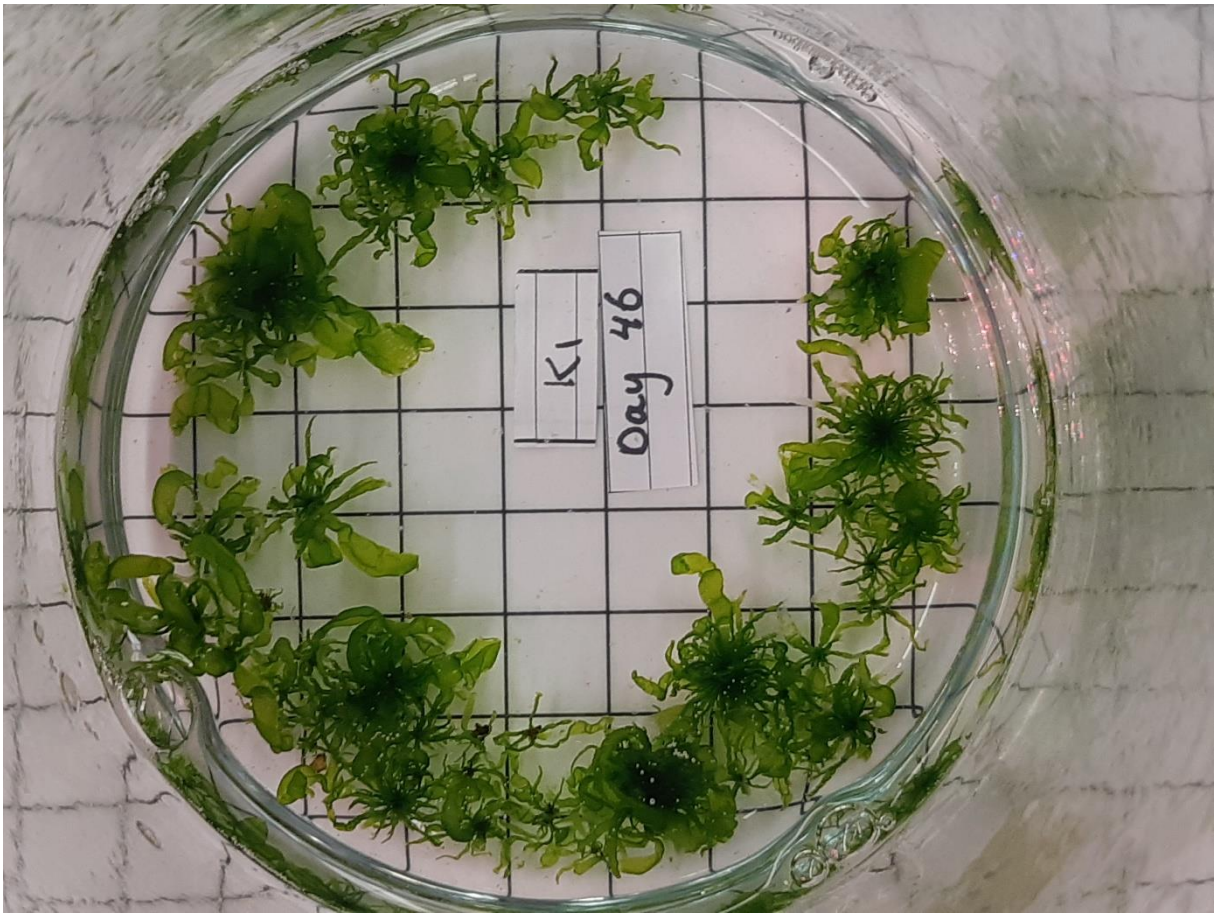

K1
Day 28


K1
Day 32


K1
Day 35


Day 39
K1


K1
Day 42



K1 20-10-12 (day 11).

K1 20-10-15 (day 14).

K1 20-10-19 (day 18).

K1 20-10-22 (day 21).

K1 20-10-26 (day 25).

K1 20-10-29 (day 28).

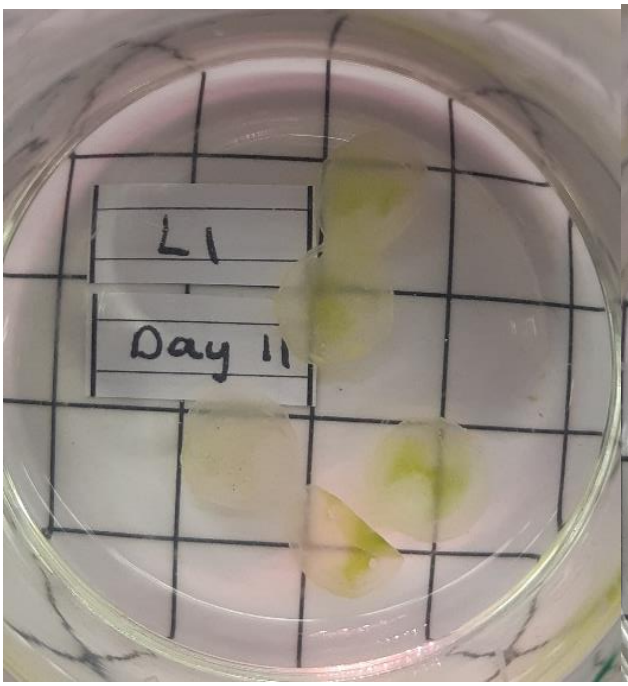
K1 20-11-02 (day 32).

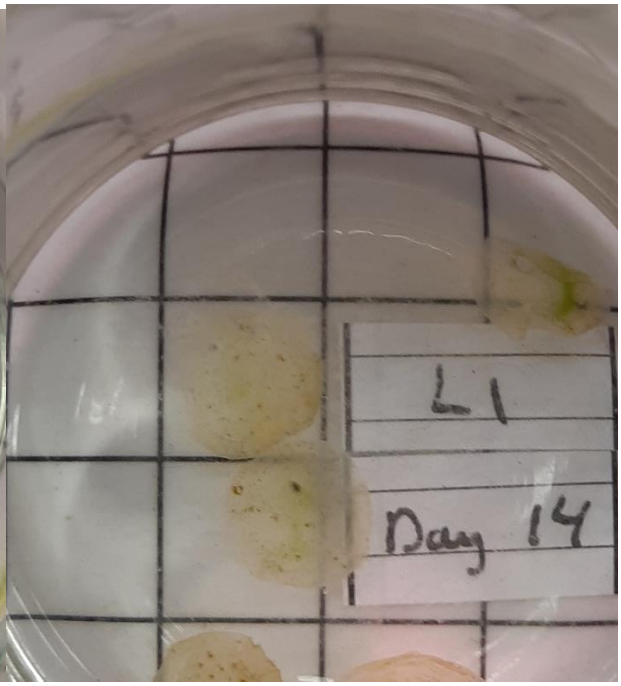
K1 20-11-05 (day 35).

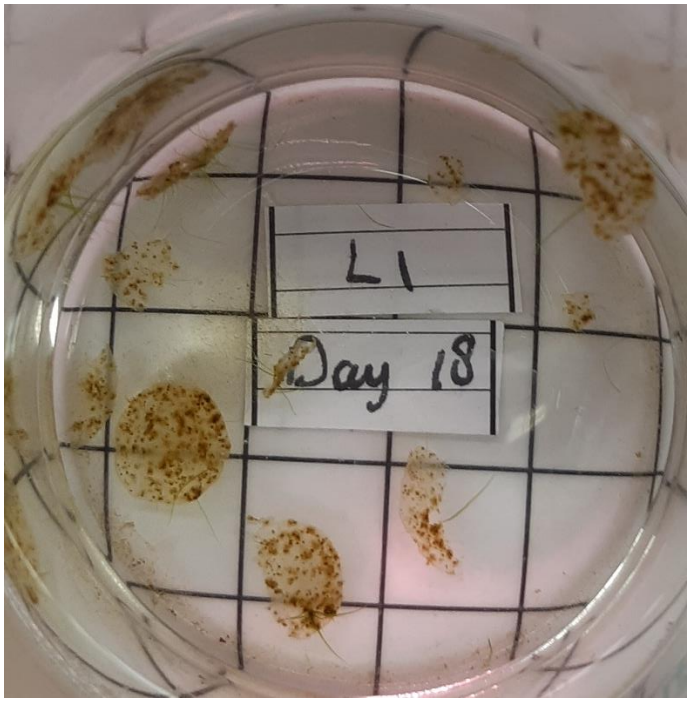
K1 20-11-09 (day 39).

K1 20-11-12 (day 42).

K1 20-11-16 (day 46).


L1
Day 11


L1
Day 14

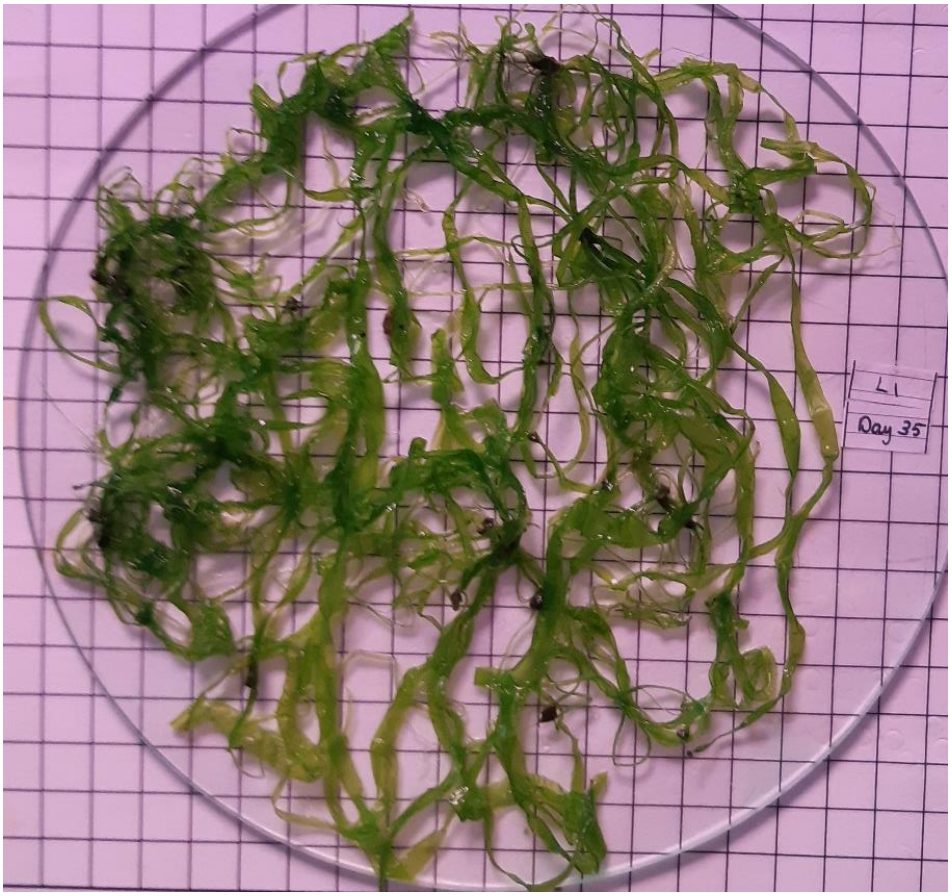

L1
Day 18

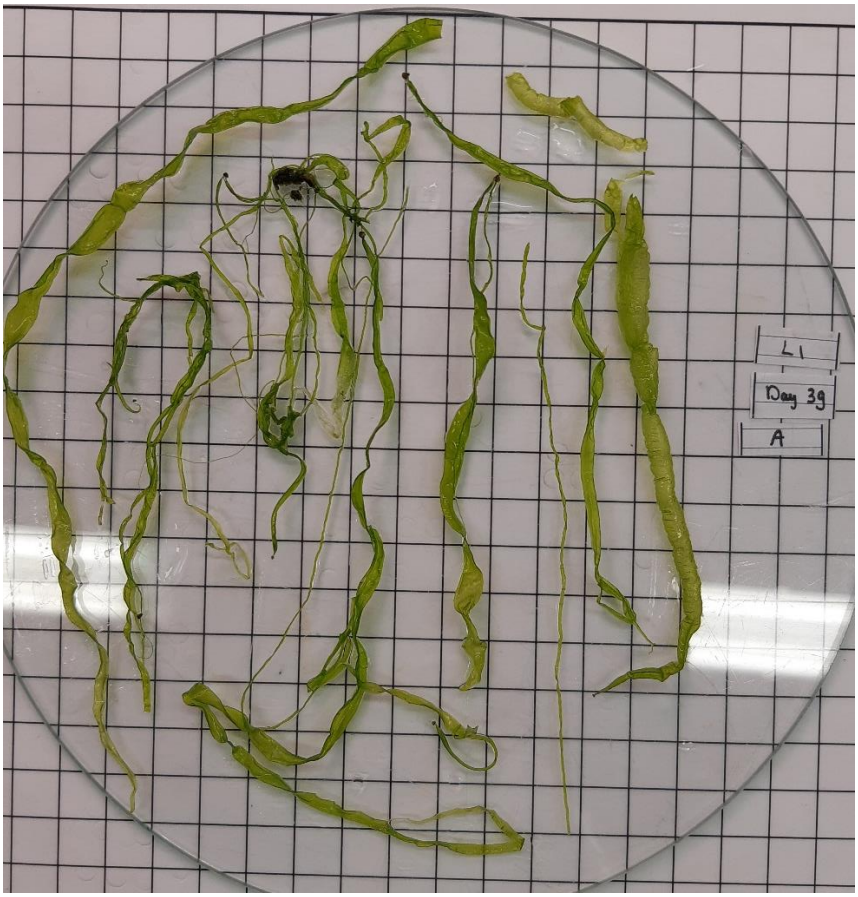

L1
Day 21

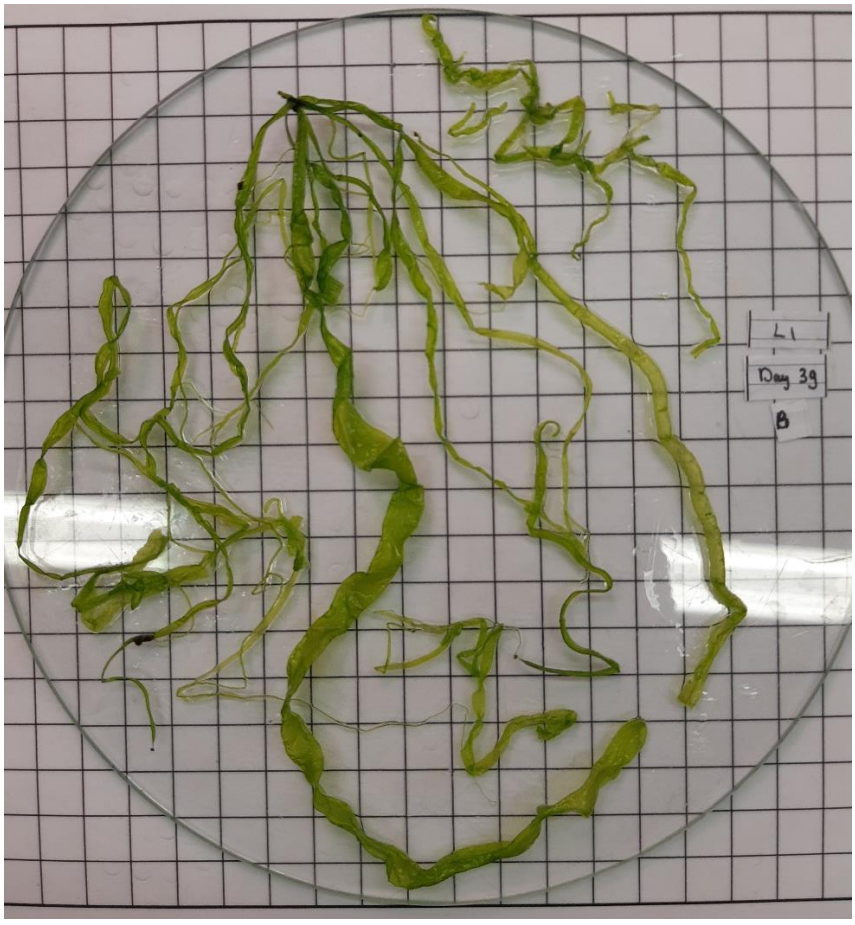

L1
Day 25

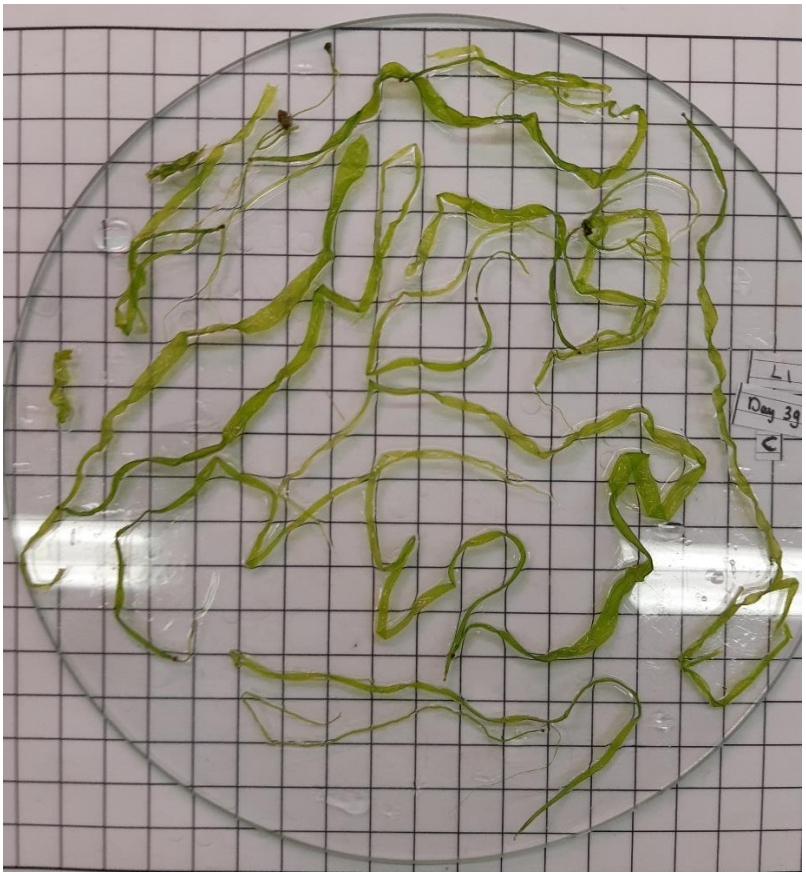

Day 28


Day 32

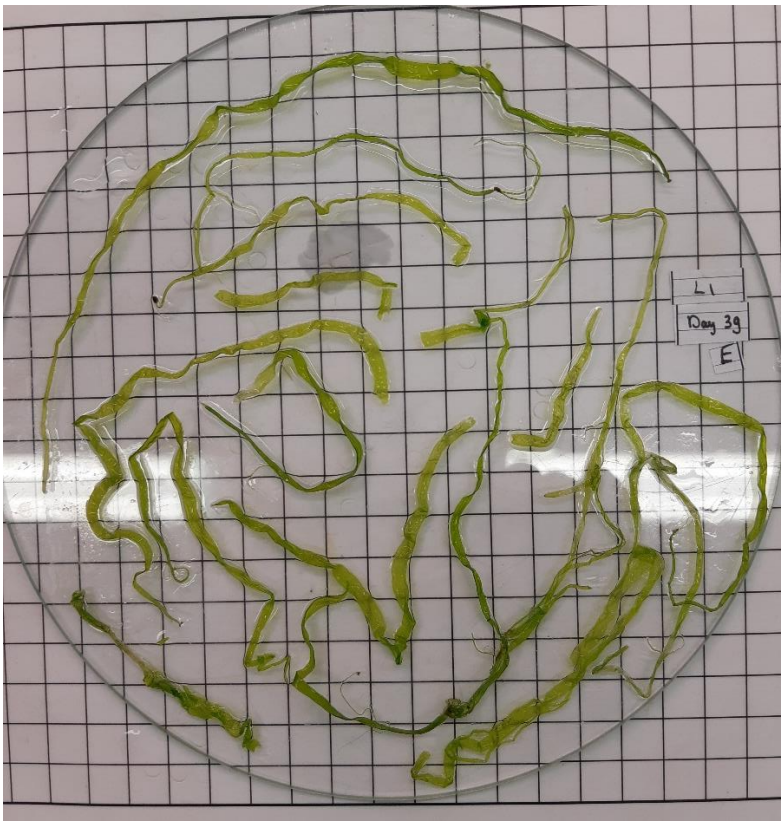

LL
Day 35

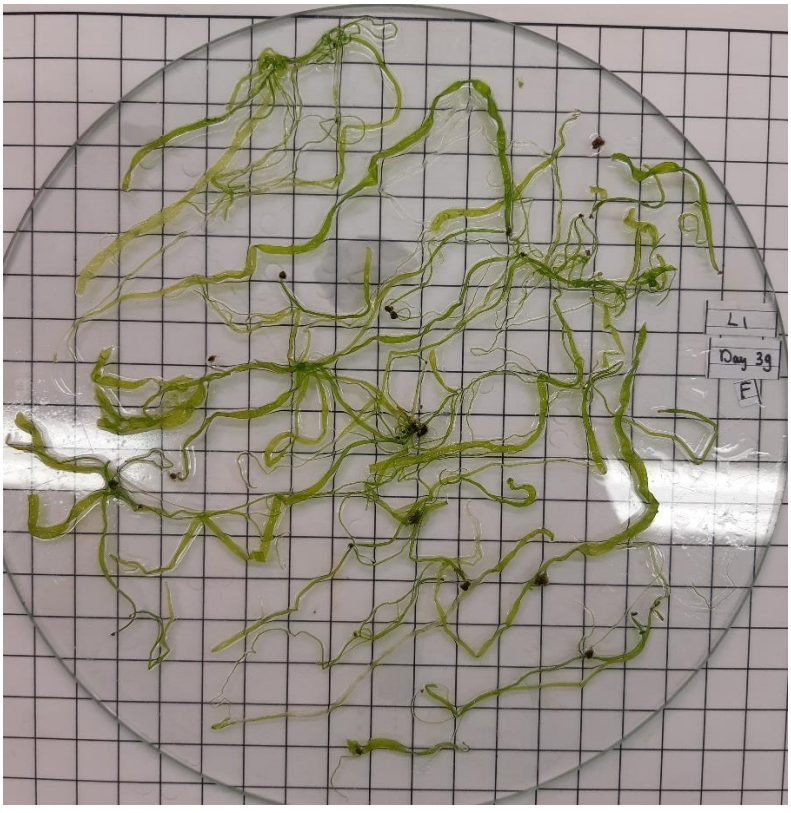

L1
Day 39
A

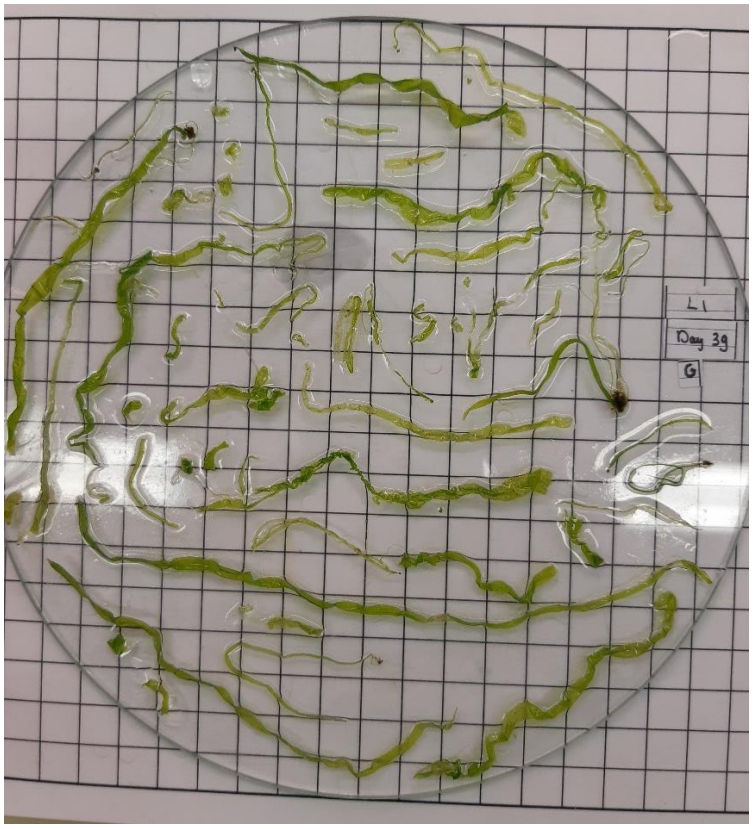

L1
Day 39
B

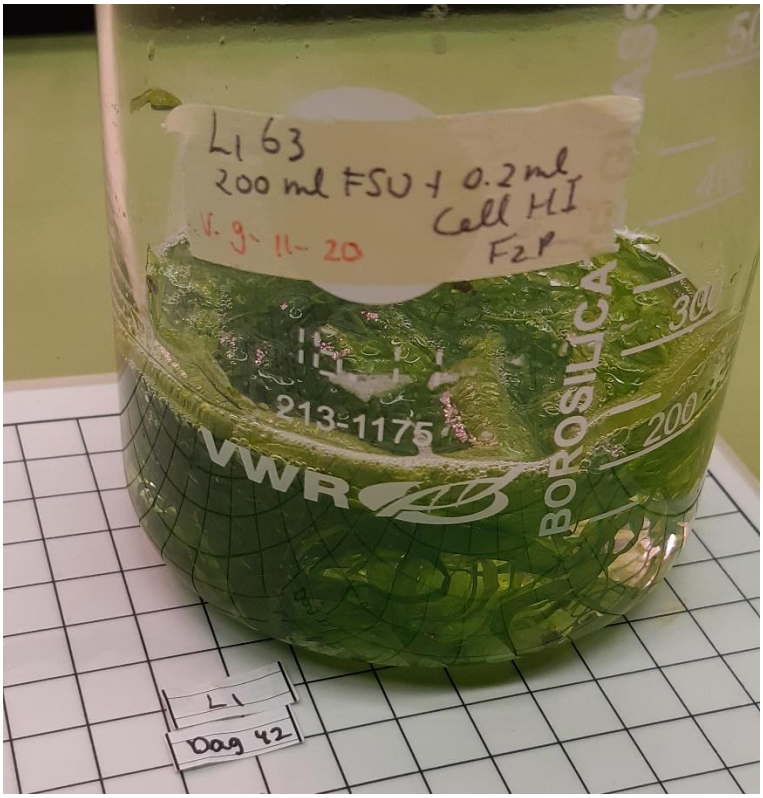

L1
Day 3g
C


L1
Day 3g
D


L1
Day 3g
E


L1
Day 3g
E


L1
Day 39
G


L1 63
200 ml FSU + 0.2 ml
Cell HI
F2P
V. 9- 11- 20
213-1175
VWR
BOROSILICA
L1
Dag 42

L1 20-10-01 (day 0). 5 cm²

L1 20-10-12 (day 11). 1 cm²

L1 20-10-15 (day 14).

L1 20-10-19 (day 18).

L1 20-10-22 (day 21).

L1 20-10-26 (day 25).

L1 20-10-29 (day 28).

L1 20-11-02 (day 32).

L1 20-11-05 (day 35). App. 11 cm x13 cm = 143 cm²

143 cm² - 1 cm²= 142 cm² increase in 24 days, the **RGR is** (ln 143 – ln 1)/24=4.96/24=**0.21**

$$RGR = \frac{\ln W_t - \ln W_0}{t}$$

L1 20-11-09 (day 39). 7 photos.

Each photo area app. 8cm x 12 cm=96 cm²

7 photos x 96 cm²=672 cm²

672 cm² - 1 cm²= 671 cm² increase in 28 days, the **RGR is** (ln 672 – ln 1)/28=6.51/28=**0.23**

$$RGR = \frac{\ln W_t - \ln W_0}{t}$$

L1 20-11-12 (day 42).

L1 20-11-16 (day 46).

Annex 3 Data *Ulva* experiment 4

Data experiment 4

Table 4.1 5 Ulva spp. population in duplo frost in liquid Nitrogen -196 °C

Code	pop.	Code GeniAlg	from storage
E1	62	UsNEE-2-200605	6 °C
E2	62	Us NEE-2-200605	6 °C
G1	60	UsZUI200207	6 °C
G2	60	Us ZUI200207	6 °C
H1	31	Us FRANCE190429(Coll.SAM)	6 °C
H2	31	Us FRANCE190429(Coll.SAM)	6 °C
K1	64	UsYER200605	6 °C
K2	64	Us YER200605	6 °C
L1	63	UsKAM200605	6 °C
L2	63	Us KAM200605	6 °C

Table 4.2 5 Ulva spp. population 5 discs per vial, in duplo frost in liquid Nitrogen -196 °C, from one or more thallus blades.

Box	Code	pop.	from storage	Date in -196 °C N	Thallus	from one thallesblade	Cryopres_FreezeControl
AB2	E2	62	6 °C	200622	5 cut discs per vial, Ø 1 cm no. 6	yes	PROTO2
AB2	G2	60	6 °C	200622	5 cut discs per vial, Ø 1 cm no. 6	NA	PROTO2
AB2	H2	31	6 °C	200622	5 cut discs per vial, Ø 1 cm no. 6	no	PROTO2
AB3	K2	64	6 °C	200629	5 cut discs per vial, Ø 1 cm no. 6	yes	PROTO2
AB3	L2	63	6 °C	200629	5 cut discs per vial, Ø 1 cm no. 6	yes	PROTO2

Table 4.3 Fresh Weight (FW) and Dry Weight (DW, 60 °C 24 h) of 5 Ulva spp cryopreserved, after 46 days of regrowth.

Code	pop.	FW (g) day46	DW (g) day46
E2	62	1.759	0.208
G2	60	1.572	0.283
H2	31	2.981	0.353
K2	64	1.089	0.130
L2	63	6.001	0.553

- *On day 8 (8dec2020) judgement on sporulation with microscope 400x magnification (see photo's)*

Table 4.4 Judgement on sporulation on day 8

Code	pop.	Sporulation / Gametes free on day 8
E2	62	no
G2	60	no
H2	31	no
K2	64	no
L2	63	no

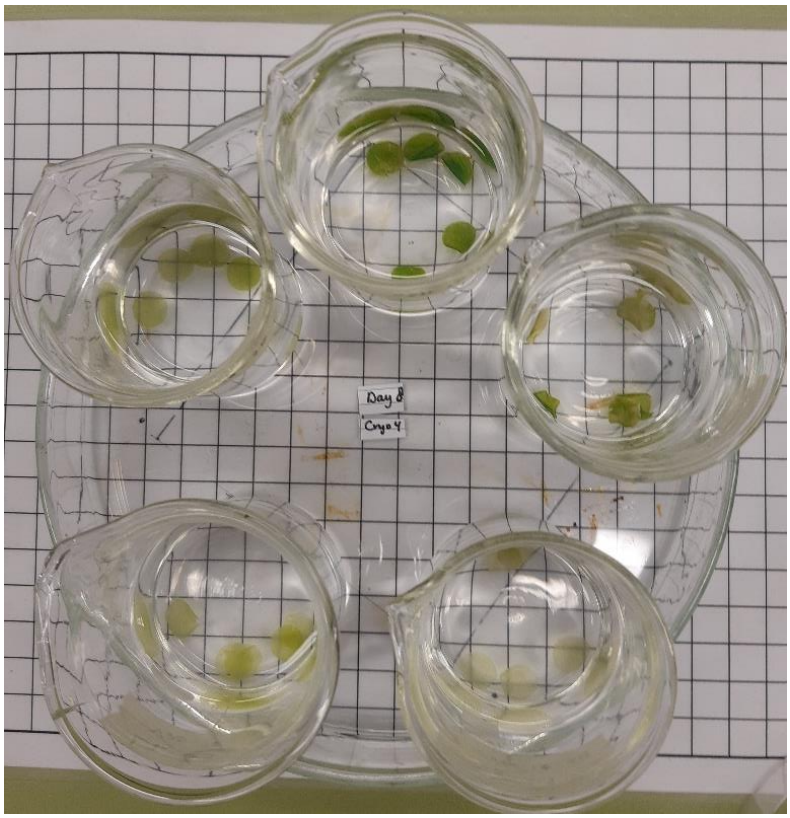
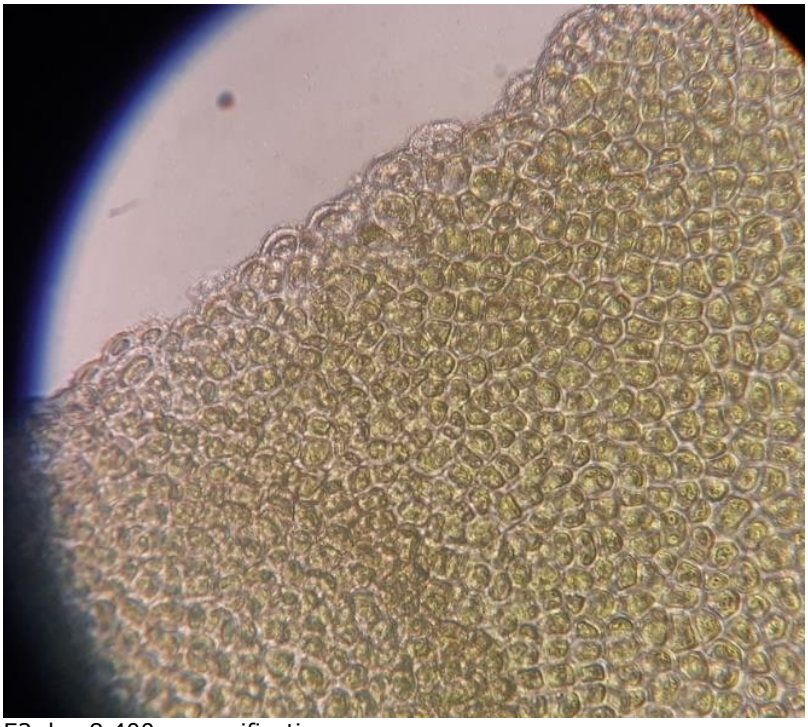
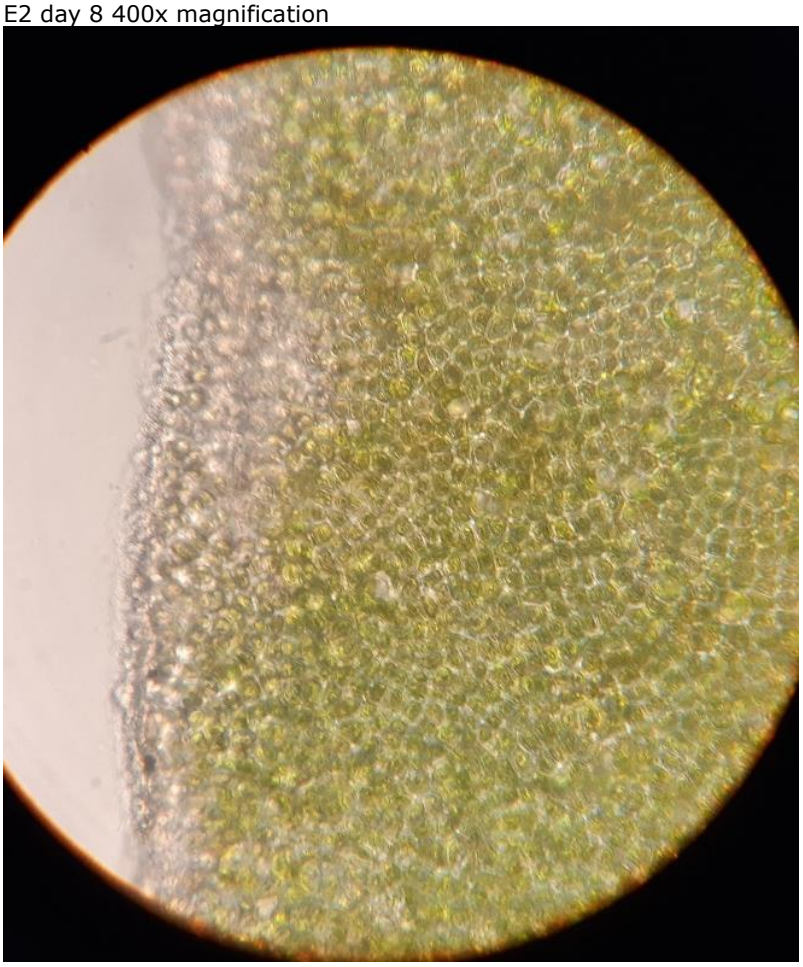


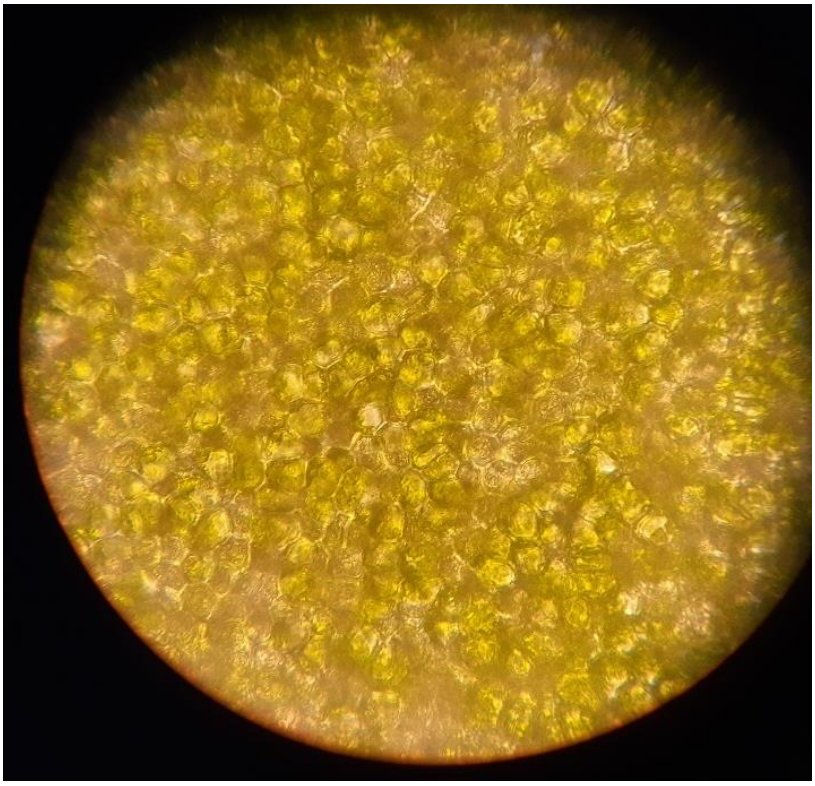
Photo Overview of 5 Ulva populations on day 8: Left Up E2, Next Up Middle G2, Right Up H2, Right Down K2, Left Down L2



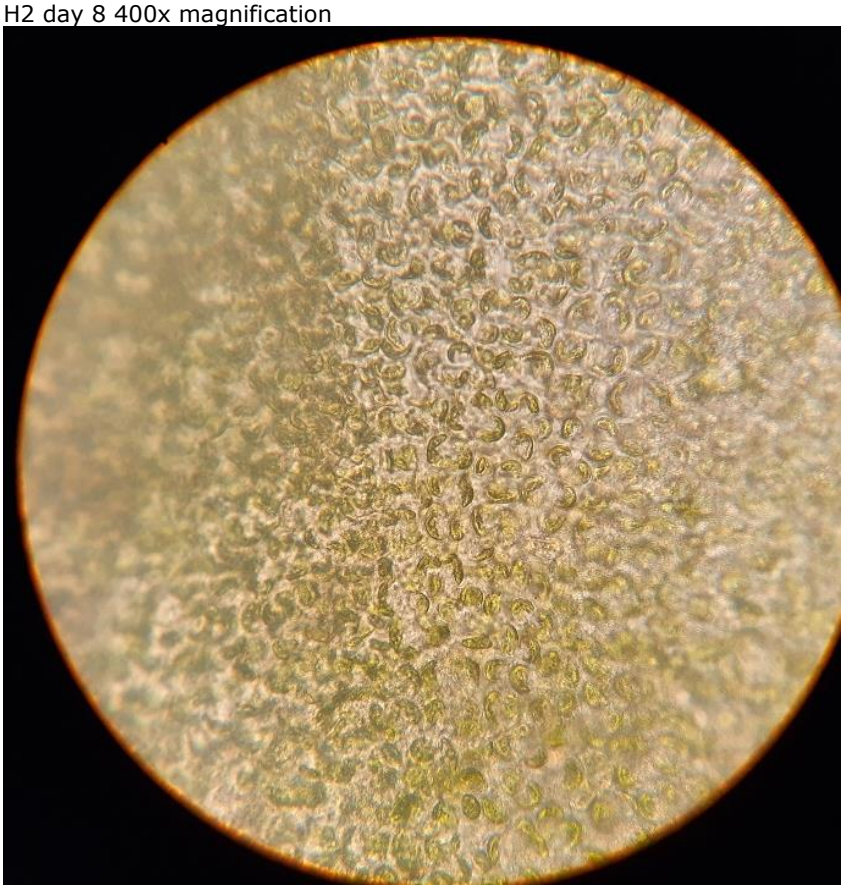
E2 day 8 400x magnification



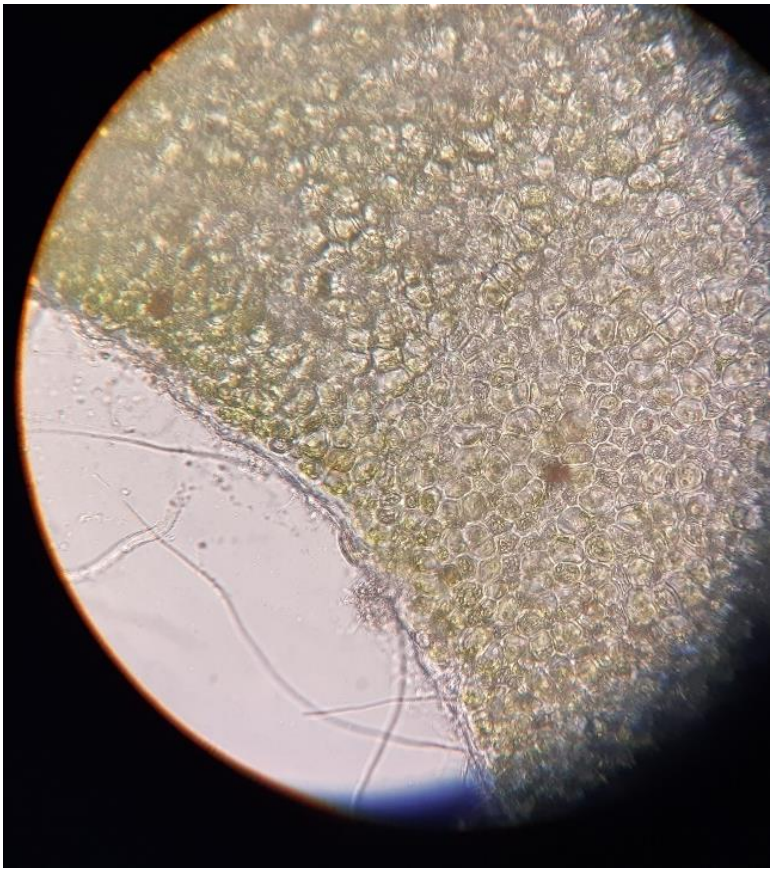
G2 day 8 400x magnification



H2 day 8 400x magnification



K2 day 8 400x magnification

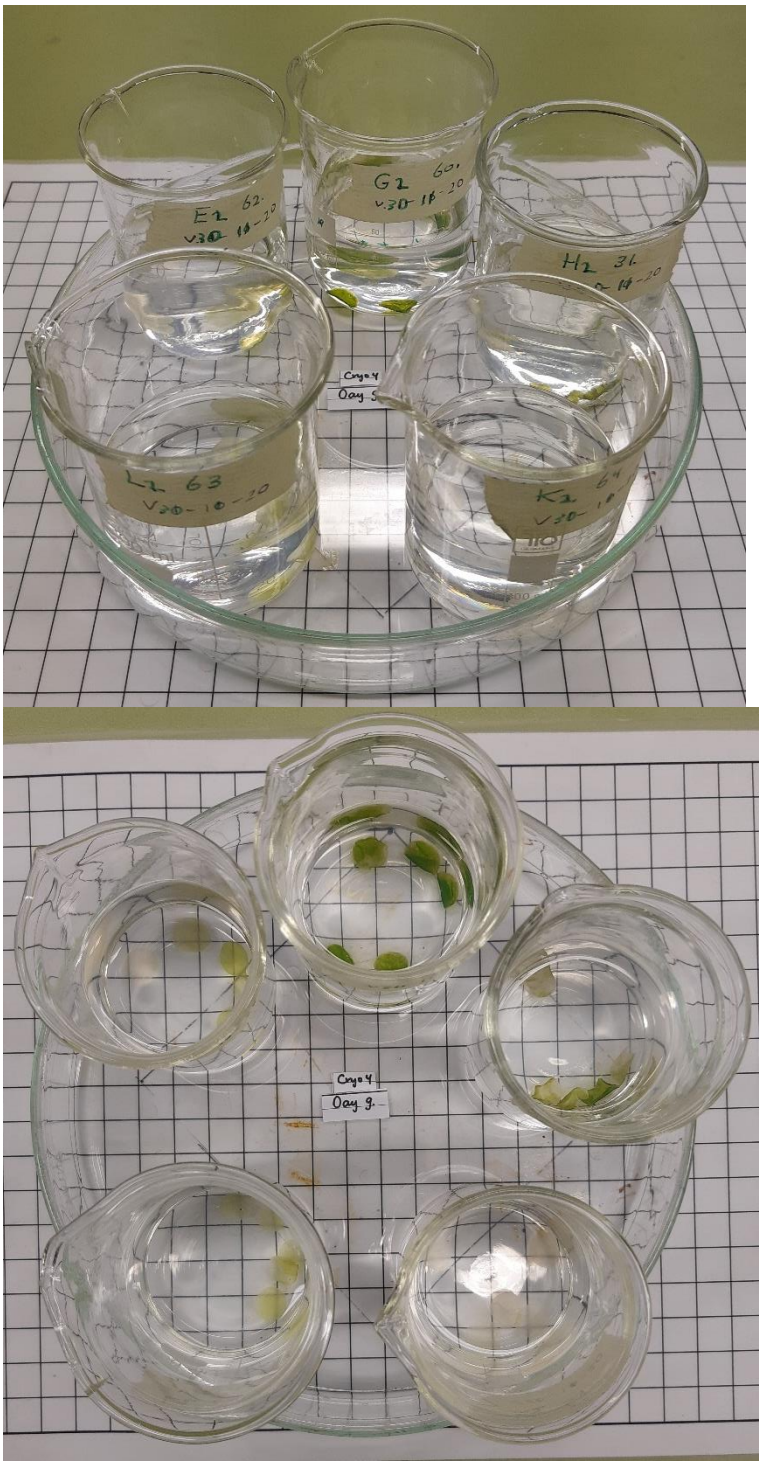


L2 day 8 400x magnification

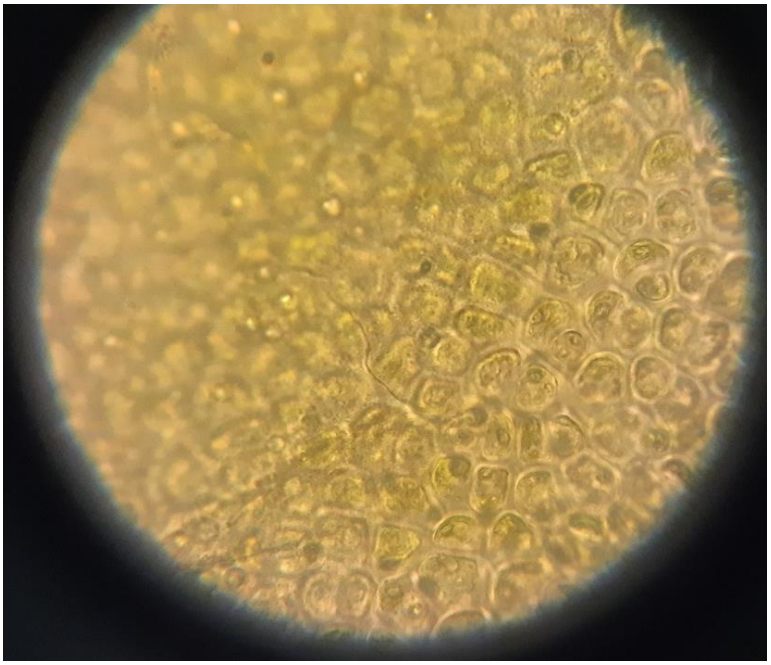
- *On day 9 (9dec2020) judgement on sporulation/ gametes free with microscope 400x and 1000x magnification (see photo's)*

Table 5. Judgement on sporulation on day 9

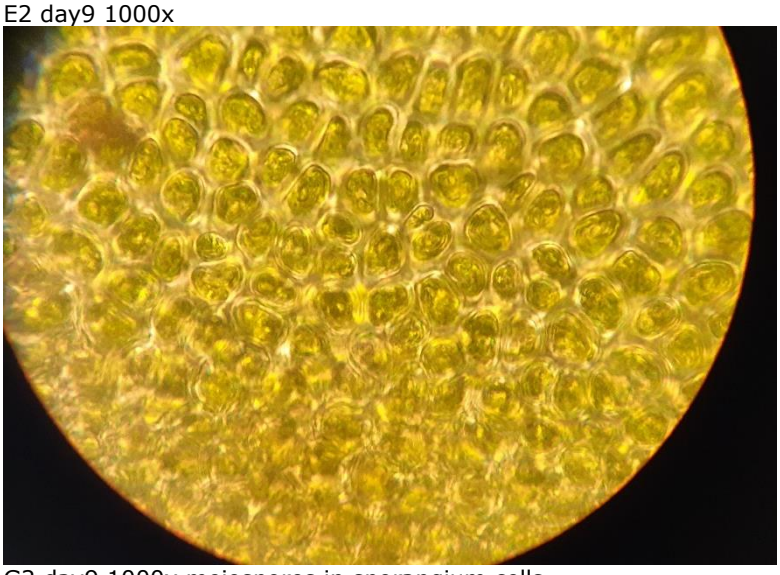
Code	pop.	Sporulation/ Gametes free on day 9
E2	62	no
G2	60	no
H2	31	no
K2	64	no
L2	63	no



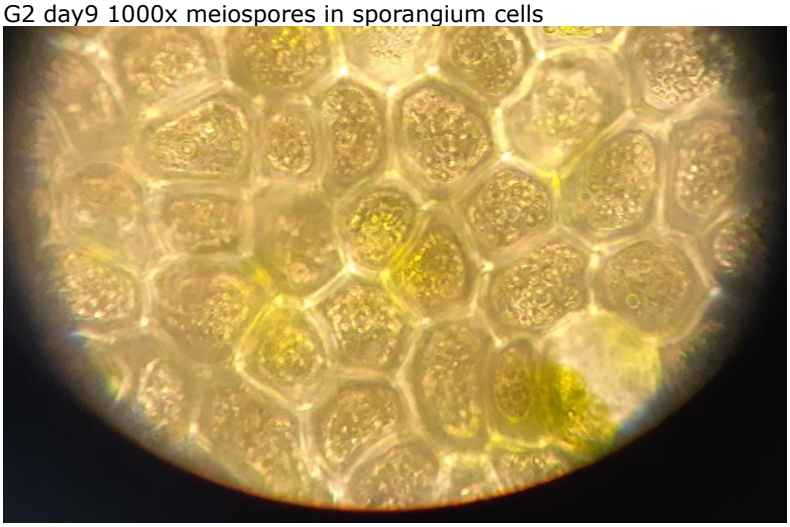
Photo’s Overview of 5 Ulva populations on day 9: Left Up E2, Next Up Middle G2, Right Up H2, Right Down K2, Left Down L2



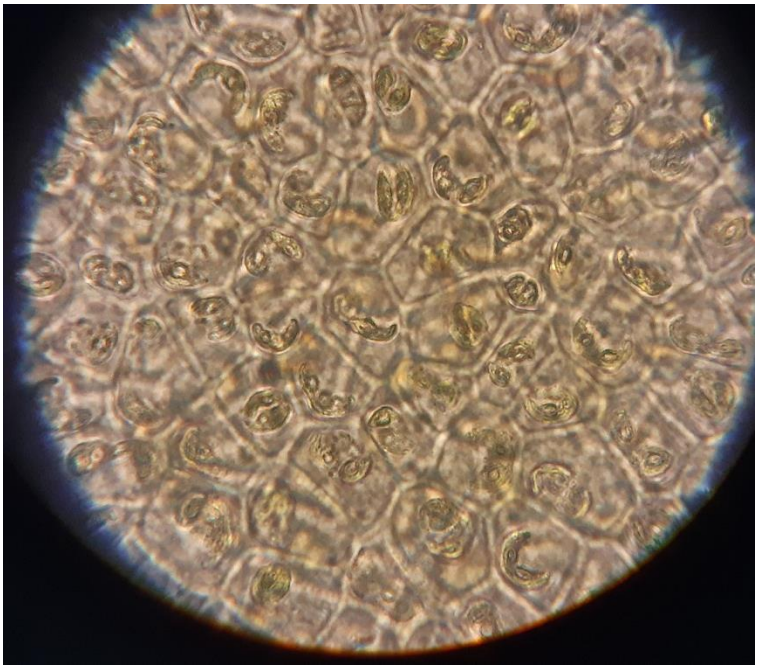
E2 day9 1000x



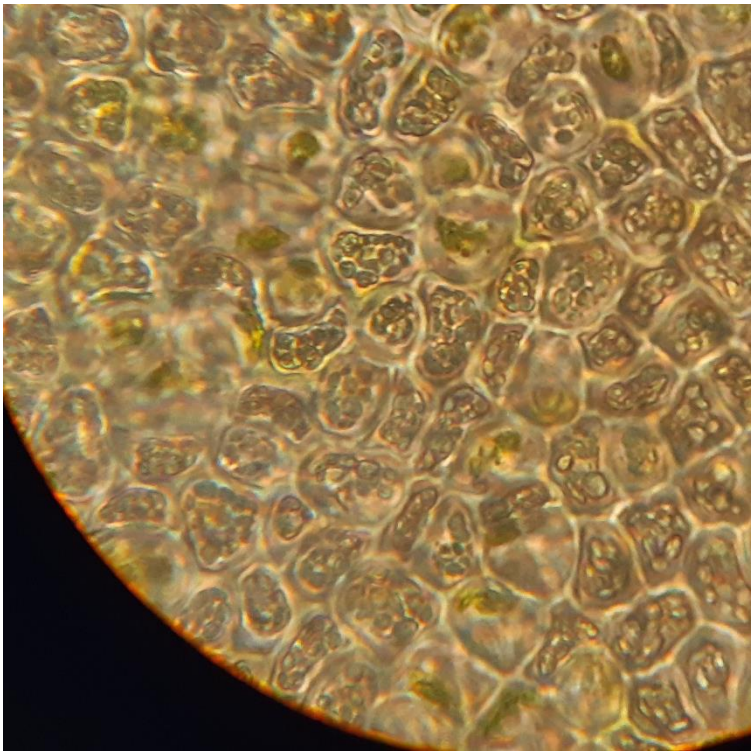
G2 day9 1000x meiospores in sporangium cells



H2 day9 1000x gametes in gametangia cells.



K2 day 9 1000x gametes in gametangia cells.



L2 day 9 1000x gametes in gametangia cells.

- *On day 10 (10dec2020) judgement on sporulation with microscope 400x and 1000x magnification (see photo's and video's)*

Table 6. Judgement on sporulation on day 10

•

Code	pop.	Sporulation/ Gametes free on day 10
E2	62	no

G2	60	no
H2	31	yes
K2	64	yes
L2	63	no

•

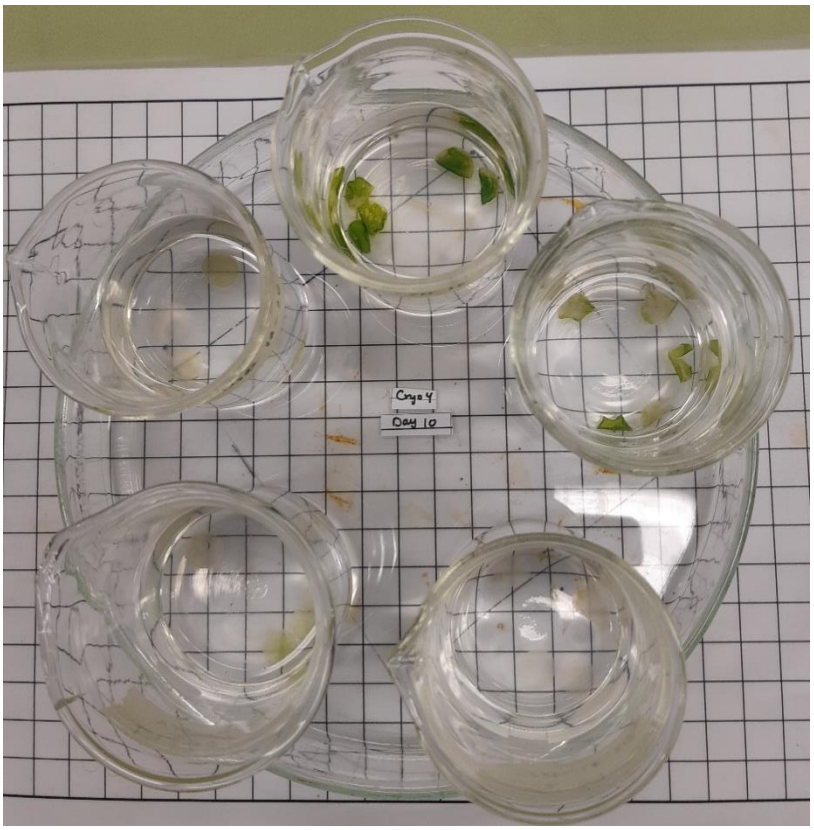
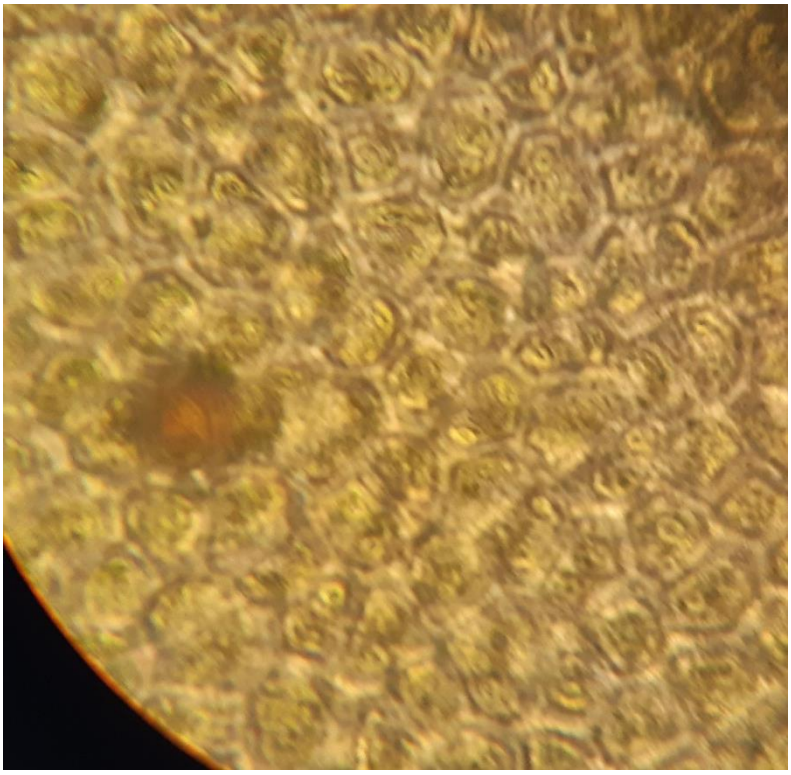
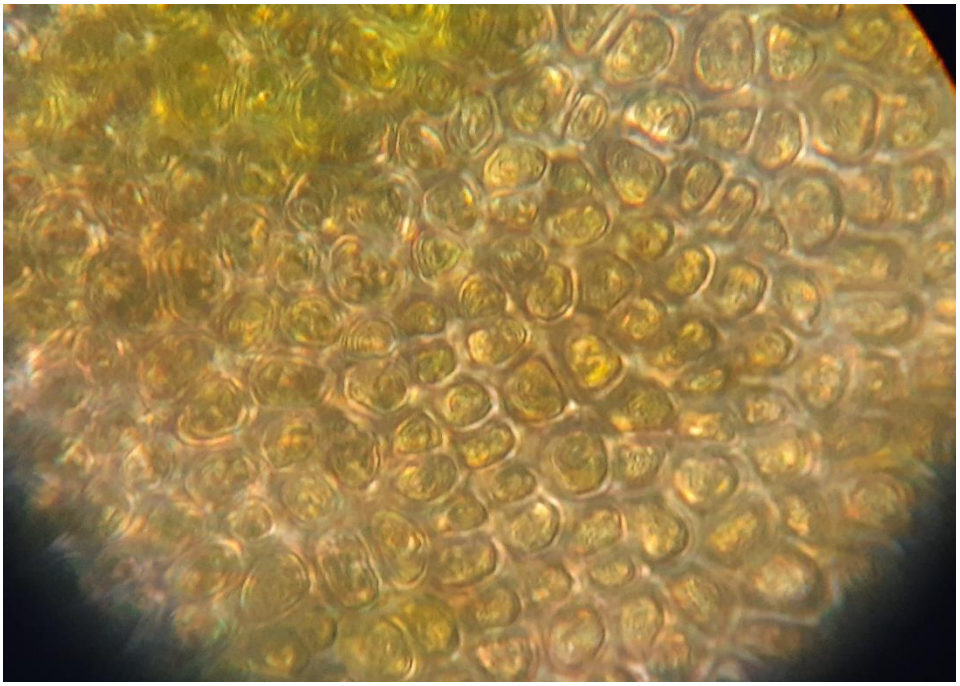


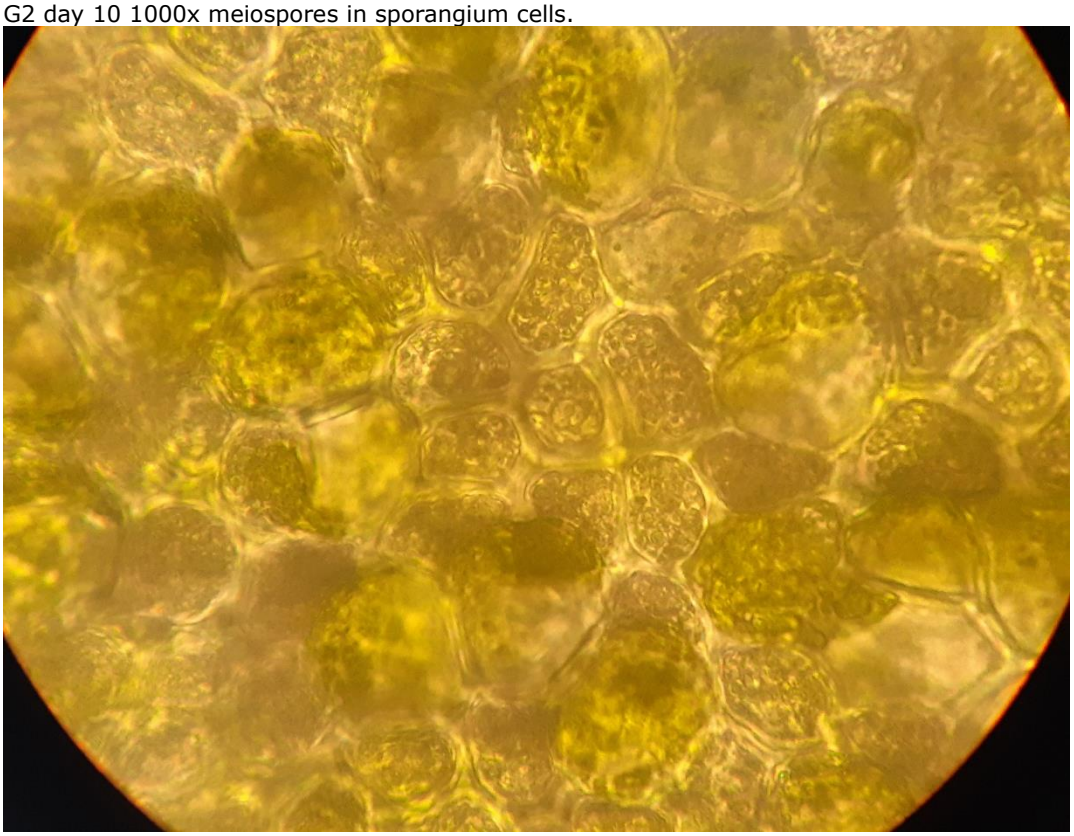
Photo Overview of 5 Ulva populations on day 10: Left Up E2, Next Up Middle G2, Right Up H2, Right Down K2, Left Down L2



E2 day 10 1000x



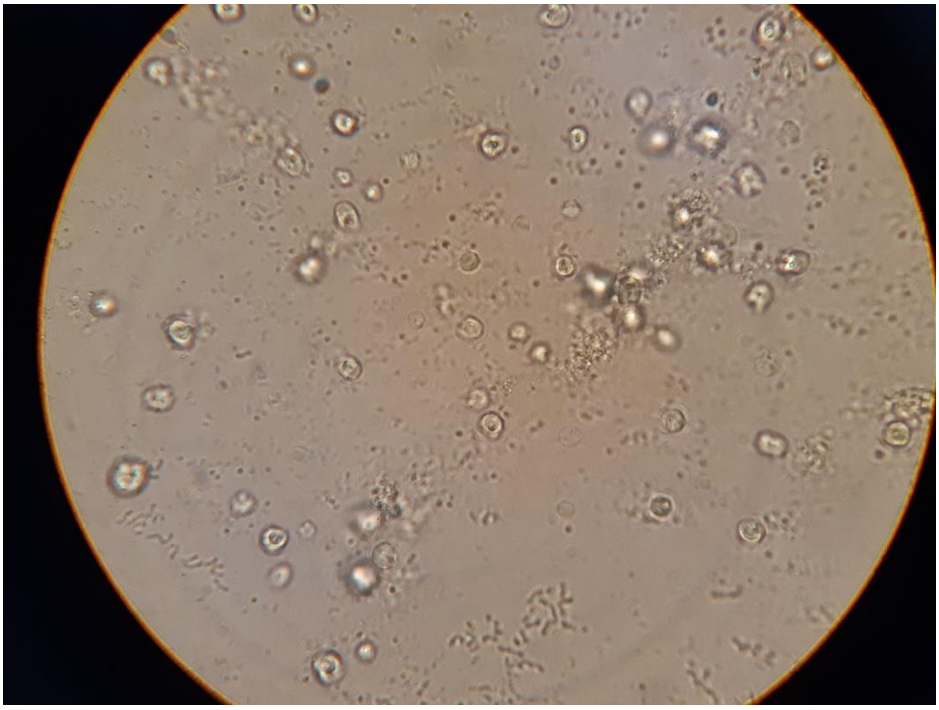
G2 day 10 1000x meiospores in sporangium cells.



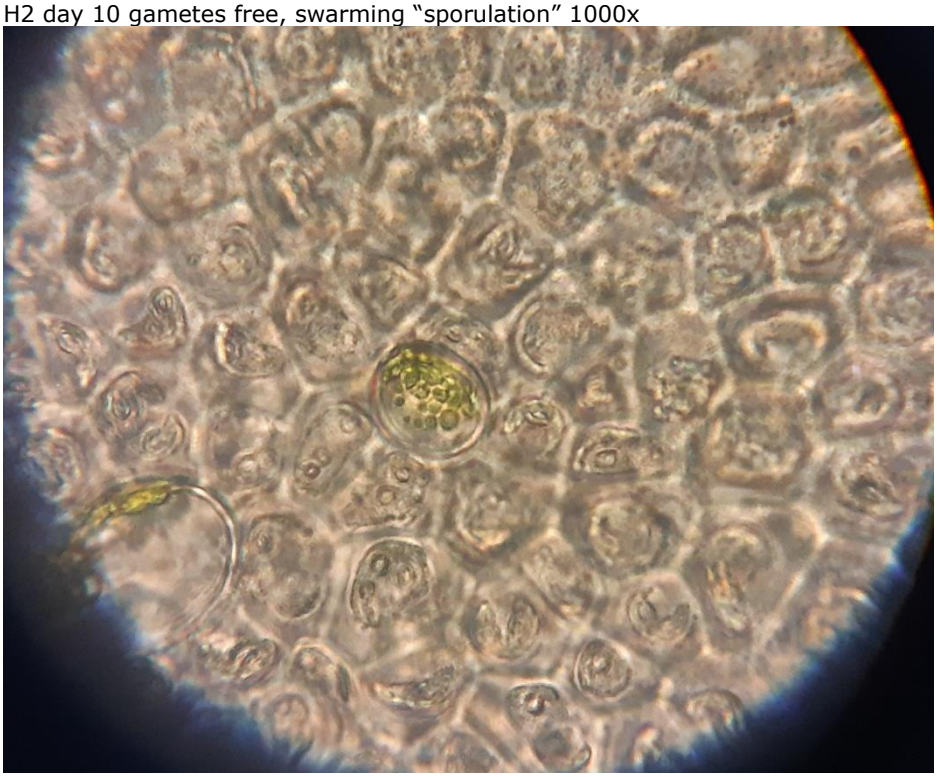
H2 day 10 Cells 1000x gametes in gametangia cells.

20201210_135314.mp4

H2 day 10 gametes free, swarming “sporulation” 1000x



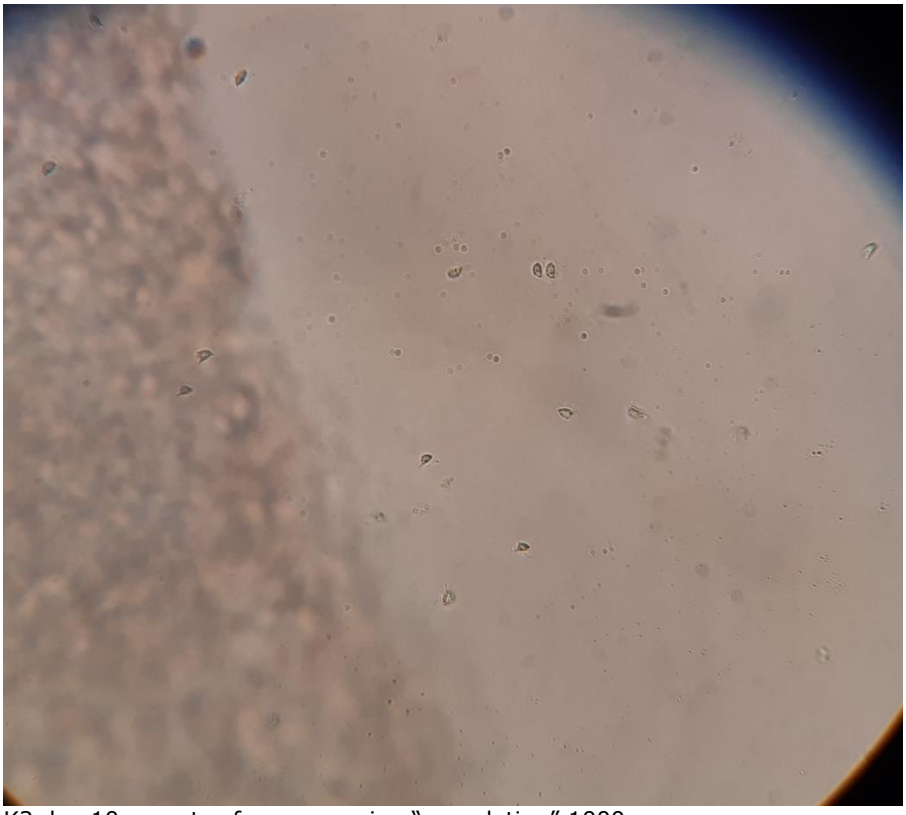
H2 day 10 gametes free, swarming “sporulation” 1000x



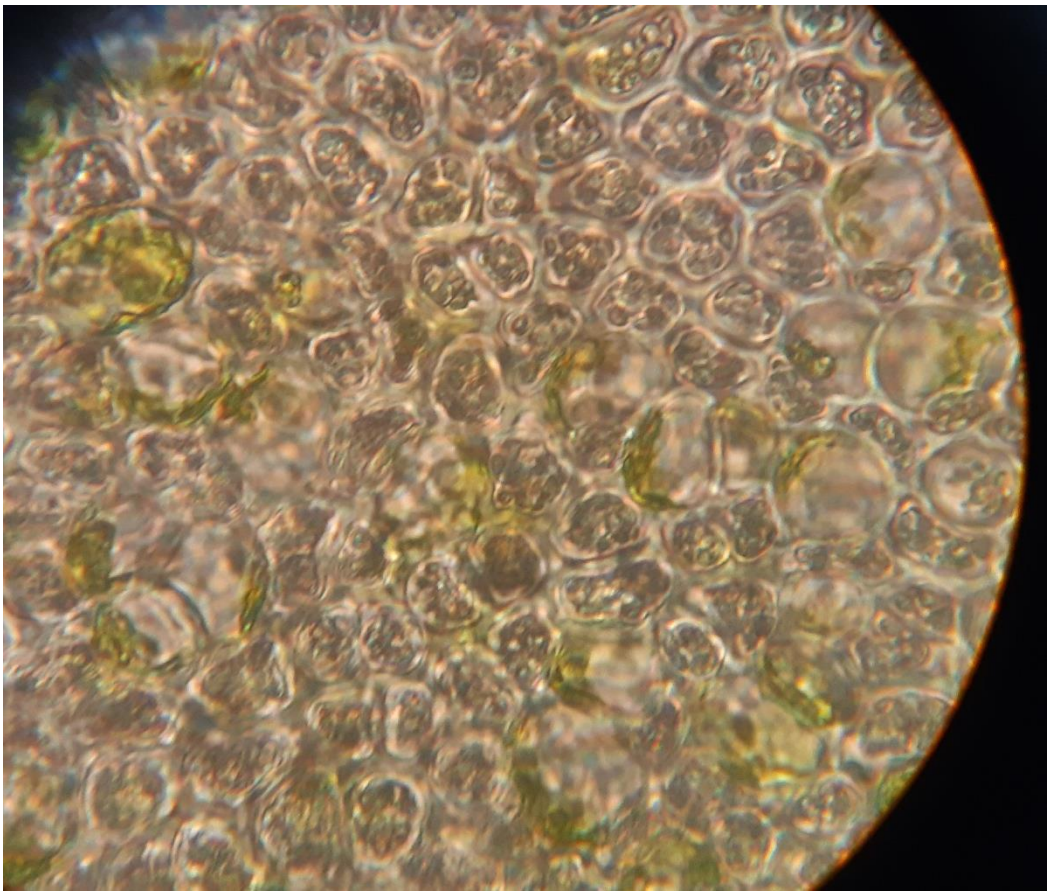
K2 day 10 Cells 1000x gametes in gametangia cells.

20201210_151045.mp4

K2 day 10 gametes free, swarming “sporulation” 1000x



K2 day 10 gametes free, swarming "sporulation" 1000x



L2 day 10 1000x gametes in gametangia cells.

- *On day 11 (11dec2020) judgement on sporulation with microscope 400x and 1000x magnification (see photo's)*

Table 7. Judgement on sporulation on day 11

Code	pop.	Sporulation/ Gametes free on day 11
E2	62	no
G2	60	no
H2	31	yes
K2	64	yes
L2	63	yes

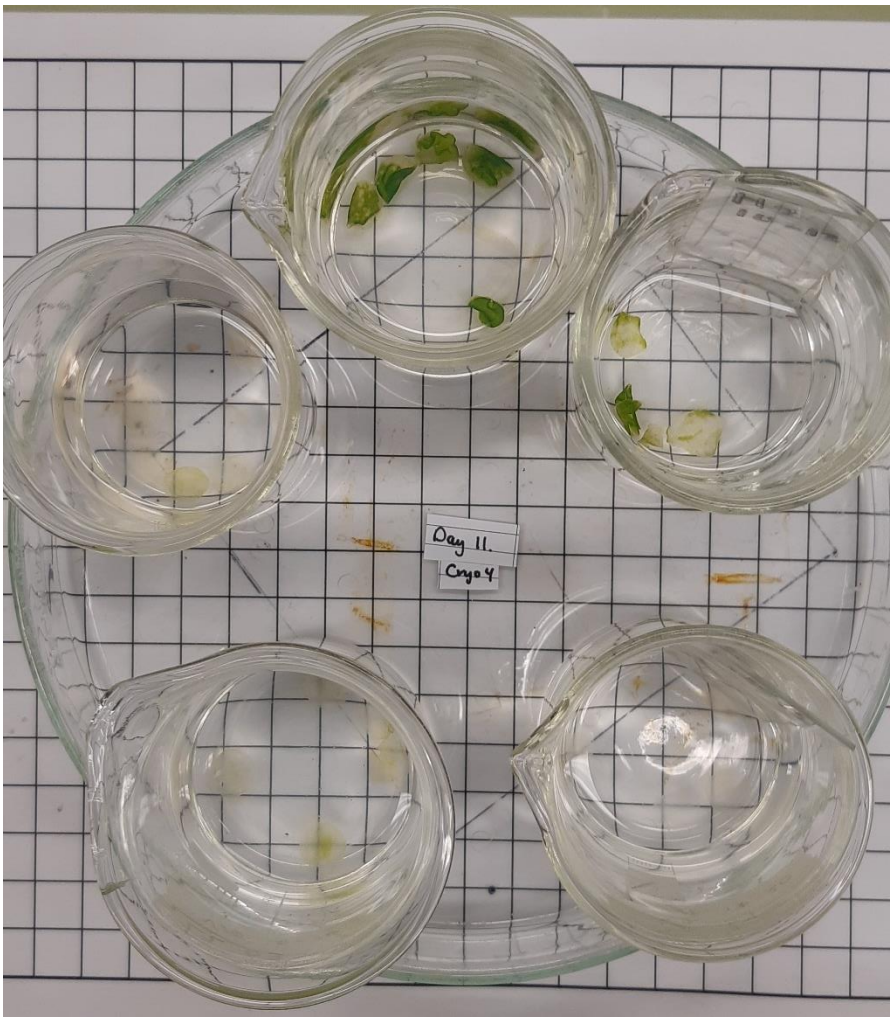
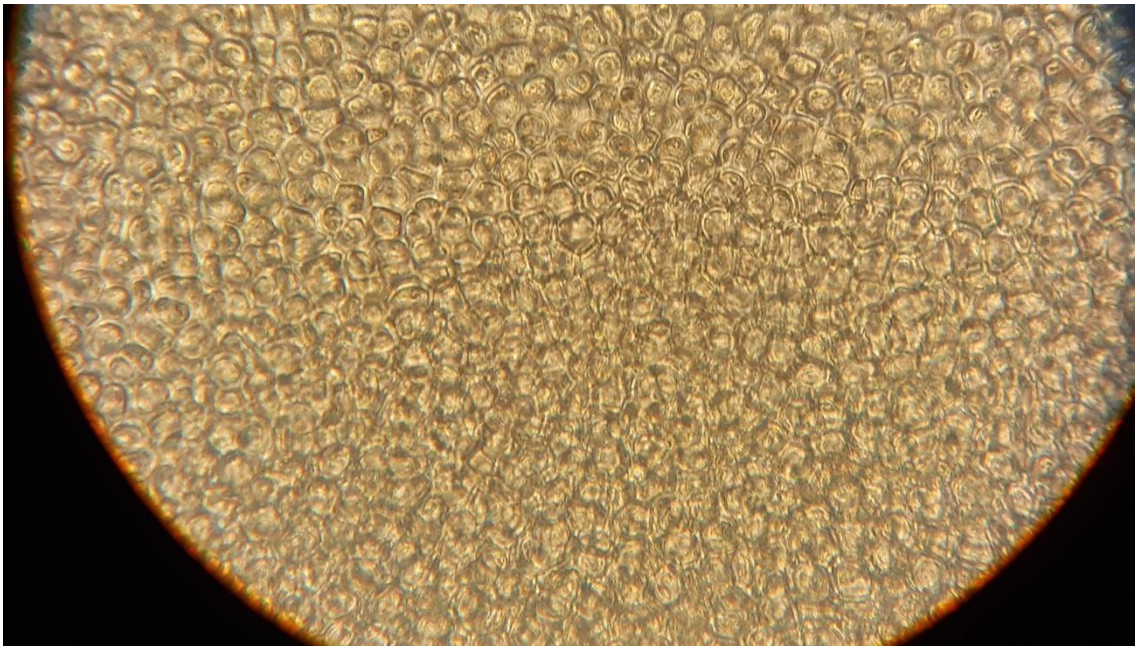
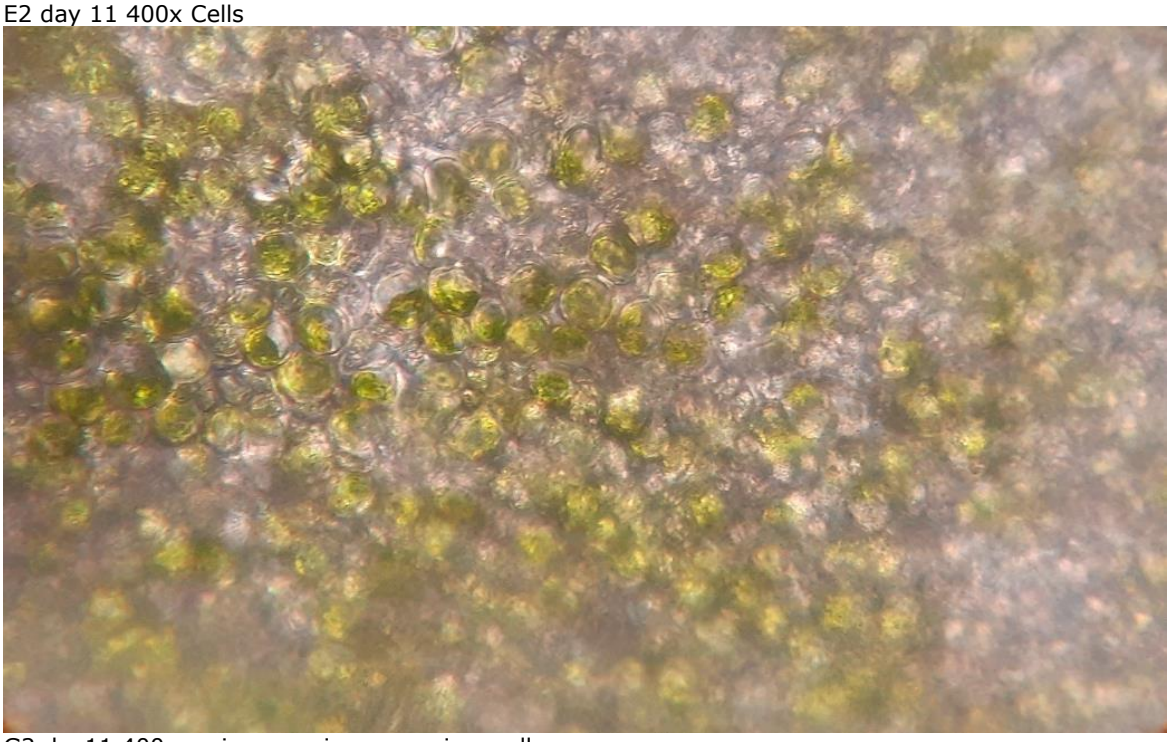


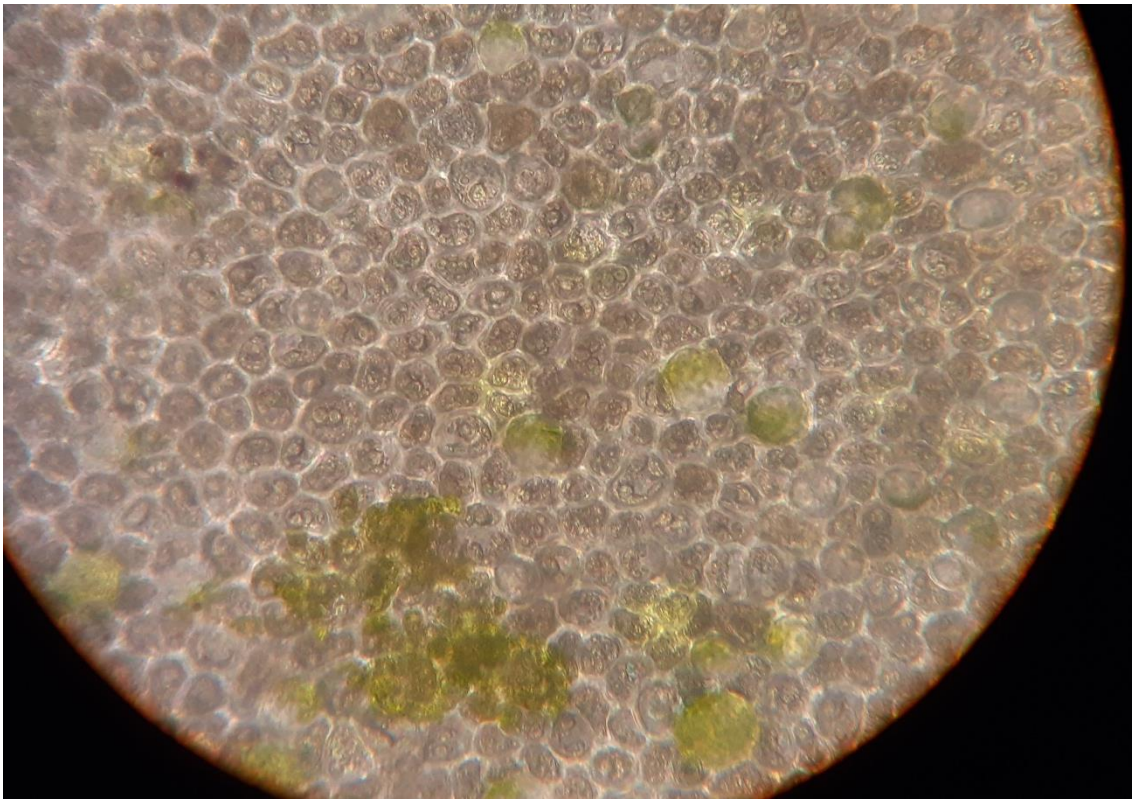
Photo Overview of 5 Ulva populations on day 11: Left Up E2, Next Up Middle G2, Right Up H2, Right Down K2, Left Down L2



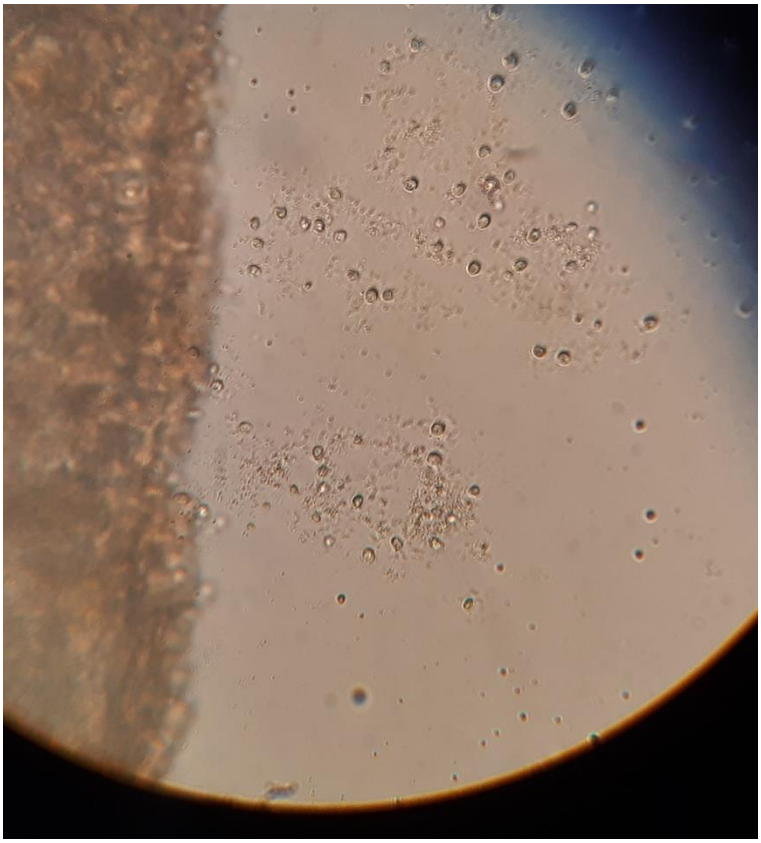
E2 day 11 400x Cells



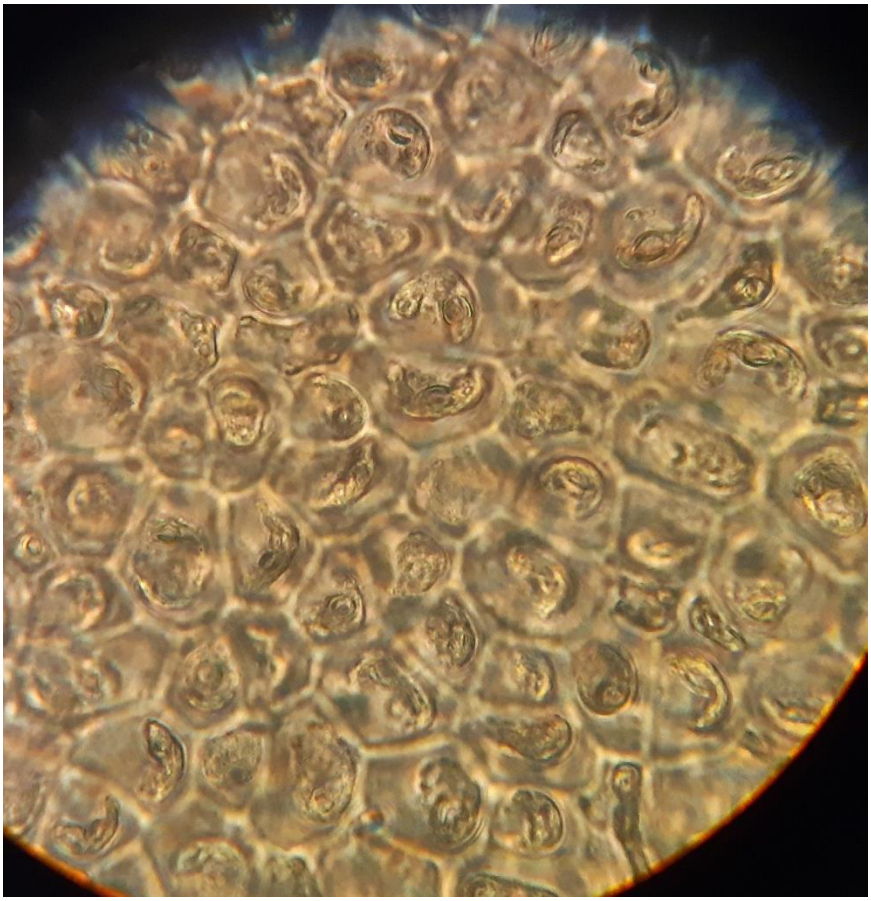
G2 day11 400x meiospores in sporangium cells



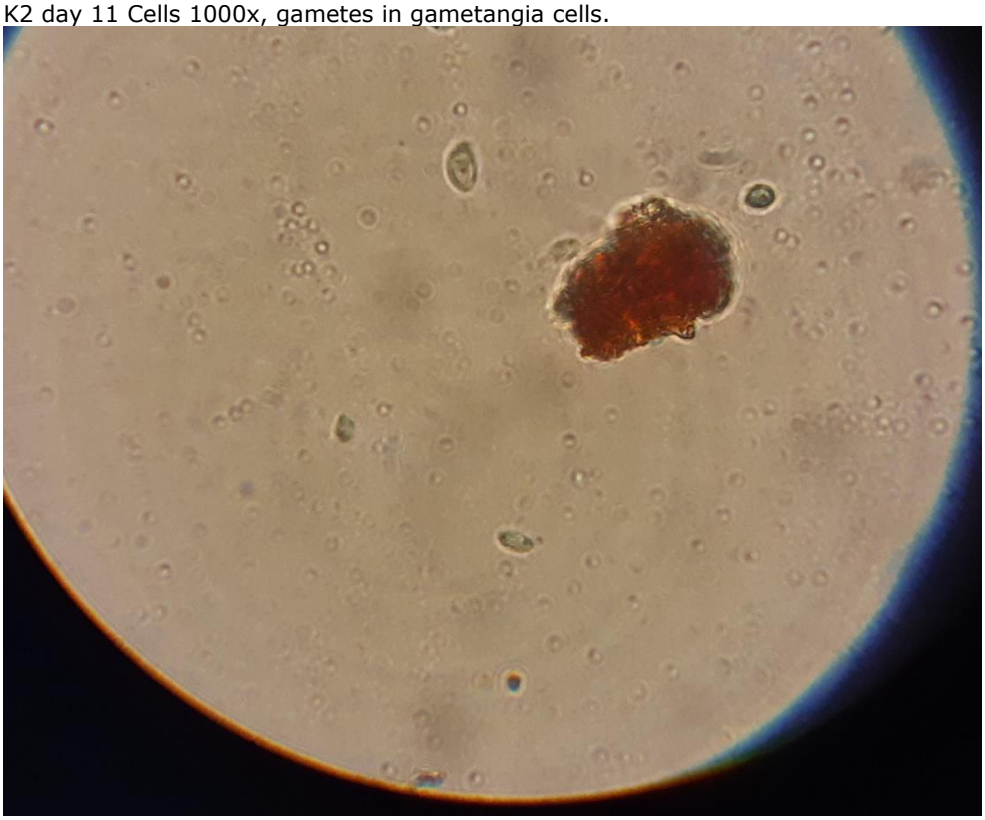
H2 day 11 Cells 400x, gametes in gametangia cells.



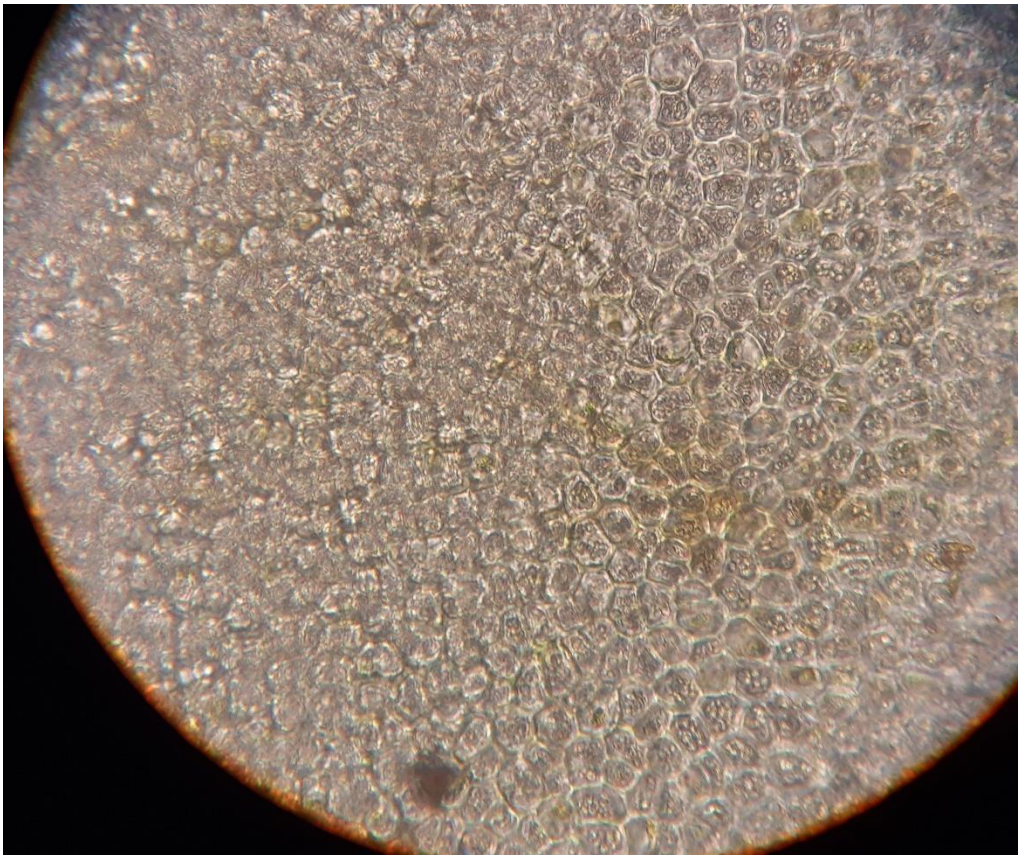
H2 day 11 gametes free, swarming “sporulation” 400x



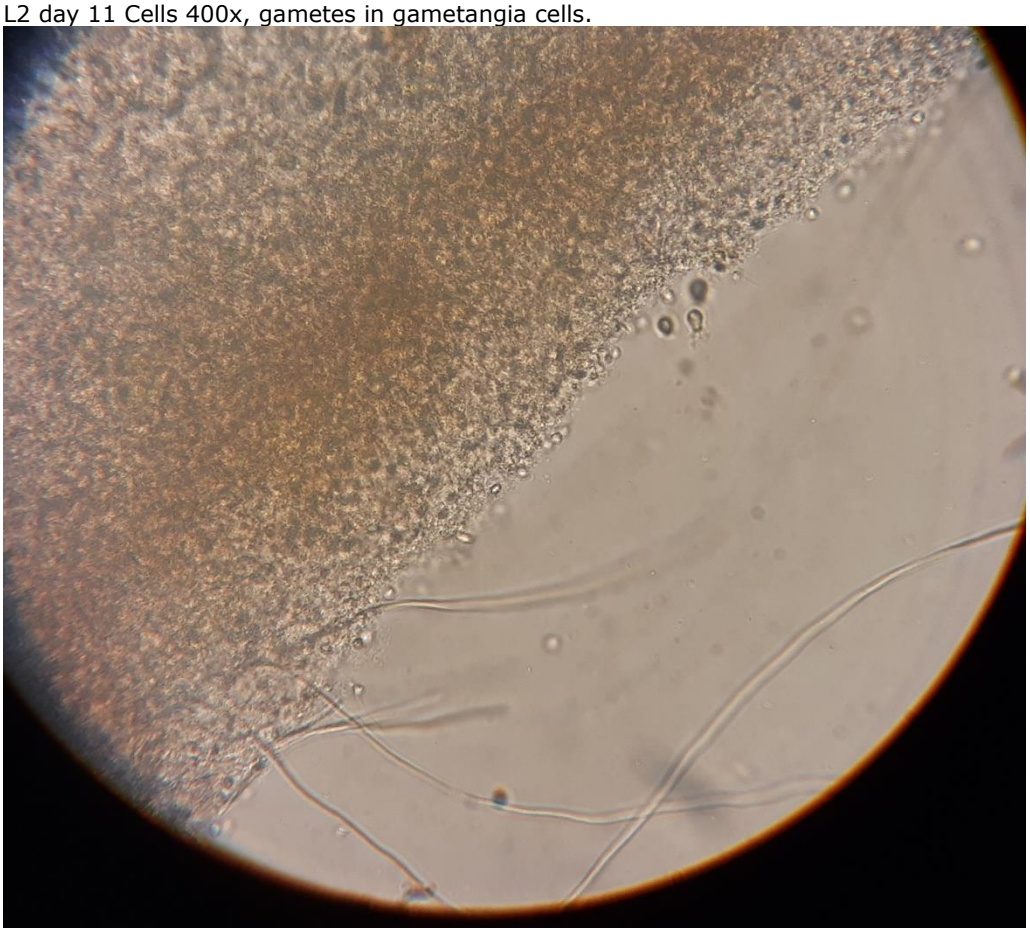
K2 day 11 Cells 1000x, gametes in gametangia cells.



K2 day 11 gametes free, swarming "sporulation" 1000x



L2 day 11 Cells 400x, gametes in gametangia cells.



L2 day 11 Gametes free, swarming 400x

- *On day 14 (14dec2020) judgement on sporulation with microscope 100x, 400x and with microscope 600x magnification with Nikon80i Chairgroup Cell biology Norbert de Rujter (see photo's and video's)*

Table 8. Judgement on sporulation on day 14

Code	pop.	Sporulation/ Gametes frre on day 14
E2	62	no
G2	60	yes
H2	31	yes
K2	64	yes
L2	63	No, see outgrow of gametes

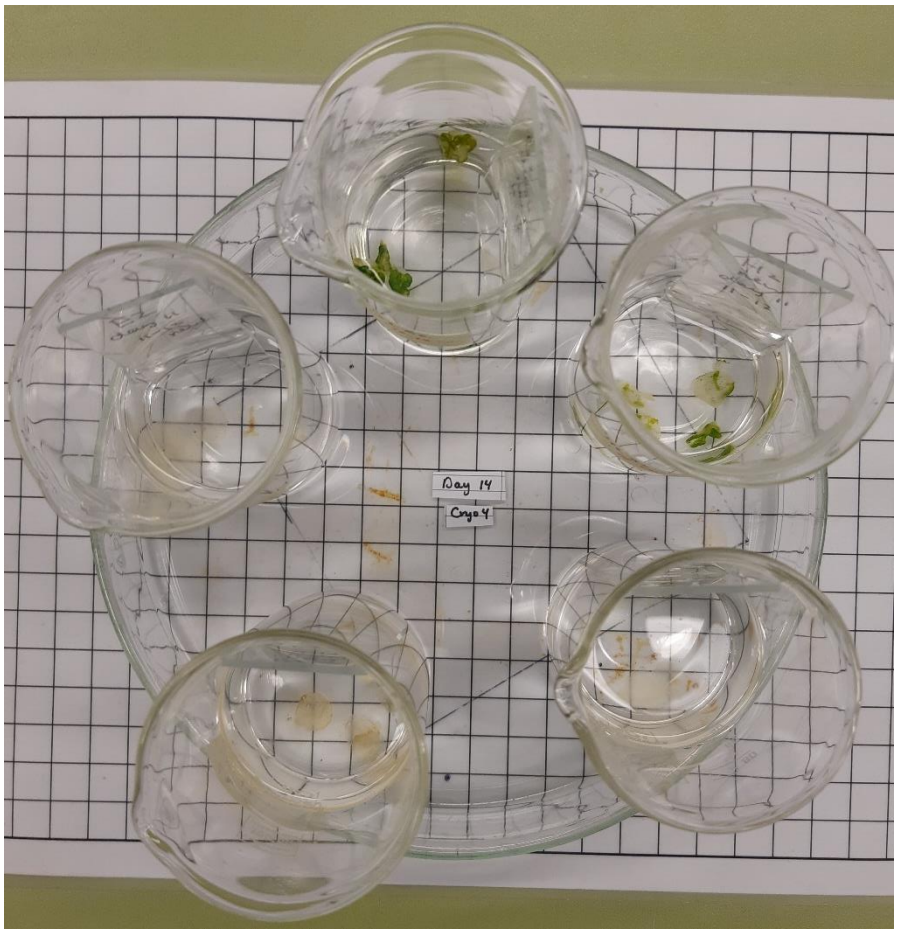
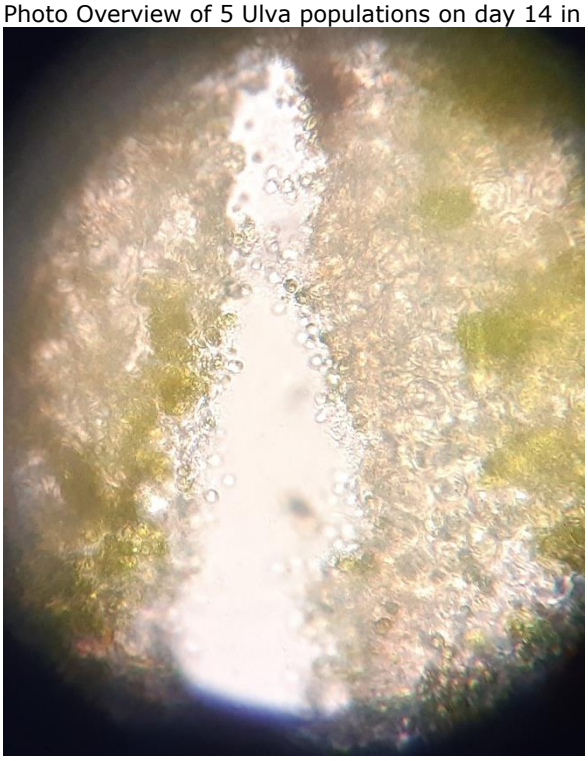
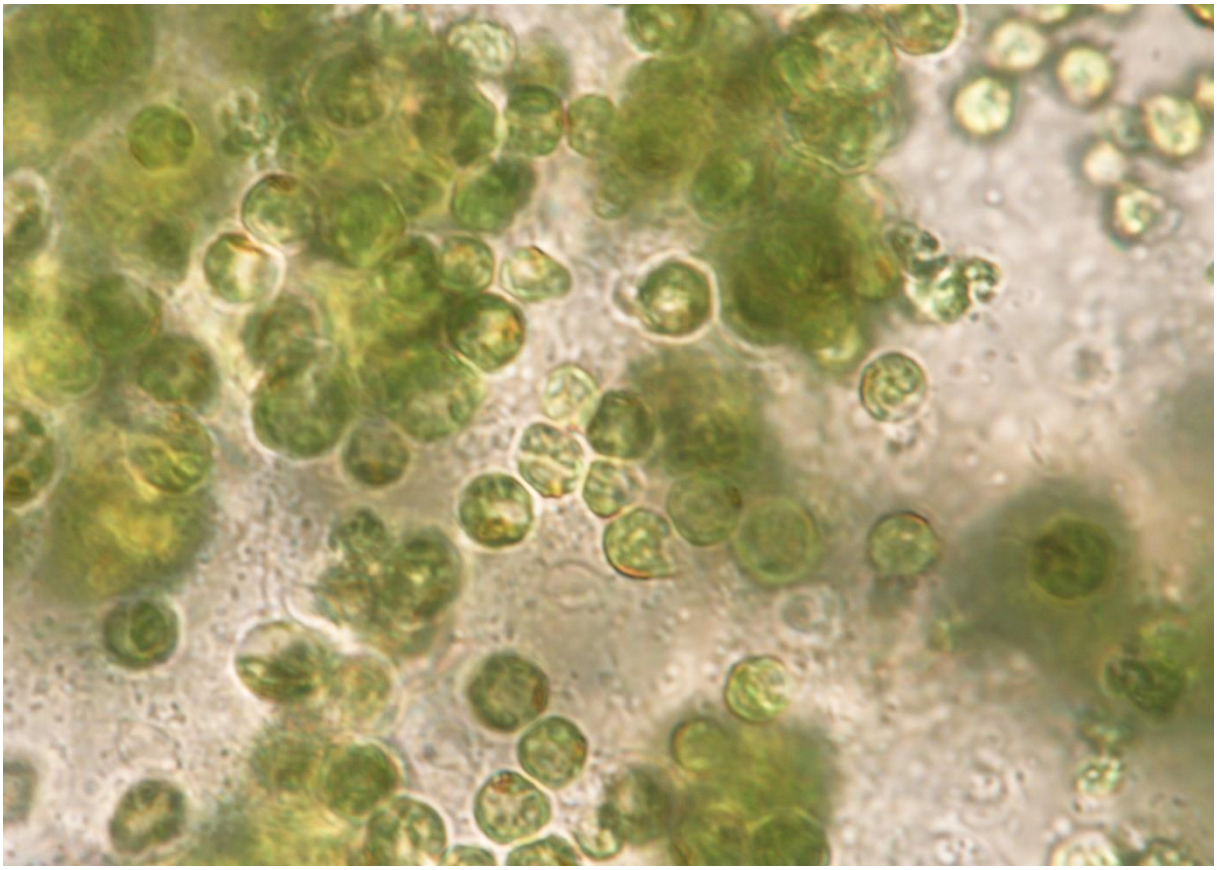


Photo Overview of 5 Ulva populations on day 14: Left Up E2, Next Up Middle G2, Right Up H2, Right Down K2, Left Down L2

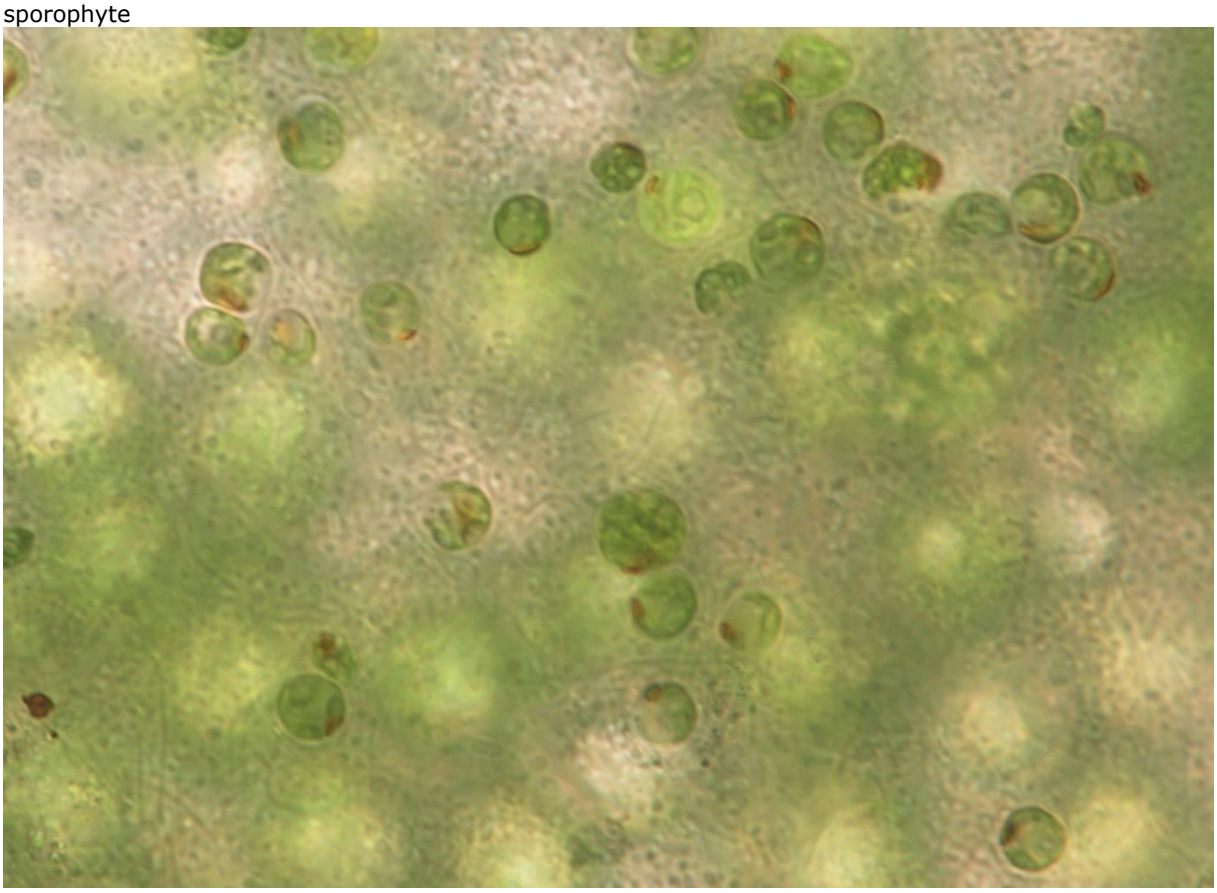
Photo Overview of 5 Ulva populations on day 14 in grow chamber 2



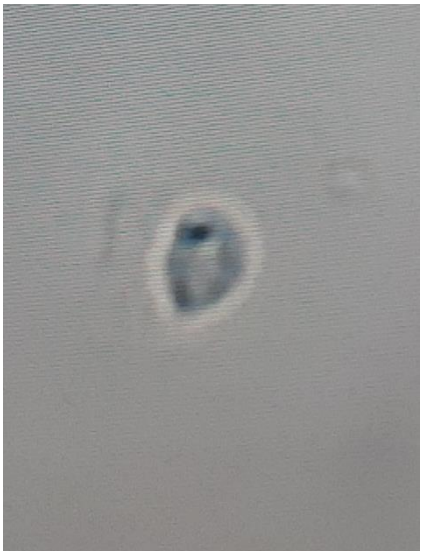
G2 day 14 Sporulation 400x, meiospores.



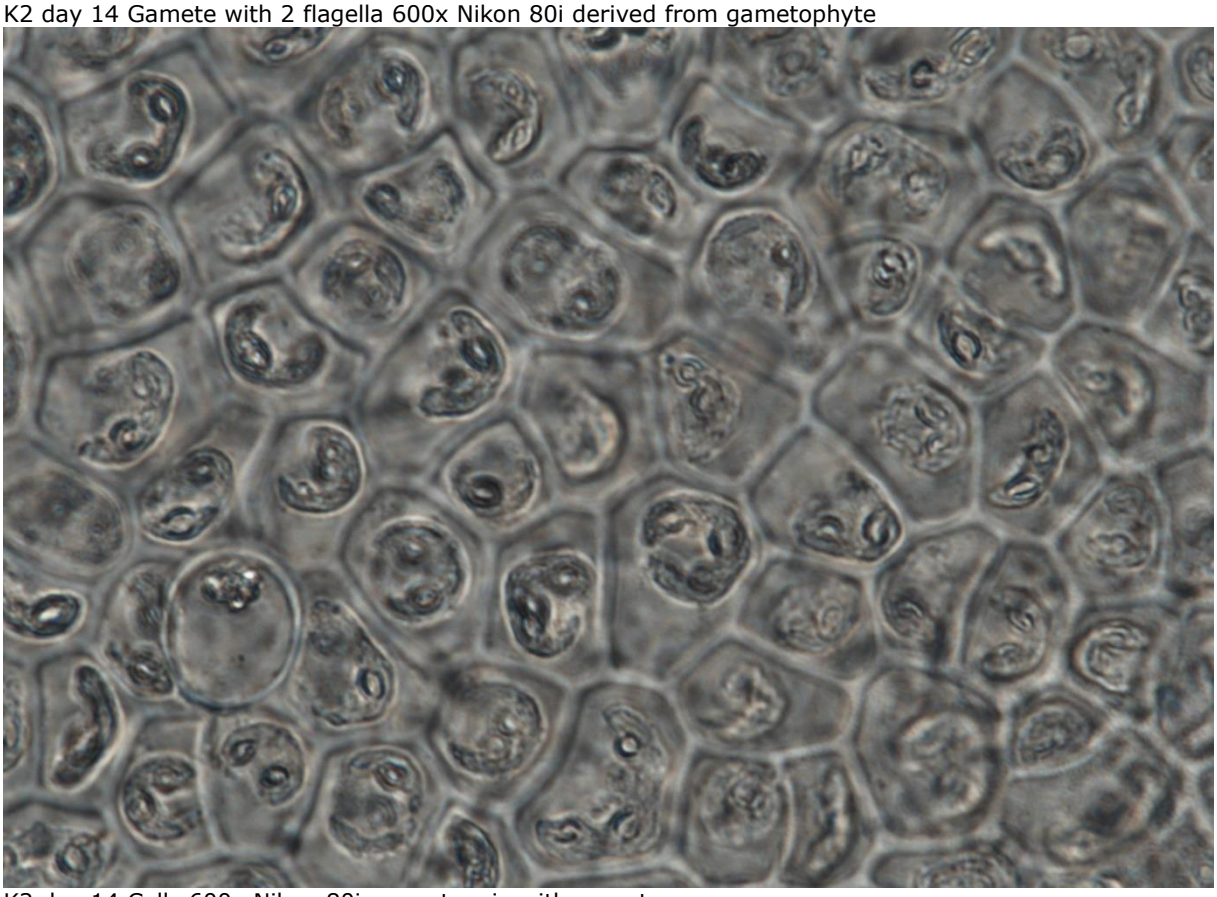
G2 day 14 Sporulation 600x, Nikon 80i, meiospores with stigma (red colour, is light receptor) derived from sporophyte



G2 day 14 Sporulation 600x, Nikon 80i, meiospores with stigma (red colour, is light receptor) derived from sporophyte

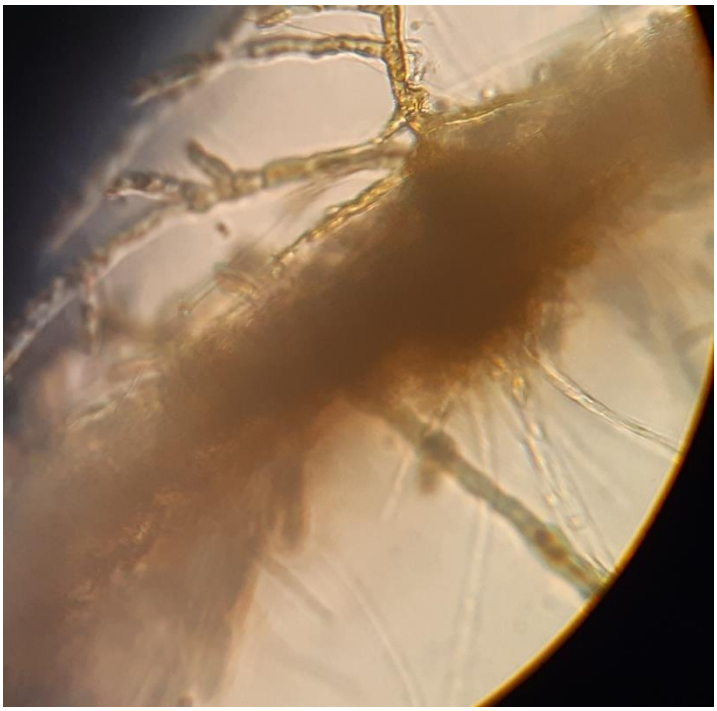


K2 day 14 Gamete with 2 flagella 600x Nikon 80i derived from gametophyte



K2 day 14 Cells 600x Nikon 80i, gametangia with gametes.

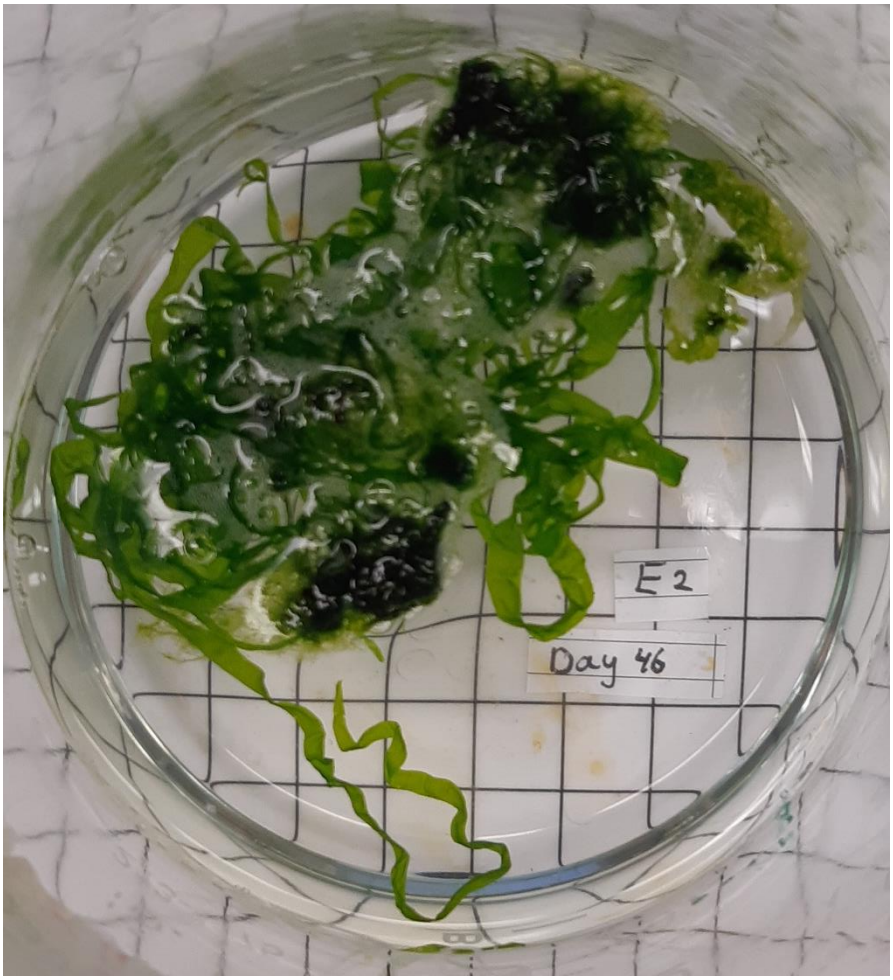
L2 day 14 Outgrow gametes 100x.



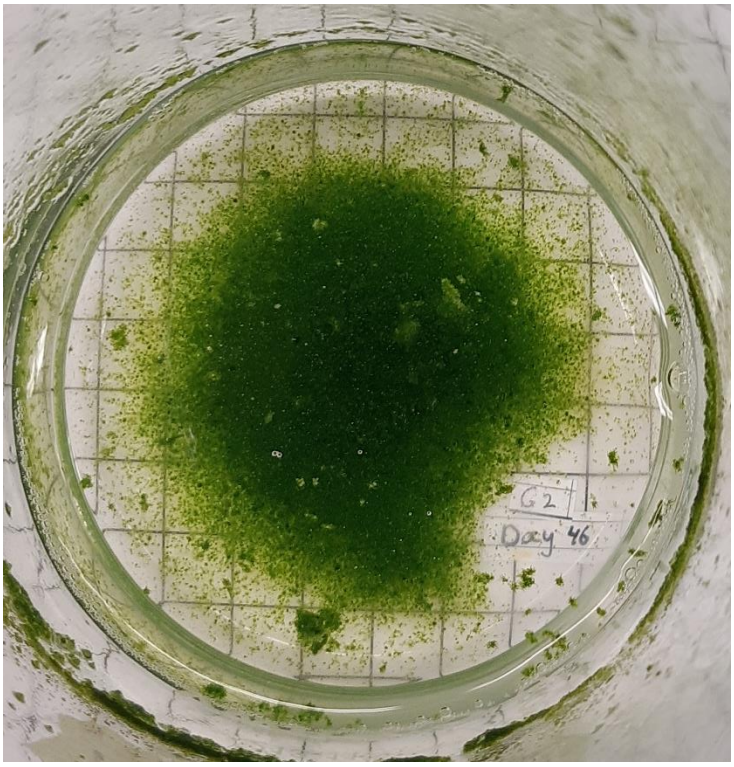
L2 day 14 Outgrow gametes 400x.

L2 day 14 Outgrow gametes 600x Nikon 80i.

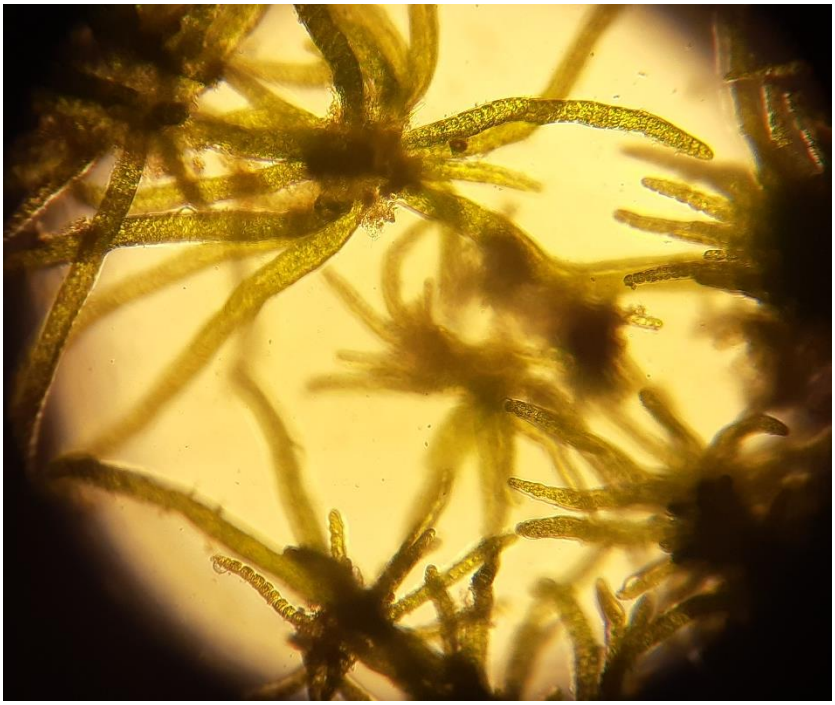
L2 day 14 Outgrow of gametes, 600x Nikon 80i.



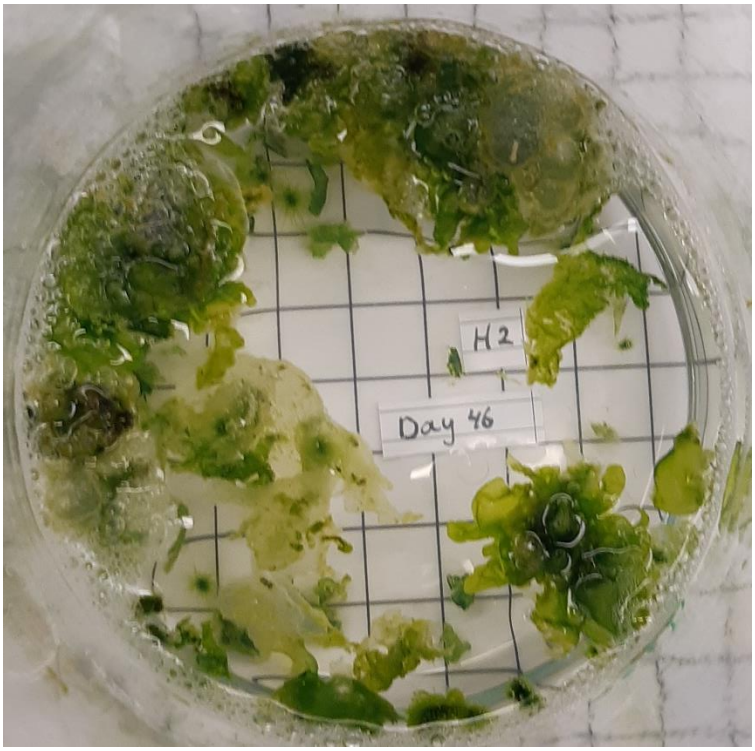
E2 21-01-15 (day 46) outgrow of gametes or meiospores.



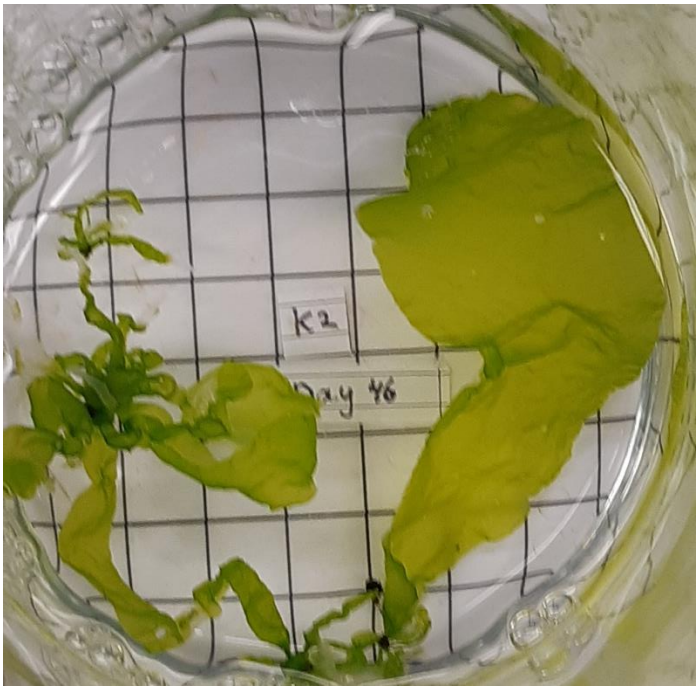
G2 21-01-15 (day 46) outgrow of meiospores.



G2 21-01-15 (day 46) outgrow of meiospores.



H2 21-01-15 (day 46) outgrow of gametes.



K2 21-01-15 (day 46) outgrow of gametes.

L2 21-01-15 (day 46) outgrow of gametes.

Annex 4 Data *Ulva* experiment 5

Data experiment 5

Table 5.1 6 Ulva spp. population in Duplo frost in liquid Nitrogen -196 °C, used in cryo-5-exp_210401, from one or more thallus blades.

Box	Code	pop.	Code GeniAlg	from storage	Date in -196 °C N	Thallus	from one thallus blade
AB1	B2	50	UsZEE191014	6 °C	200120	5 cut discs per vial, Ø 1 cm no. 6	NA
AB2	I1	66	UsHEE_STRAND200605	6 °C	200622	5 cut discs per vial, Ø 1 cm no. 6	yes
AB5	R1	62	UsNEE-2-200605	6 °C	200721	5 cut discs per vial, Ø 1 cm no. 6	yes
AB6	V2	72	UsYER-haven-200727	6 °C	200820	5 cut discs per vial, Ø 1 cm no. 6	yes
AB6	Y2	75	UsKAMP-pontje_200727	6 °C	200820	5 cut discs per vial, Ø 1 cm no. 6	yes
AB8	FA1	60	UsZUI200207	6 °C	210218	5 cut discs per vial, Ø 1 cm no. 6	no

Table 5.2 6 Ulva spp. population 5 discs per vial, in Duplo frost in liquid Nitrogen -196 °C

Code	pop.	Code GeniAlg	Cryopreservation_FreezeControl	same location as used in	Duplo of
B2	50	UsZEE191014	PROTO2		Cryo-1-exp_20-2-04
I1	66	UsHEE_STRAND200605	PROTO2	Cryo-1-exp_20-2-04	
R1	62	UsNEE-2-200605	PROTO2	Cryo-3-exp & Cryo-4-exp	
V2	72	UsYER-haven-200727	PROTO2	Cryo-3-exp & Cryo-4-exp	Cryo-2-exp_20-9-14
Y2	75	UsKAMP-pontje_200727	PROTO2	Cryo-3-exp & Cryo-4-exp	Cryo-2-exp_20-9-14
FA1	60	UsZUI200207	PROTO2	Cryo-3-exp & Cryo-4-exp	

Table 5.3 Fresh Weight (FW) of 6 Ulva spp cryopreserved, after 46 days of regrowth.

Code	pop.	Code GeniAlg	FW (g) day46
B2	50	UsZEE191014	3.043
I1	66	UsHEE_STRAND200605	3.396
R1	62	UsNEE-2-200605	3.676
V2	72	UsYER-haven-200727	4.543
Y2	75	UsKAMP-pontje_200727	8.905
FA1	60	UsZUI200207	5.193

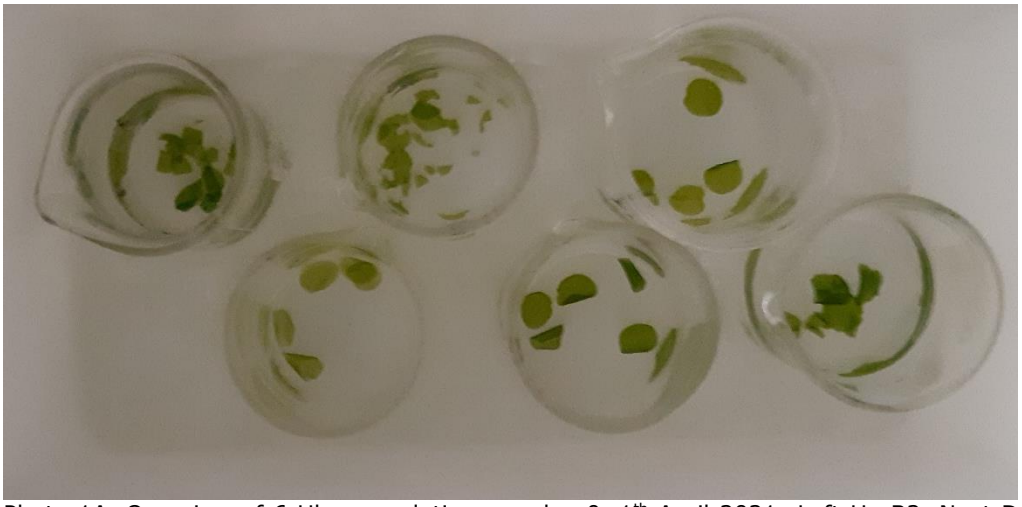


Photo 1A. Overview of 6 Ulva populations on day 0, 1th April 2021: Left Up B2, Next Down I1, Next Up R1, Next Down V2, Next Up Y2, Next Down FA1.

Photo 1B. Overview of 6 Ulva populations on day 0, 1th April 2021: Left Up B2, Next Down I1, Next Up R1, Next Down V2, Next Up Y2, Next Down FA1.

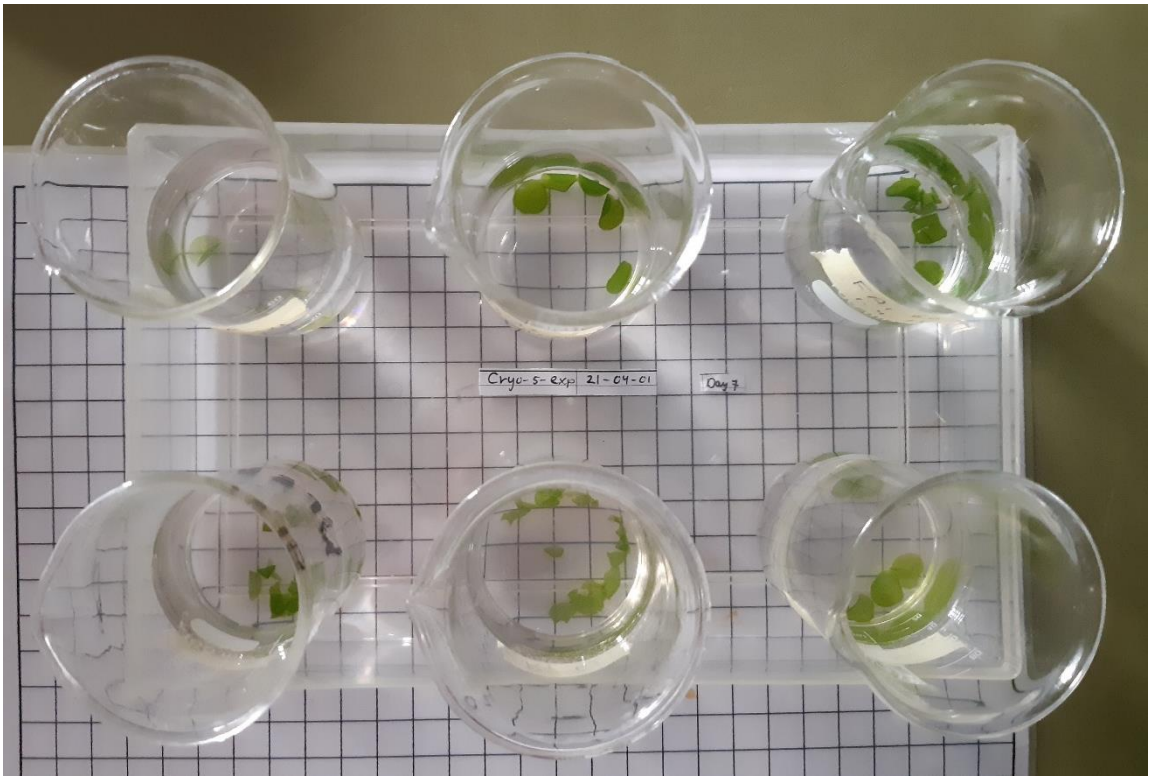


Photo 2 Overview of 6 Ulva populations on day 7, 8th April 2021: Left Down B2, Left Up I1, Middle Down R1, Middle Up V2, Right Down Y2, Right Up FA1.

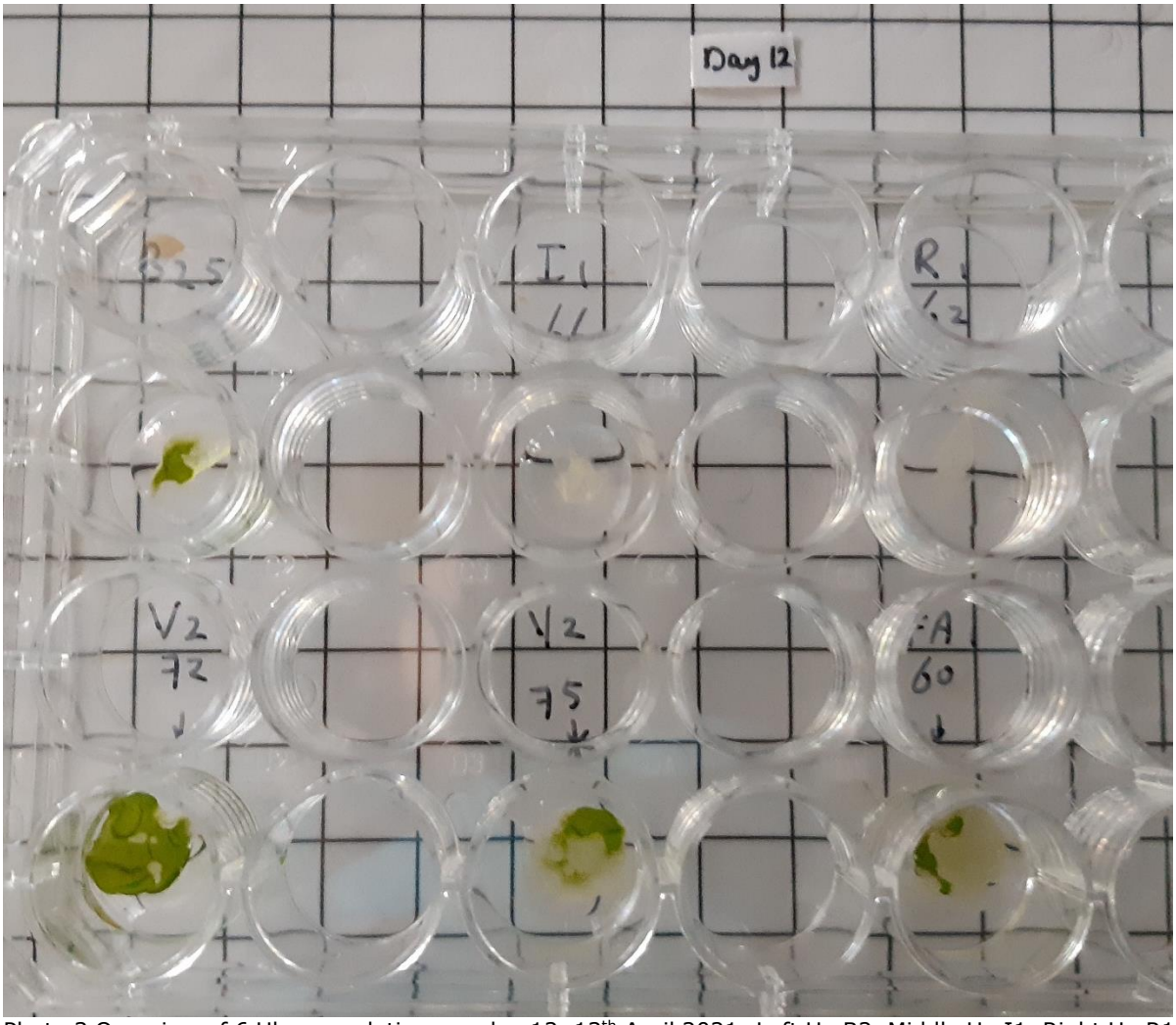


Photo 3 Overview of 6 Ulva populations on day 12, 13th April 2021: Left Up B2, Middle Up I1, Right Up R1, Left Down V2, Middle Down Y2, Right Down FA1.

- *On day 8 (9apr2021) judgement on sporulation with microscope 400x magnification (see photos)*

Table 5.4 Judgement on sporulation on day 8, 9th April 2021

Code	pop.	Sporulation / Gametes free on day 8
B2	50	no
I1	66	no
R1	62	no
V2	72	no
Y2	75	no
FA1	60	no

- *On day 11 (12apr2021) judgement on sporulation with microscope 100 x 200 x 400x magnification (see photos and video's)*

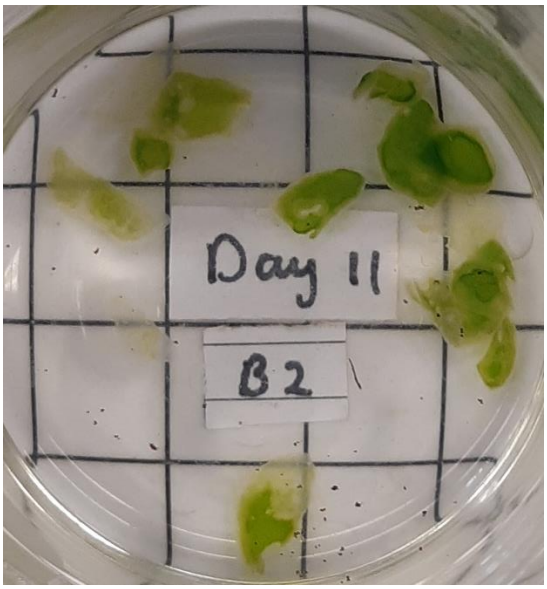
Table 5.5 Judgement on sporulation on day 11, 12th April 2021

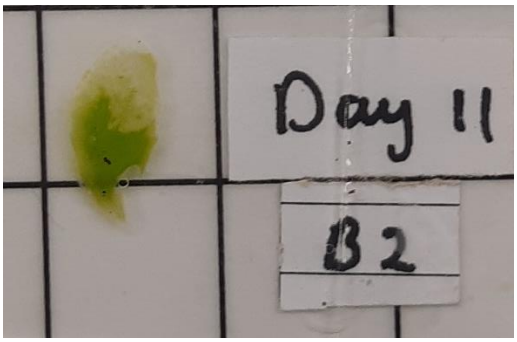
Code	pop.	Sporulation / Gametes free on day 11
B2	50	Yes, gametes, some moves
I1	66	Thallus has sporulated probably earlier, attached gametes or spores on pale thallus
R1	62	Thallus has sporulated probably earlier, attached gametes or spores on pale thallus
V2	72	Yes, lots of moving gametes
Y2	75	No
FA1	60	No

- *On day 12 (14dec2020) judgement on sporulation with microscope 200x 400x 600x magnification with Nikon80i Chair group Cell biology Norbert de Rujter (see photos)*

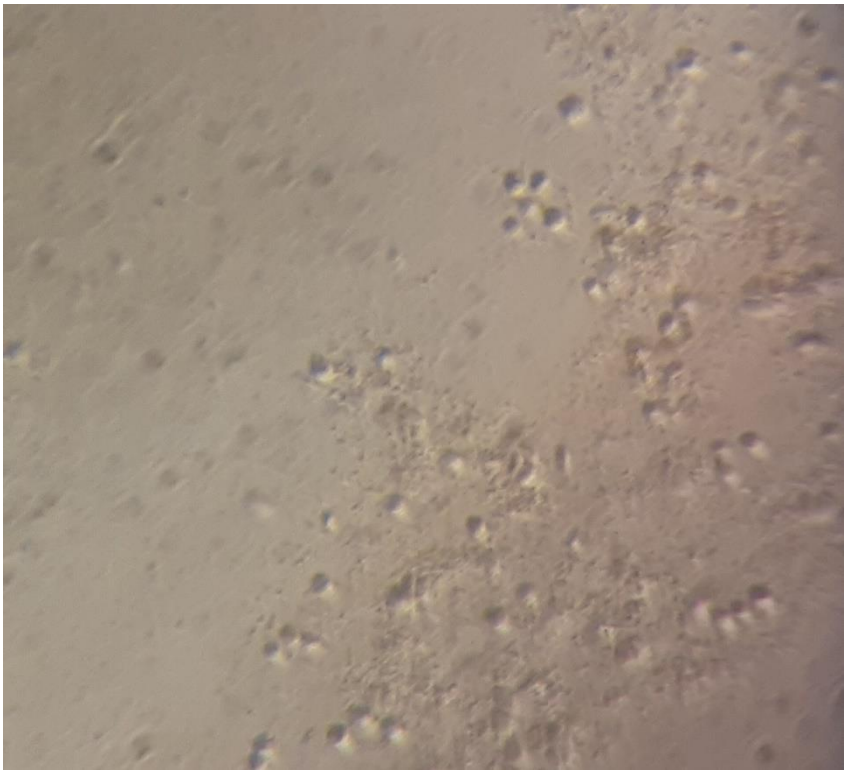
Table 5.6 Judgement on sporulation on day 12, 13th April 2021

Code	pop.	Sporulation / Gametes free on day 11
B2	50	Yes, probably gametes, lots of moving 'gametes'
I1	66	Thallus has sporulated, no moving gametes or spores
R1	62	Thallus is pale, no moving gametes or spores
V2	72	Yes, lots of moving gametes
Y2	75	No
FA1	60	No

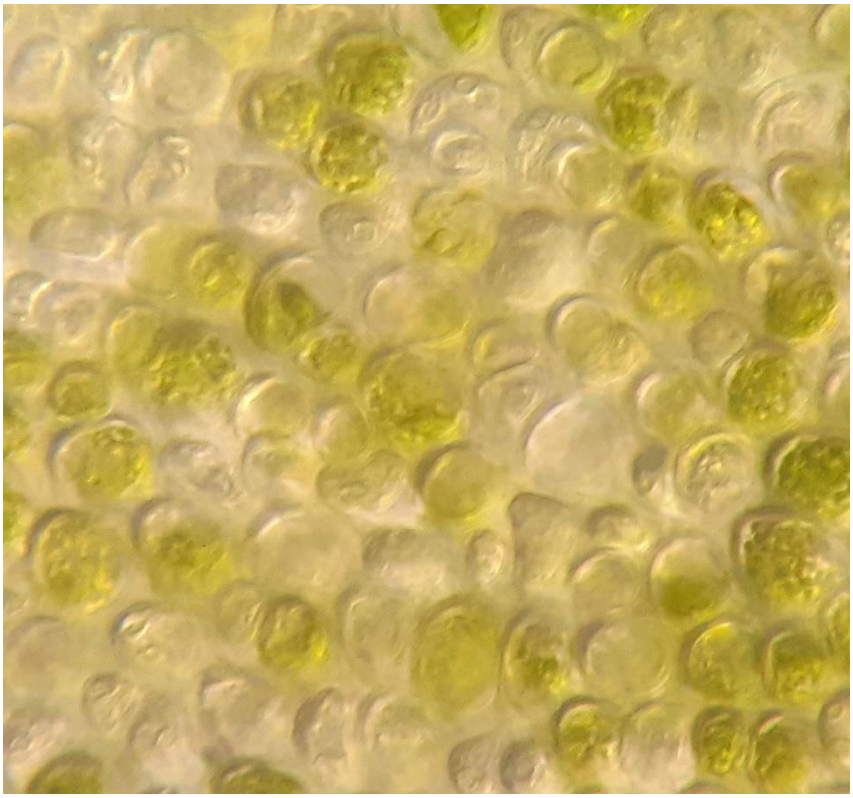




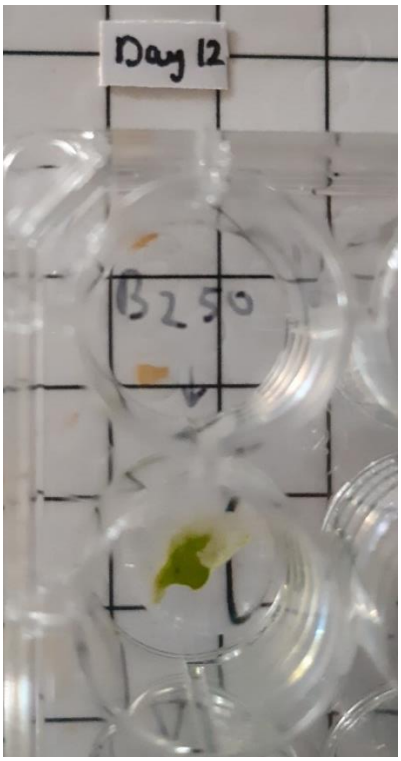
B2 21-04-12 (day 11) overview and one detail thallus, used for microscope



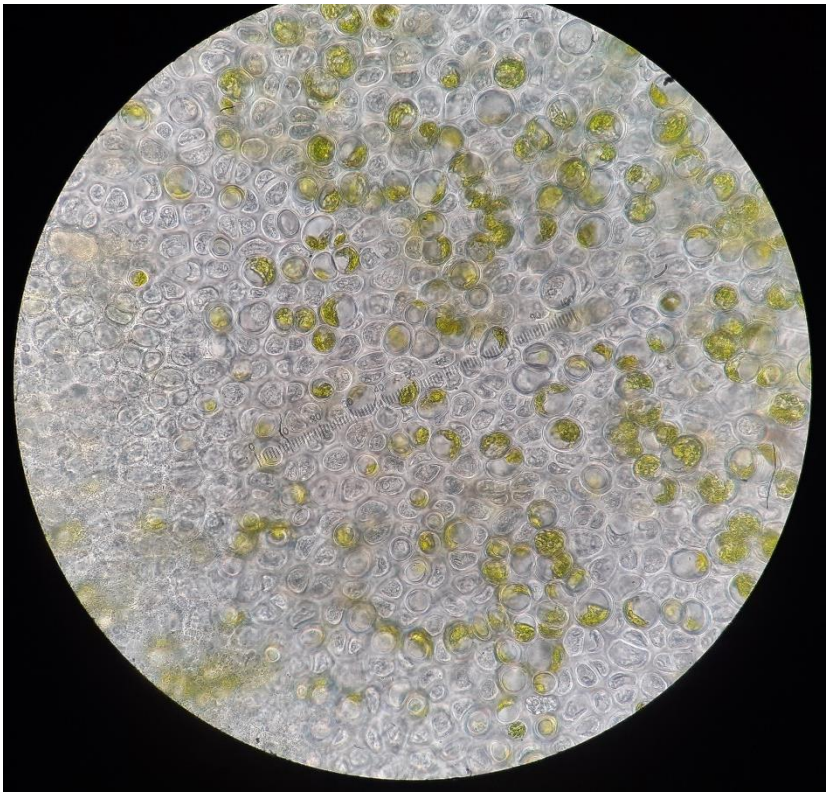
B2 21-04-12 (day 11) Gametes, stationary and some moving 400x



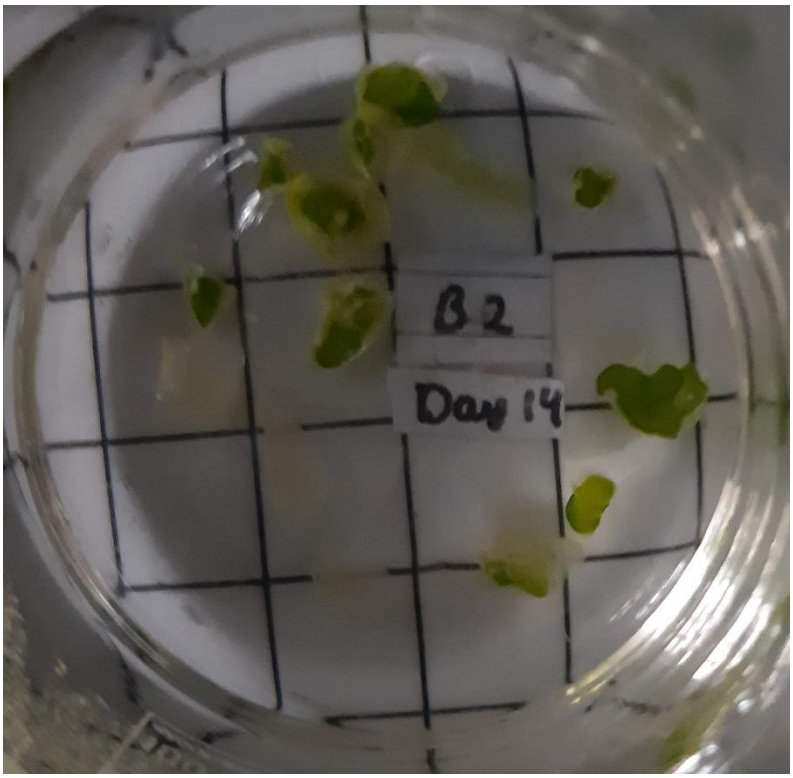
B2 21-04-12 (day 11) Thallus tissue 400x



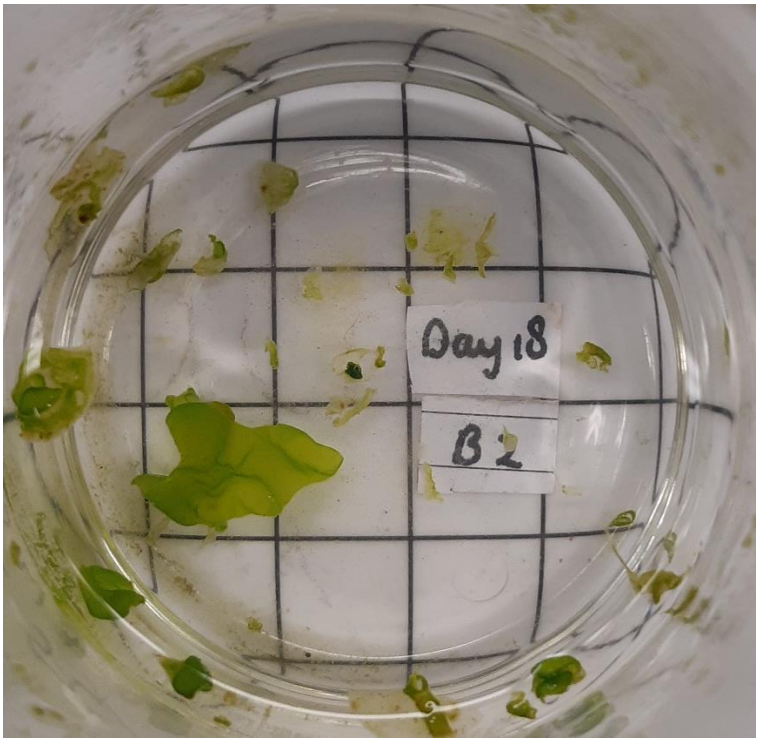
B2 21-04-13 (day 12) One detail thallus, used for microscope



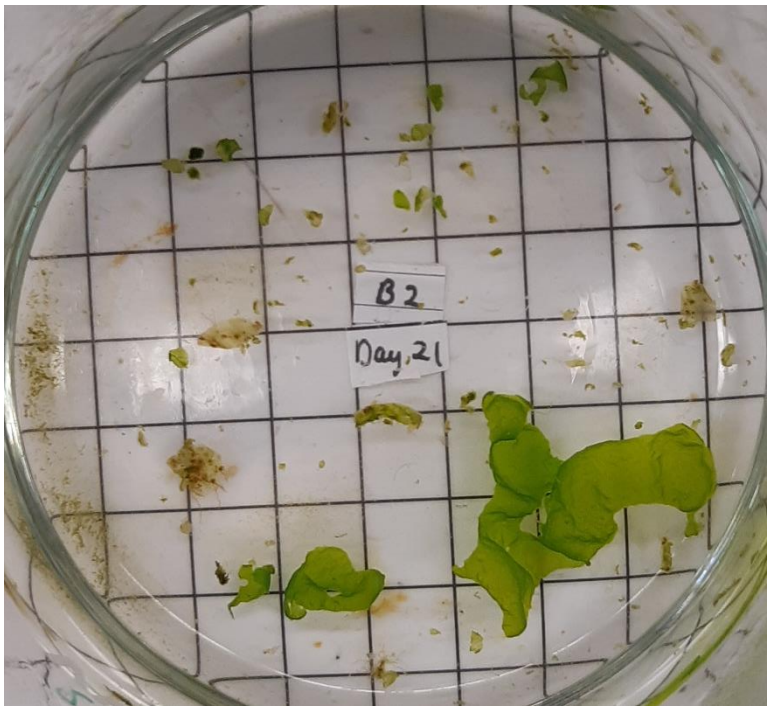
B2 21-04-13 (day 12) Thallus tissue 600x, probably gametes, a lot moves



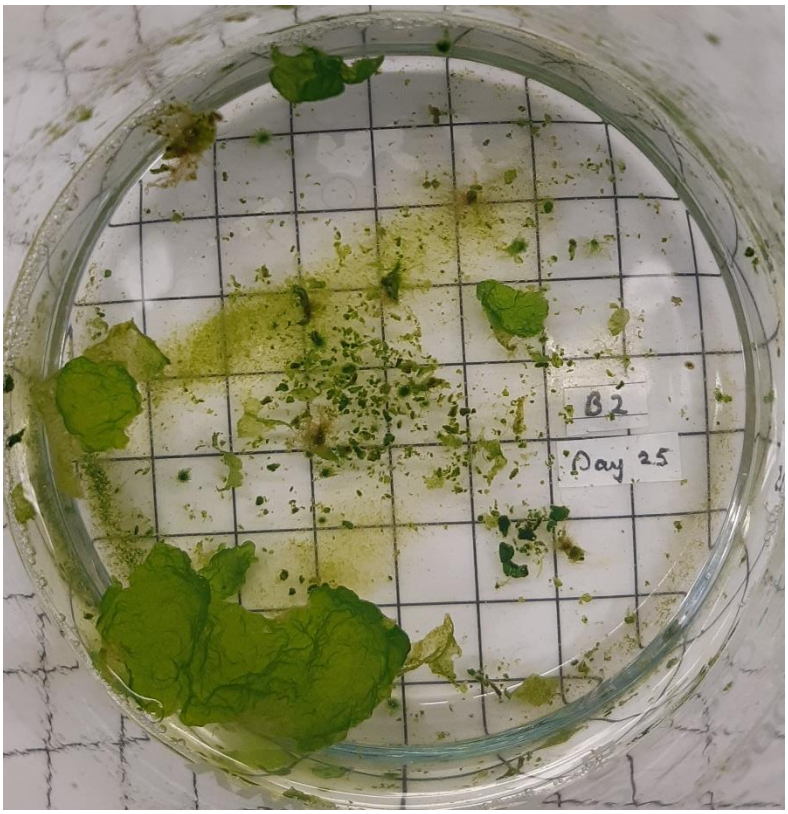
B2 21-04-15 (day 14) overview



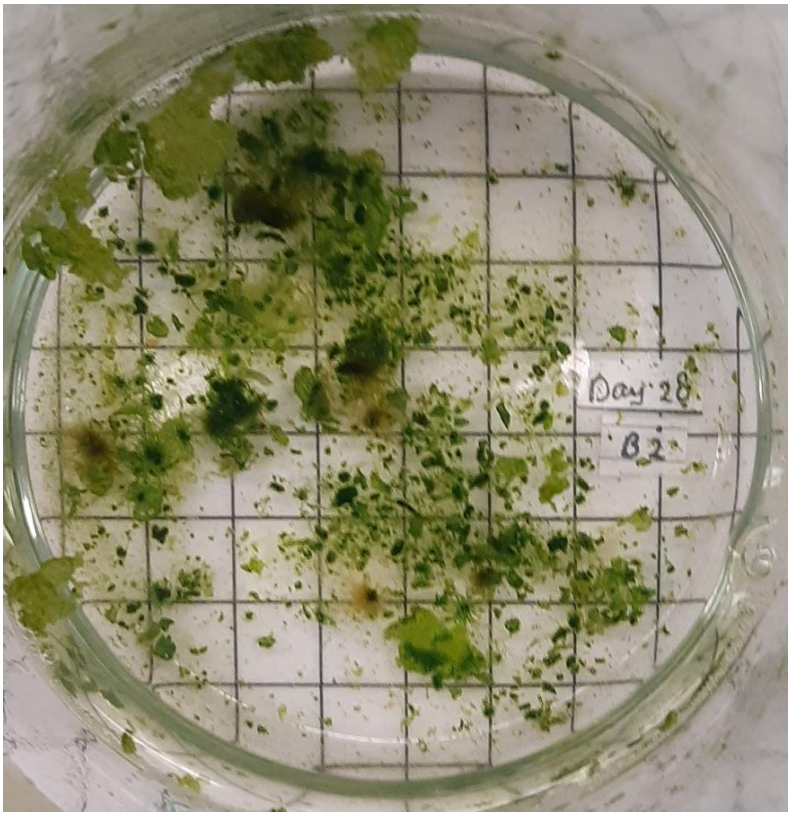
B2 21-04-19 (day 18) overview



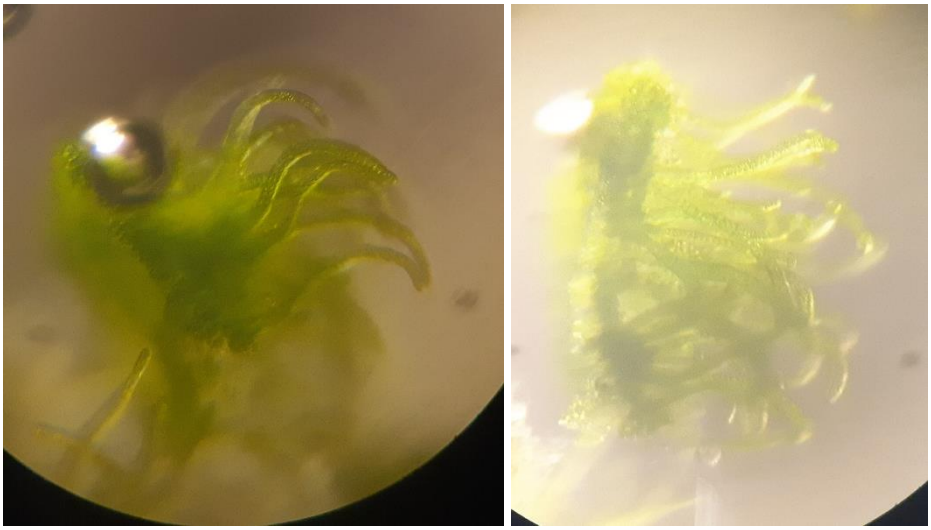
B2 21-04-22 (day 21) overview



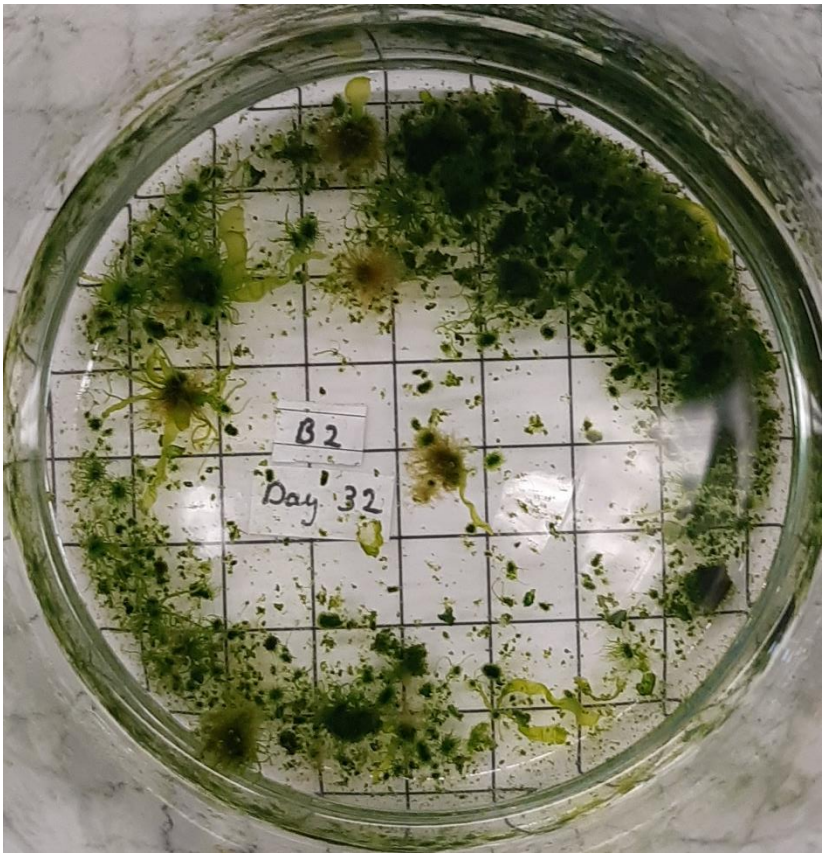
B2 21-04-26 (day 25) overview



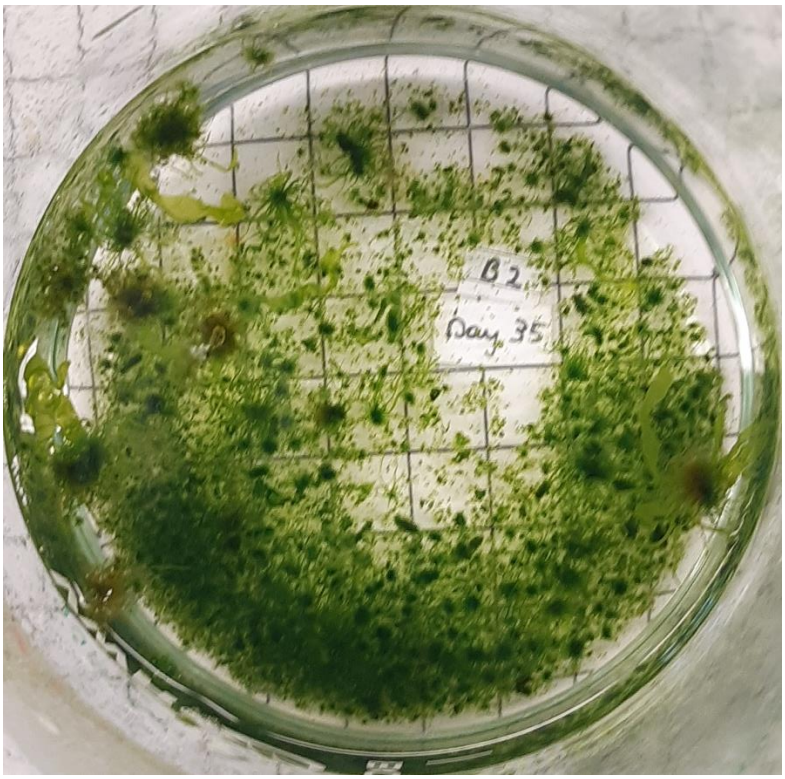
B2 21-04-29 (day 28) overview, outgrow of gametes in the form of a lot of thallus‘spiders’



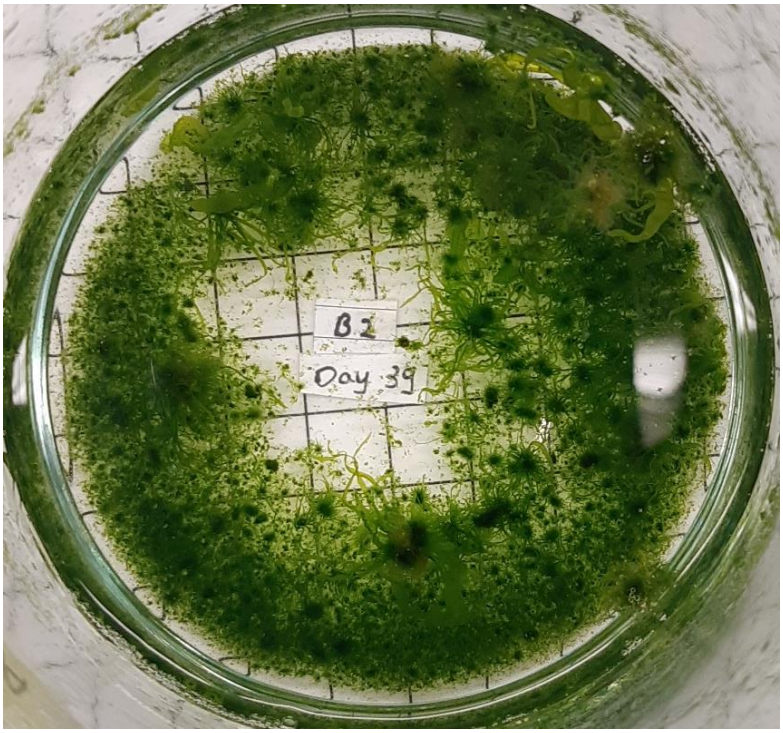
B2 21-04-29 (day 28) 100x outgrow of gametes, thallus‘spiders’



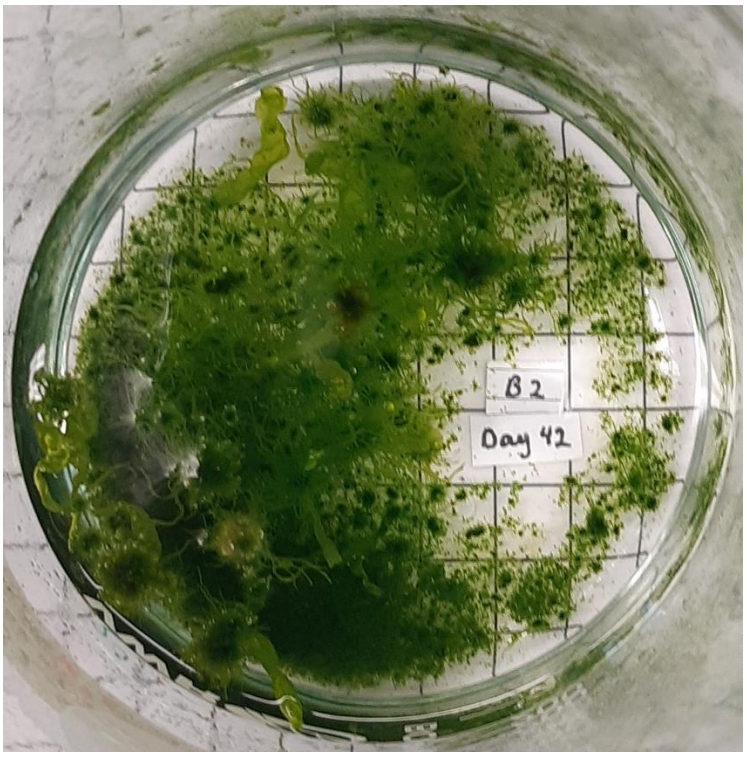
B2 21-05-03 (day 32) overview of outgrow of gametes, in the form of a lot of thallus‘spiders’



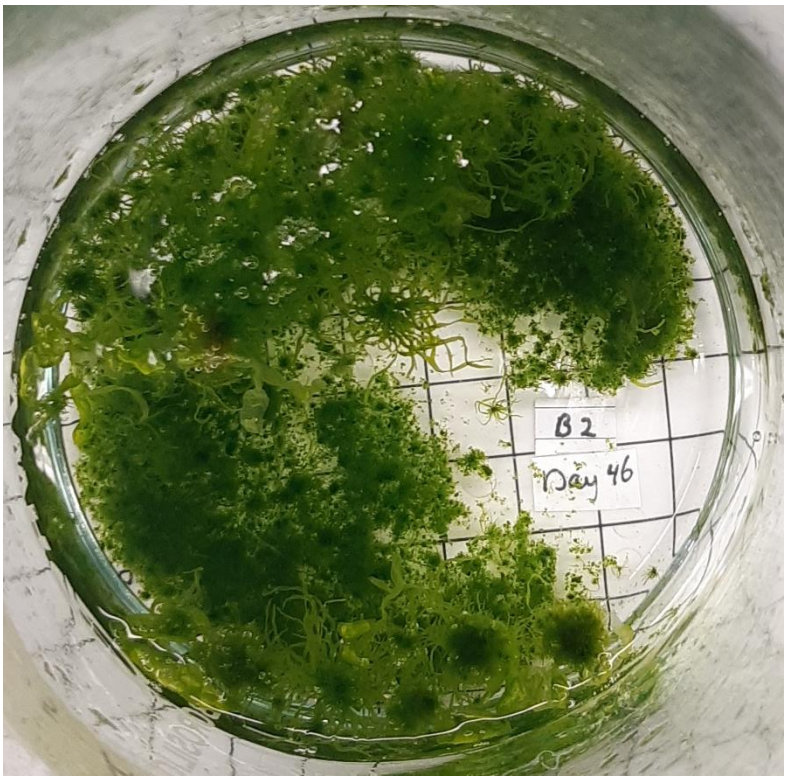
B2 21-05-06 (day 35) overview of outgrow of gametes, in the form of a lot of thallus‘spiders’



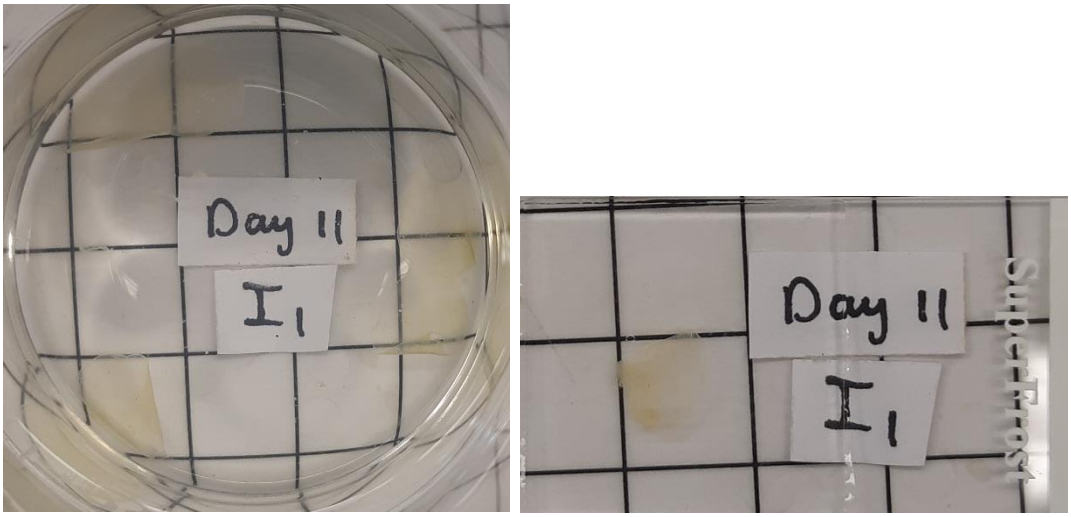
B2 21-05-10 (day 39) overview of outgrow of gametes into lots of thallus‘spiders’



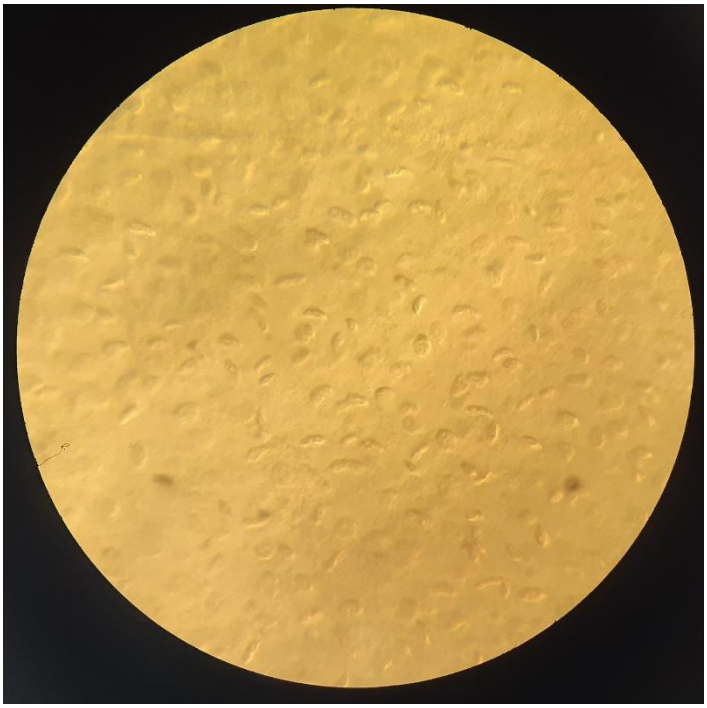
B2 21-05-13 (day 42) overview of outgrow of gametes into lots of thallus‘spiders’



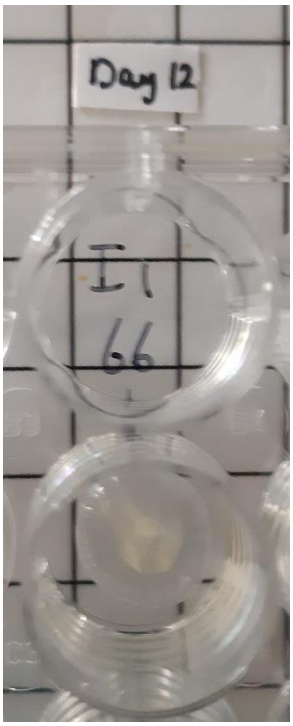
B2 21-05-17 (day 46) overview of outgrow of gametes into lots of thallus‘spiders’



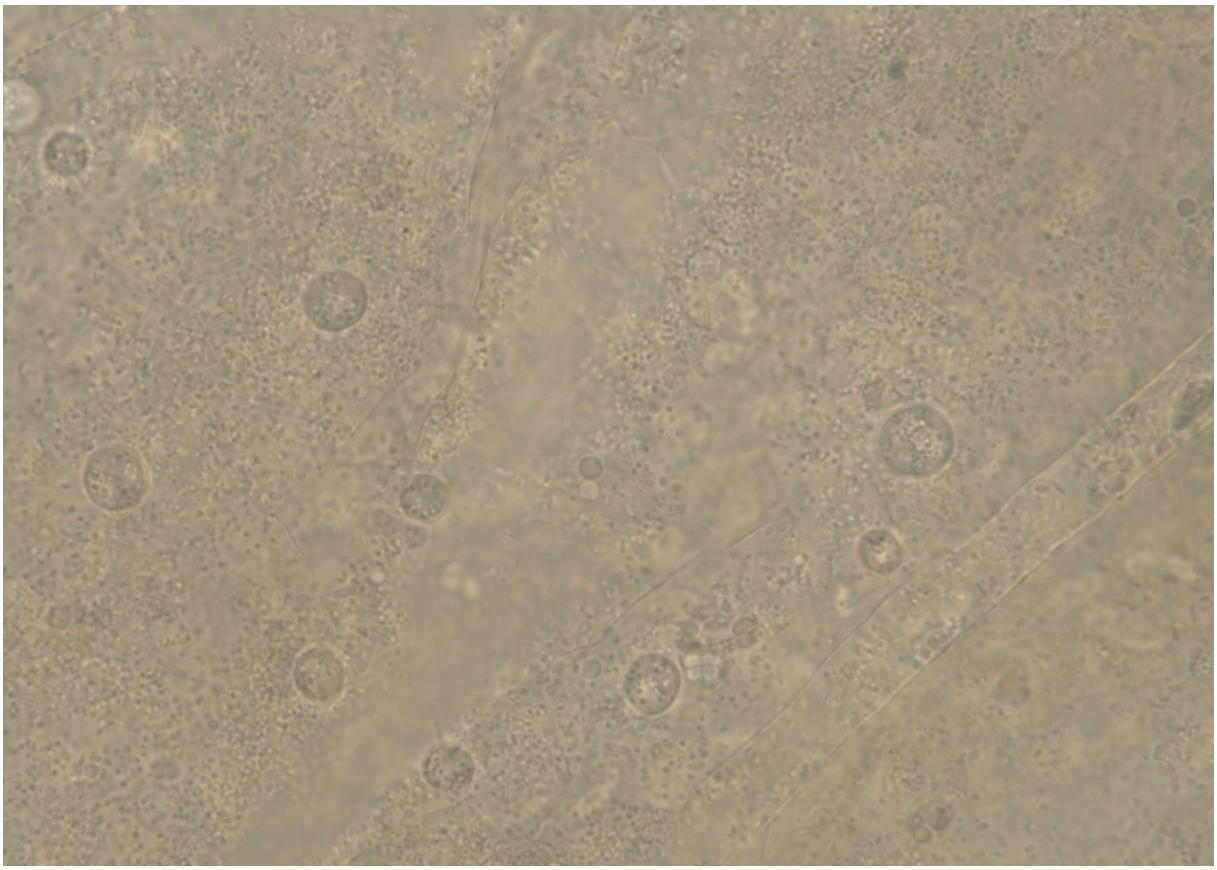
I1 21-04-12 (day 11) overview and one detail thallus, used for microscope

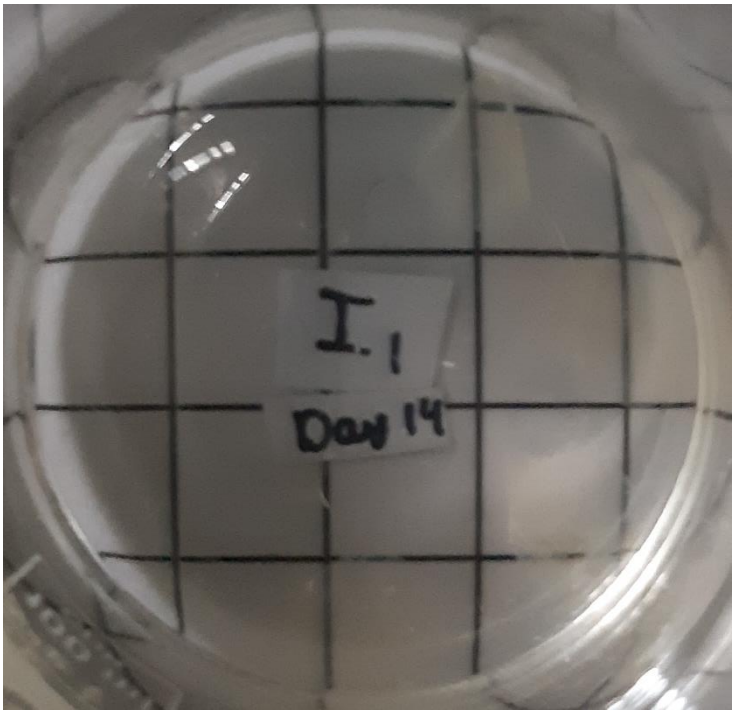


I1 21-04-12 (day 11) Probably attached gametes or spores on pale thallus 400 x.

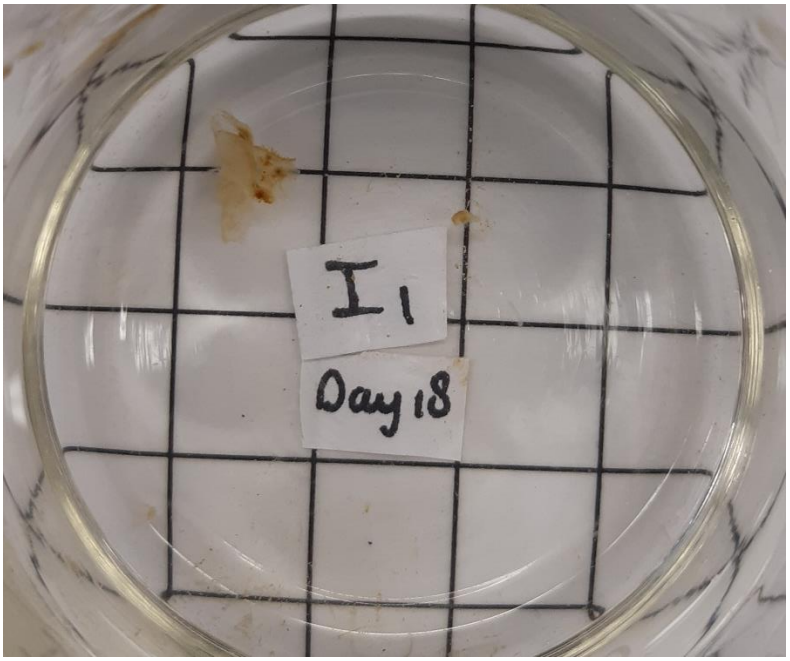


I1 21-04-13 (day 12) One detail thallus, used for microscope

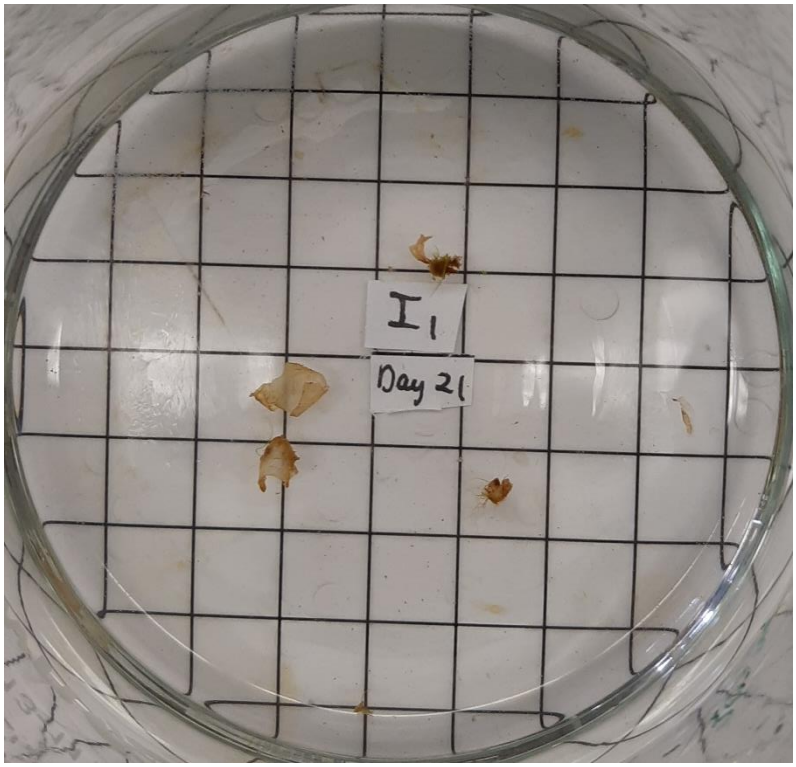

I1 21-04-13 (day 12) Thallus has sporulated probably meiospores 600x Nikon 80i



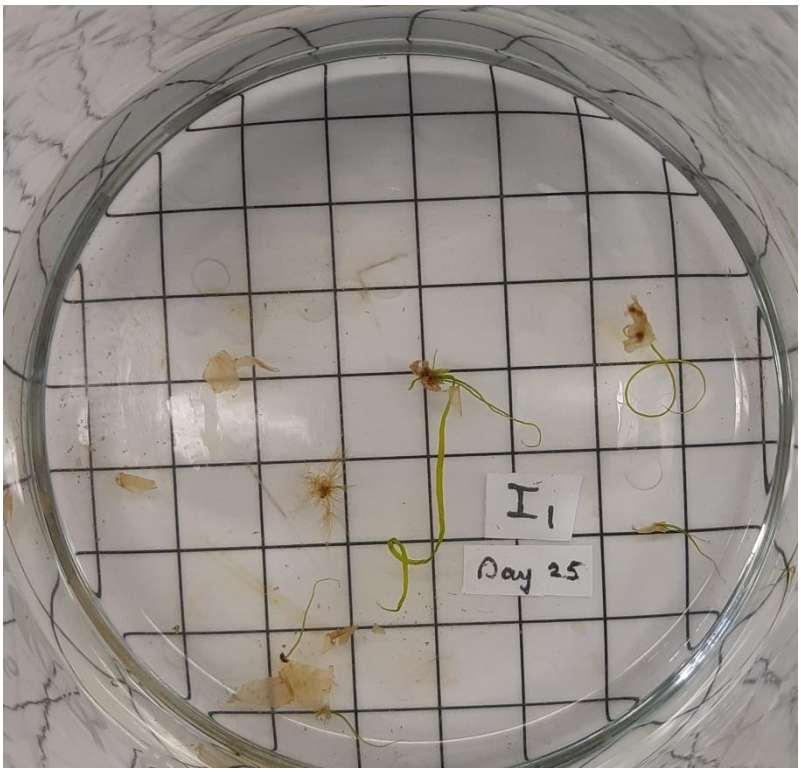
I1 21-04-15 (day 14) overview



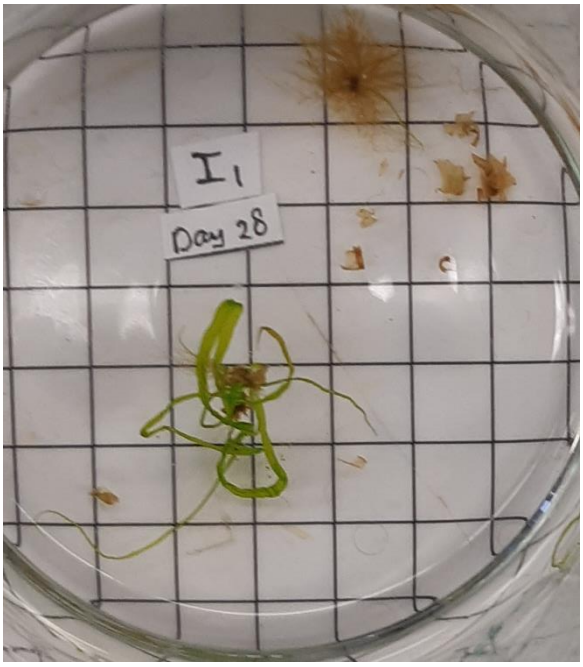
I1 21-04-19 (day 18) overview



I1 21-04-22 (day 21) overview

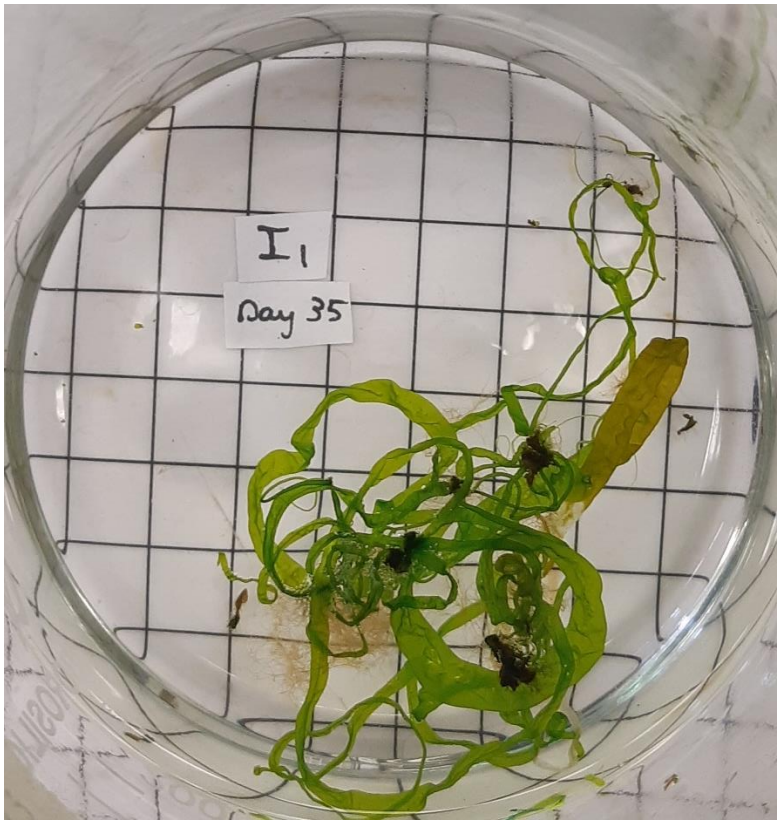


I1 21-04-26 (day 25) overview



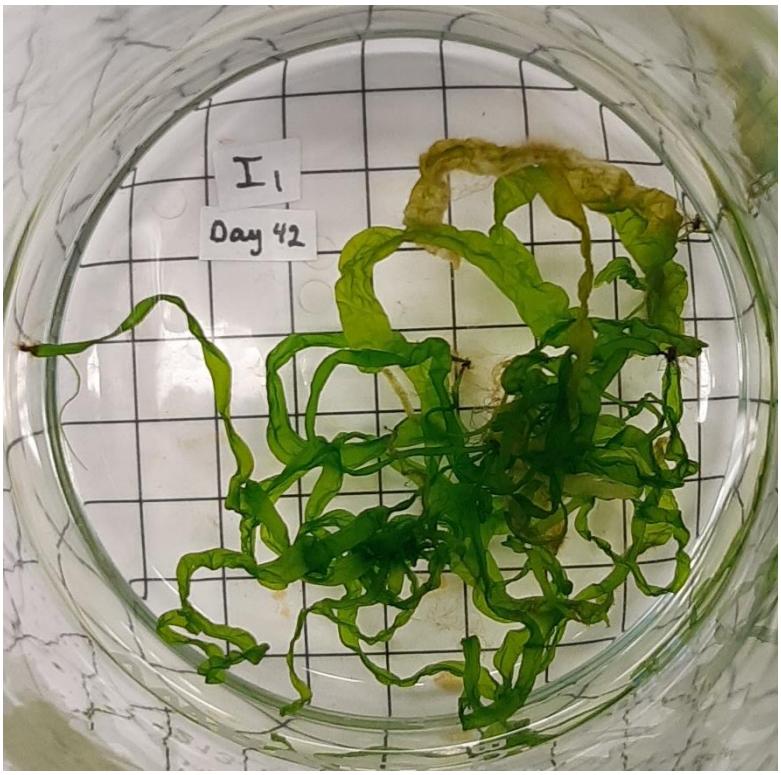
I1 21-04-29 (day 28) overview, outgrow of gametes or spores

I1 21-05-03 (day 32) overview, outgrow of gametes or spores

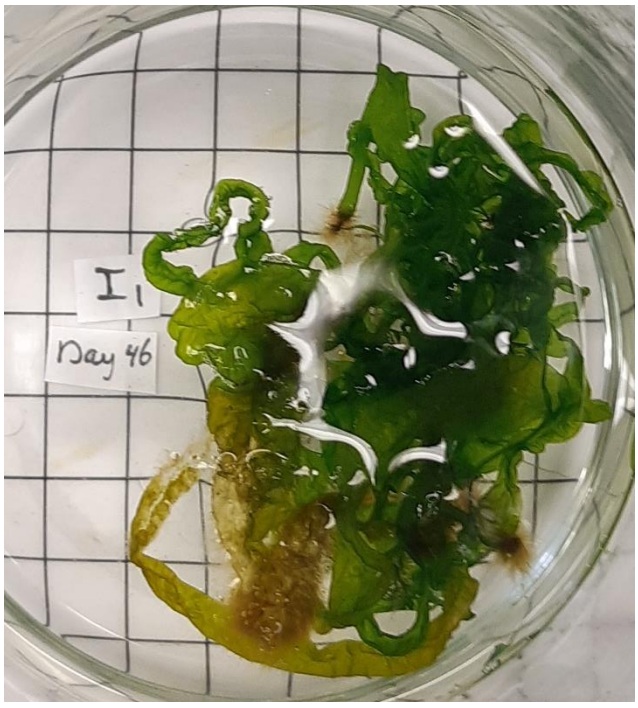


I1 21-05-06 (day 35) overview, outgrow of gametes or spores

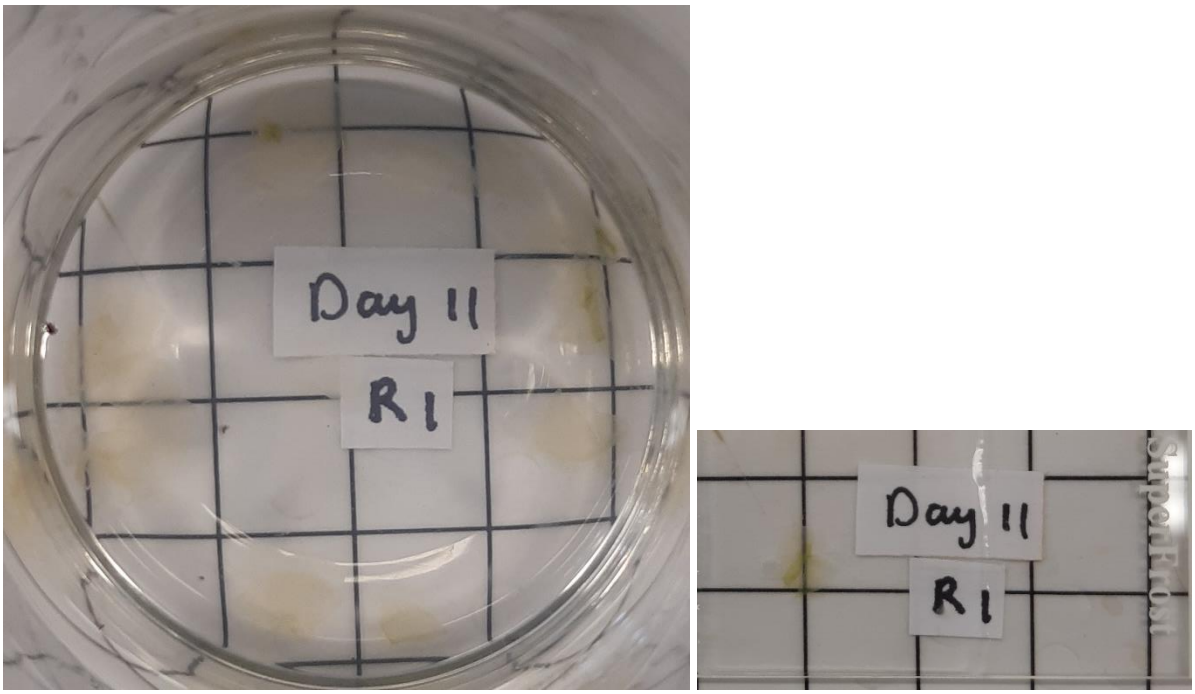
I1 21-05-10 (day 39) overview, outgrow of gametes or spores



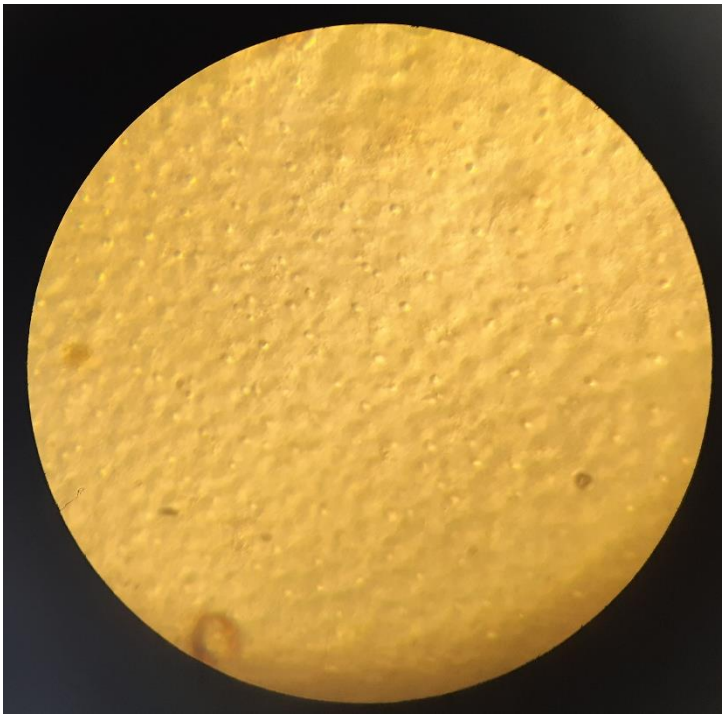
I1 21-05-13 (day 42) overview, outgrow of gametes or spores



I1 21-05-17 (day 46) overview, outgrow of gametes or spores

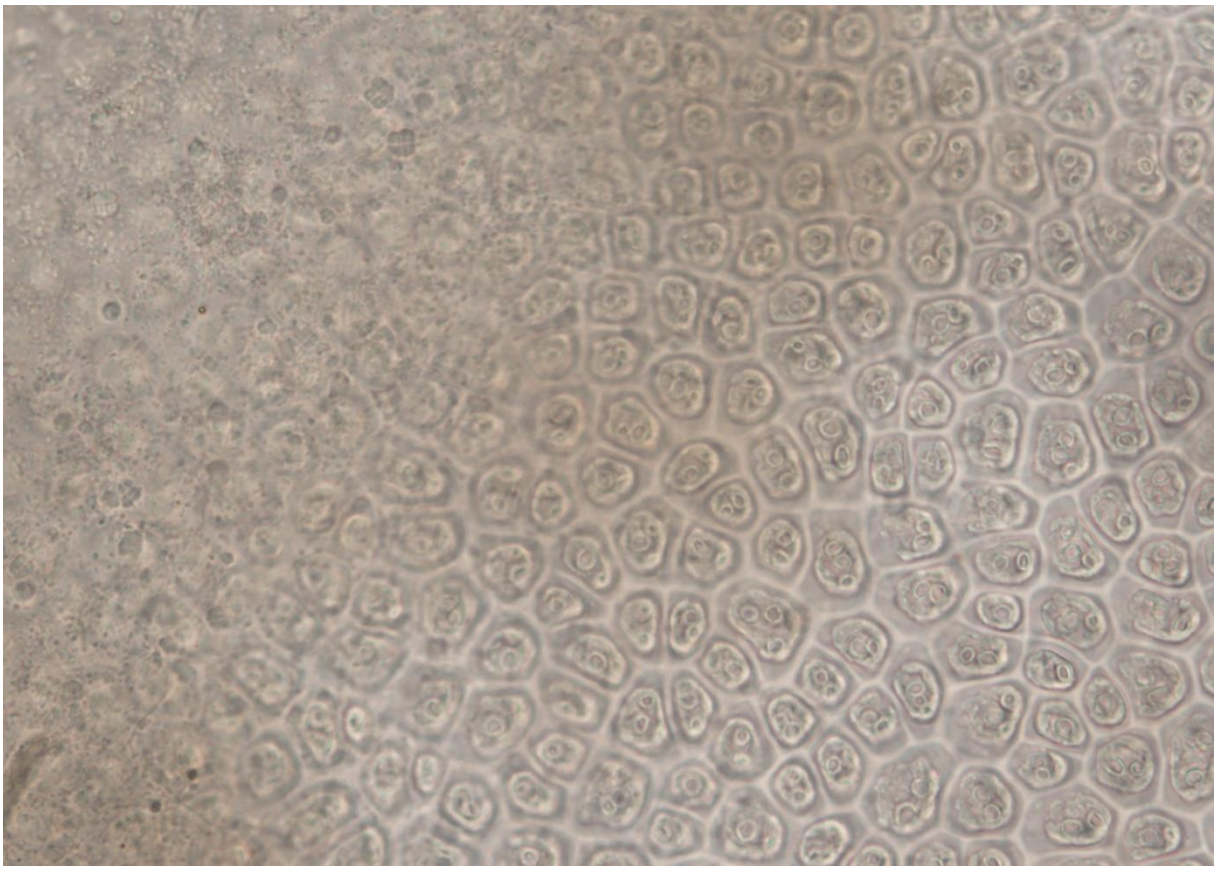


R1 21-04-12 (day 11) overview and one detail thallus, used for microscope

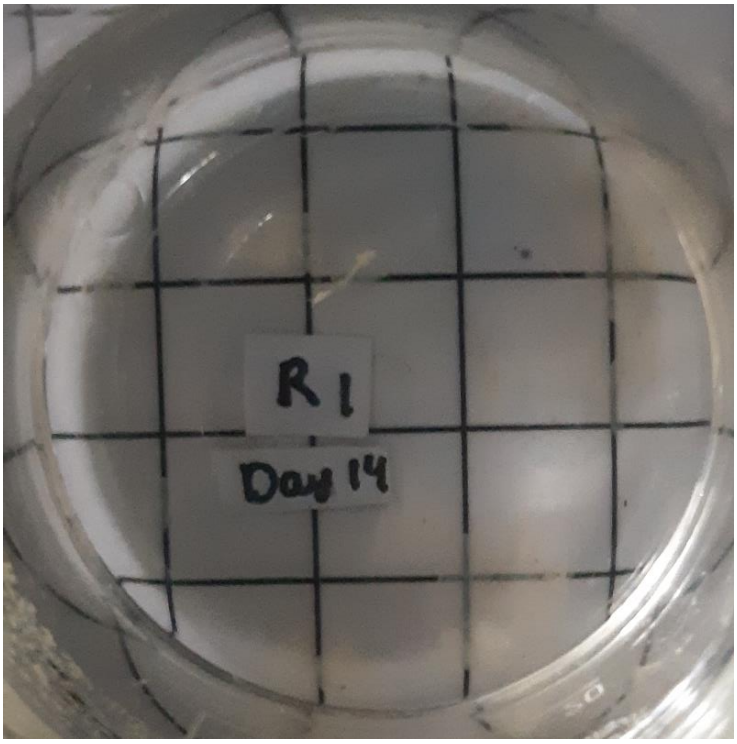


R1 21-04-12 (day 11) Probably attached gametes or spores on pale thallus 400 x.

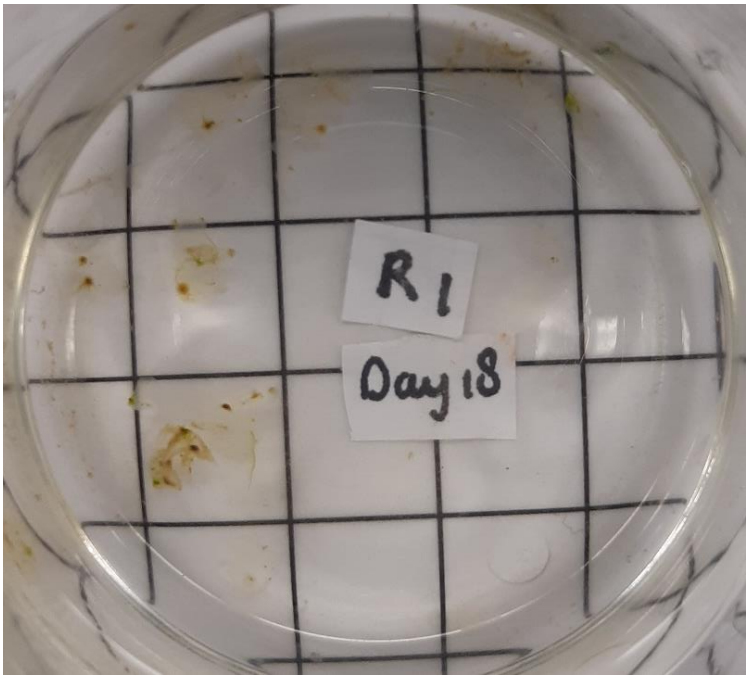
R1 21-04-13 (day 12) One detail thallus, used for microscope



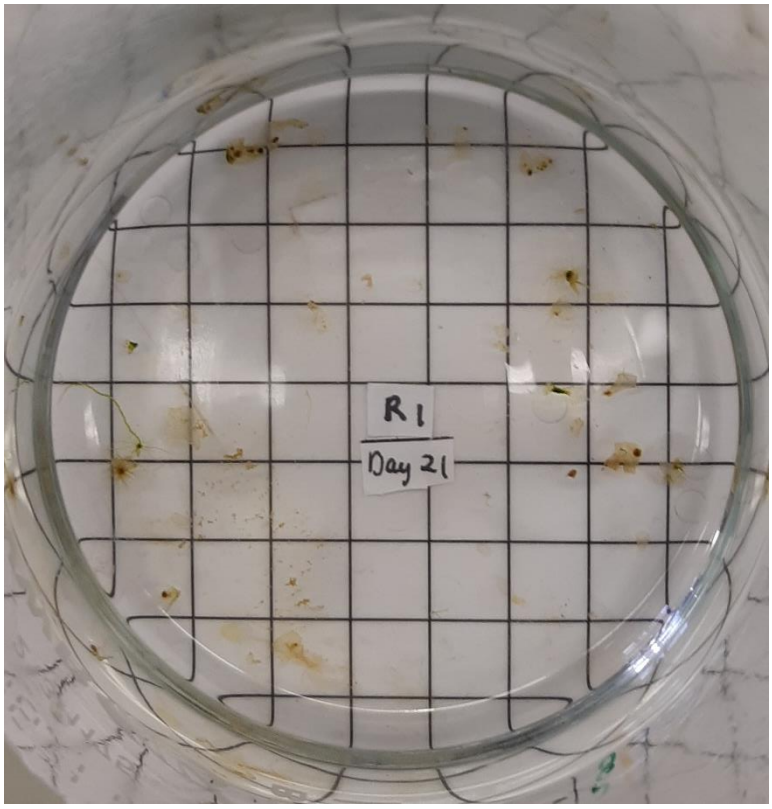
R1 21-04-13 (day 12) Pale thallus tissue, no moving gametes or spores, 400x Nikon 80i



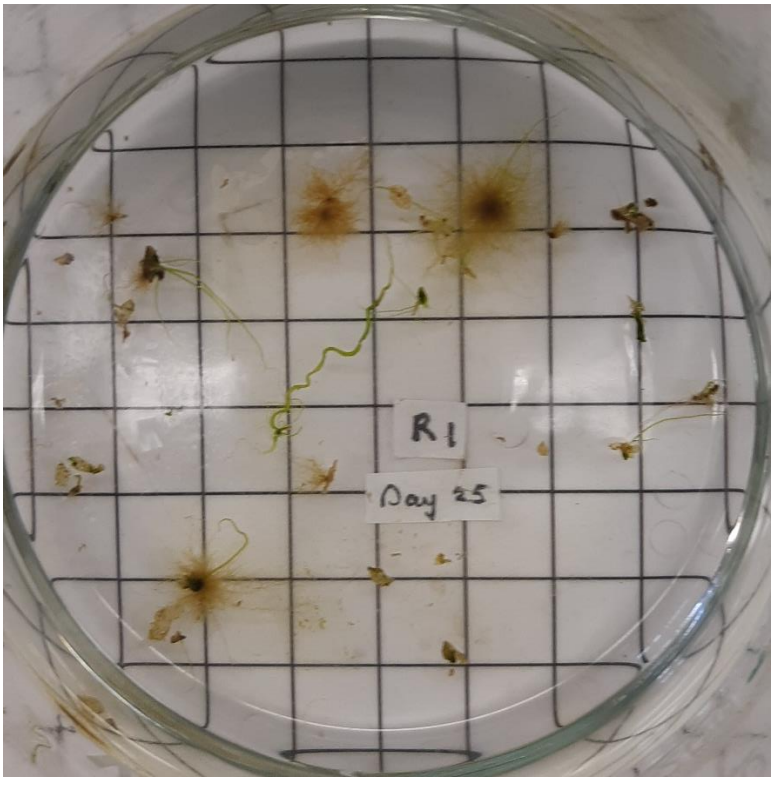
R1 21-04-15 (day 14) overview



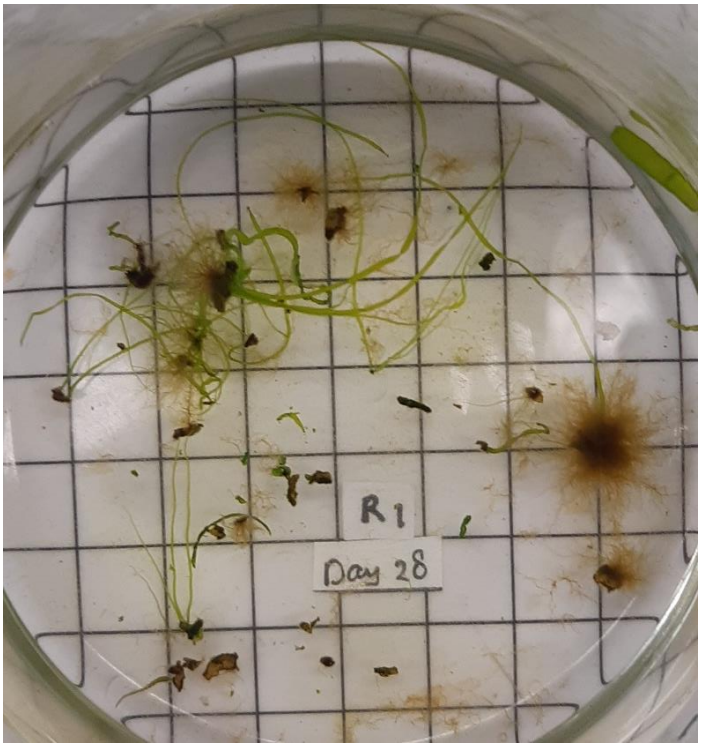
R1 21-04-19 (day 18) overview



R1 21-04-22 (day 21) overview

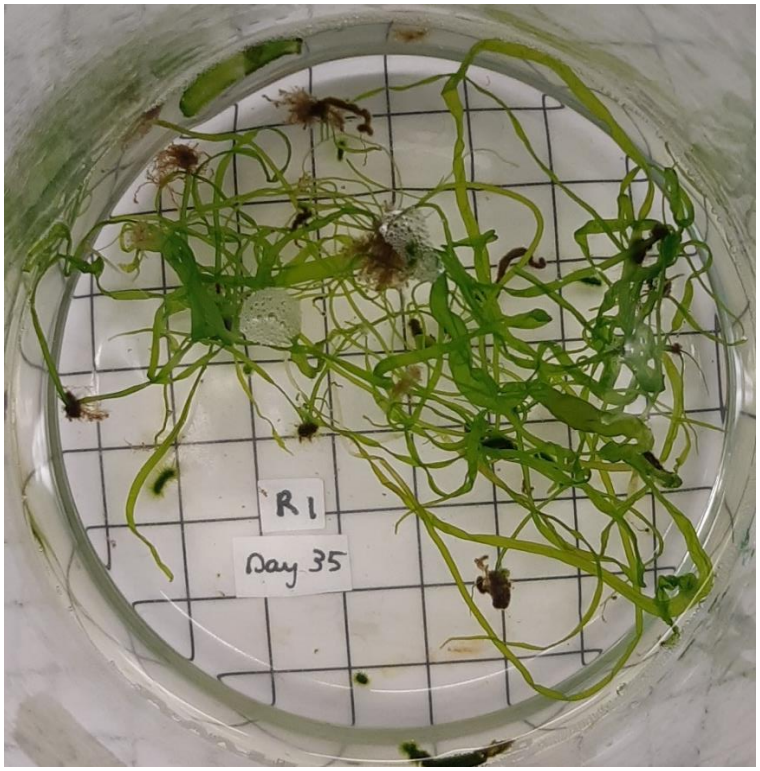


R1 21-04-26 (day 25) overview

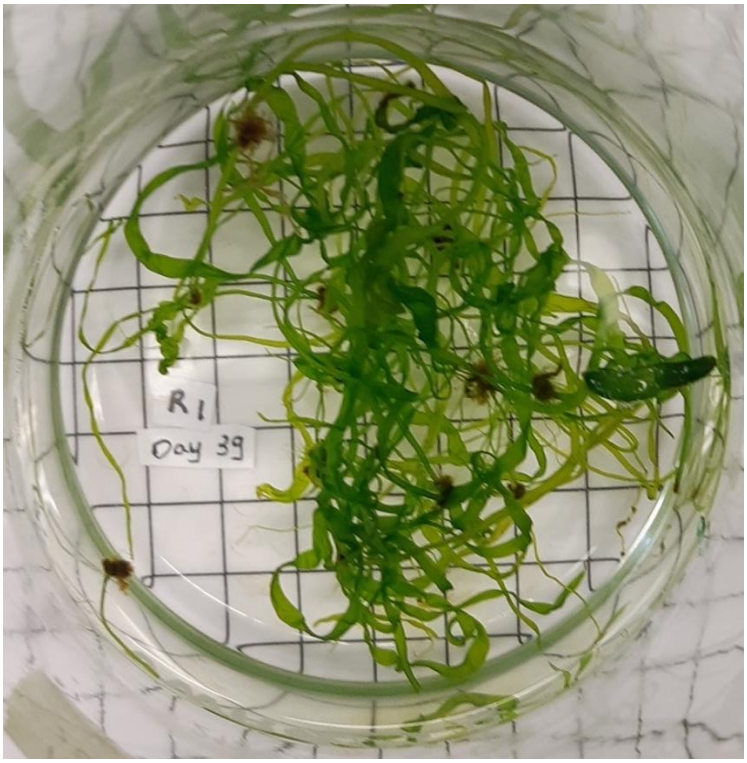


R1 21-04-29 (day 28) overview, outgrow of gametes or spores

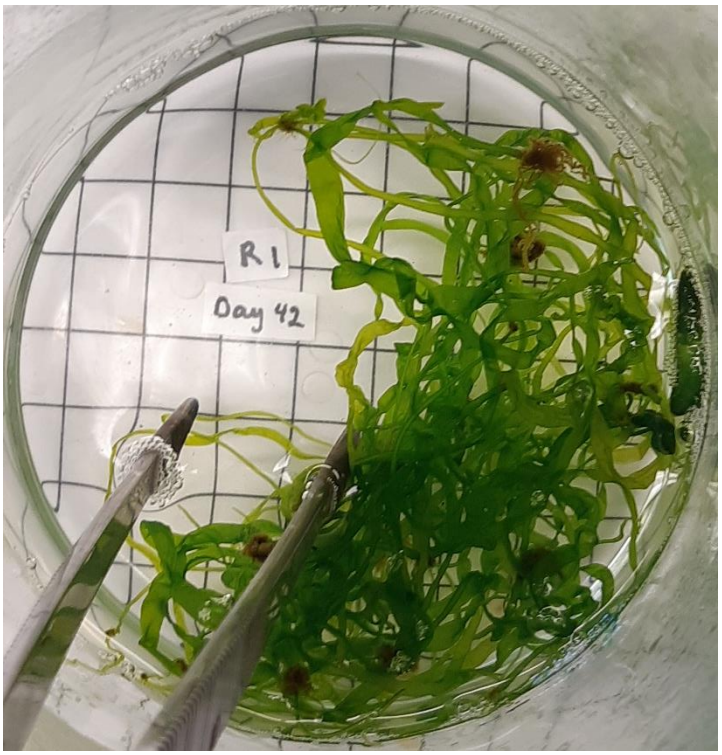
R1 21-05-03 (day 32) overview, outgrow of gametes or spores



R1 21-05-06 (day 35) overview, outgrow of gametes or spores

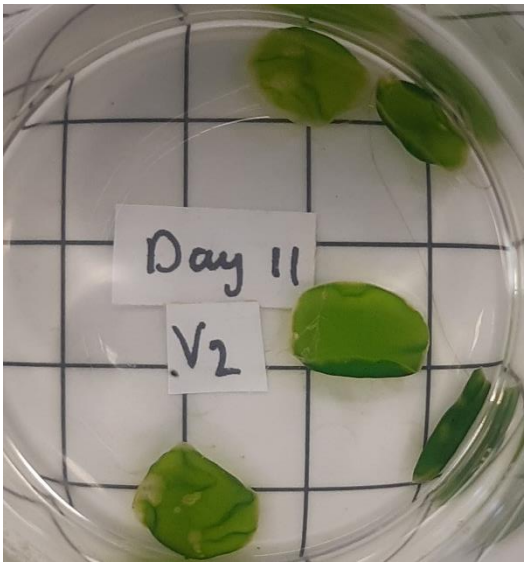


R1 21-05-10 (day 39) overview, outgrow of gametes or spores



R1 21-05-13 (day 42) overview, outgrow of gametes or spores

R1 21-05-17 (day 46) overview, outgrow of gametes or spores



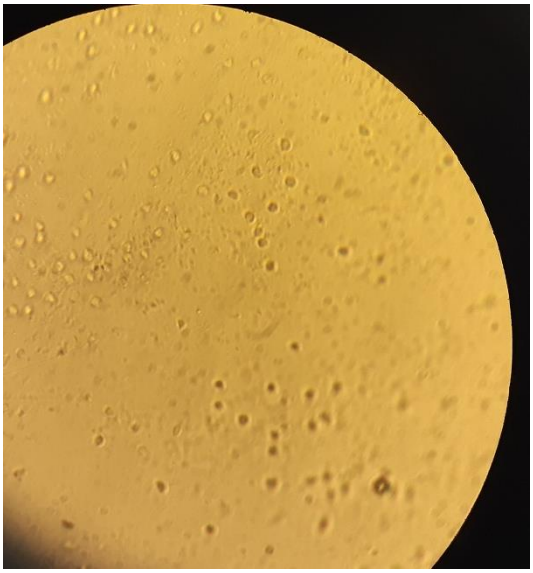
V2 21-04-12 (day 11) overview and one detail thallus, used for microscope

20210412_112903.mp4

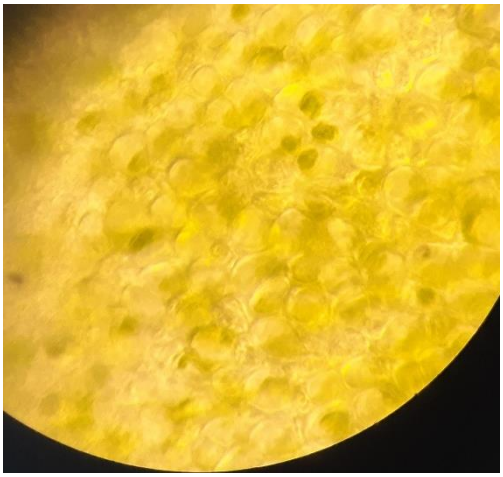
V2 21-04-12 (day 11) Video of lots of moving gametes, 100 x

20210412_113158.mp4

V2 21-04-12 (day 11) Video of lots of moving gametes, 400 x

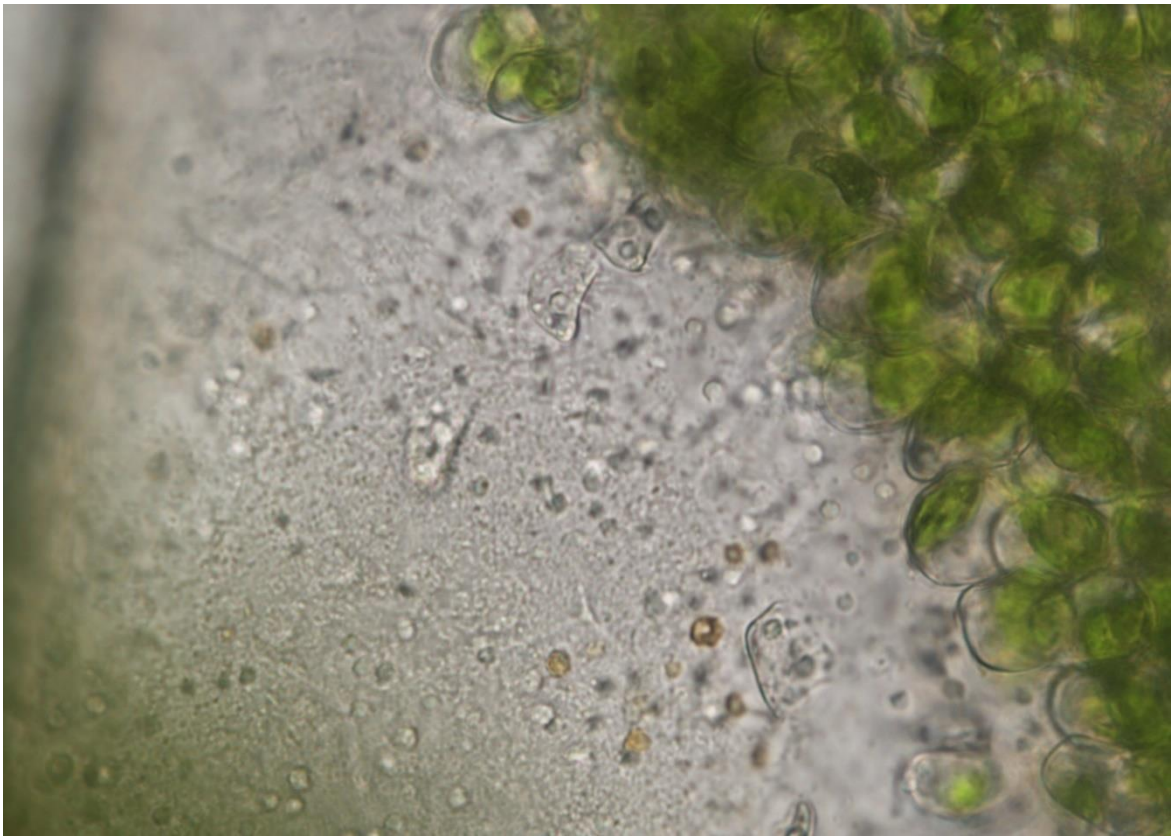


V2 21-04-12 (day 11) Photo of lots of moving gametes, 400 x

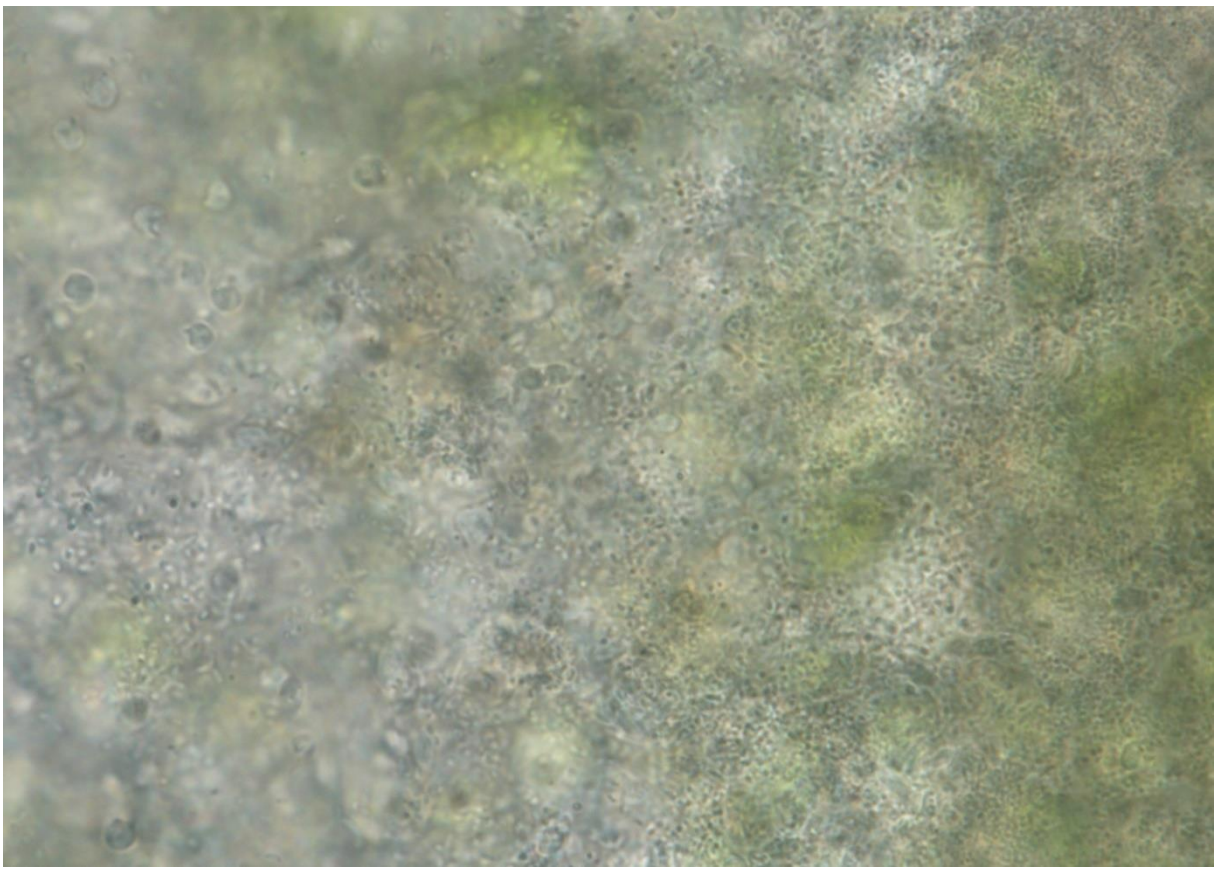


V2 21-04-12 (day 11) Thallus tissue 400x

V2 21-04-13 (day 12) One detail thallus, used for microscope

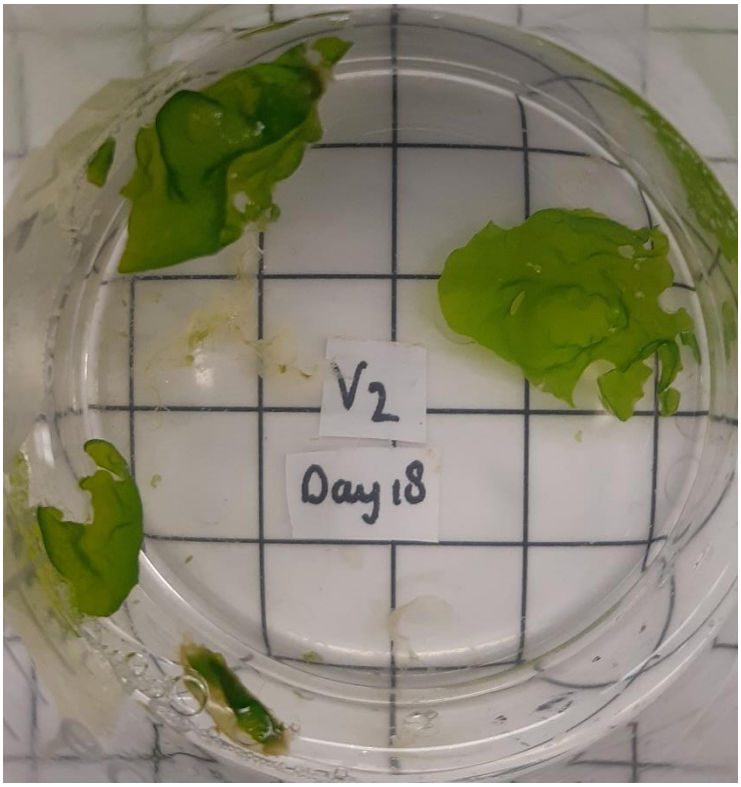


V2 21-04-13 (day 12) Photo of lots of moving gametes, 400 x Nikon 80i

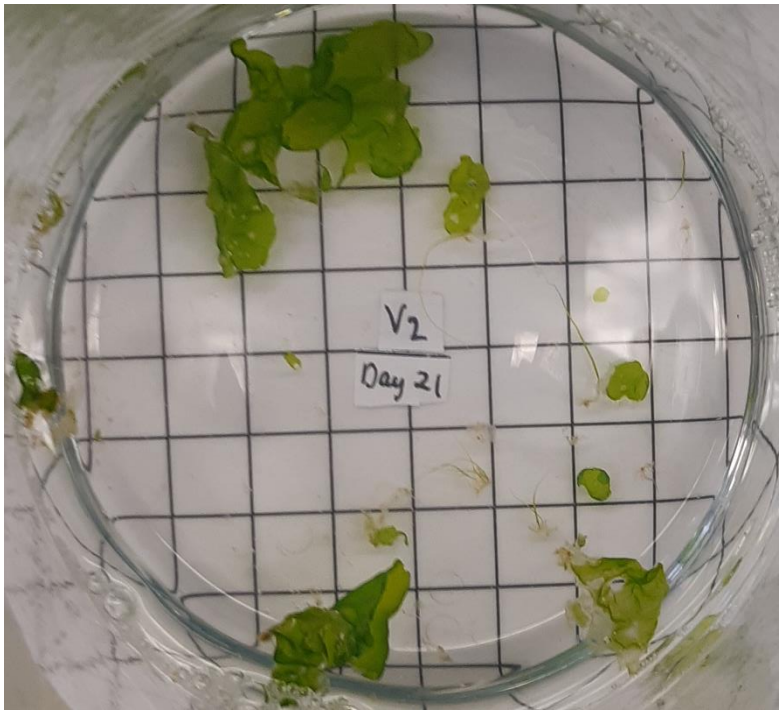


V2 21-04-13 (day 12) Photo of lots of moving gametes, 600 x Nikon 80i

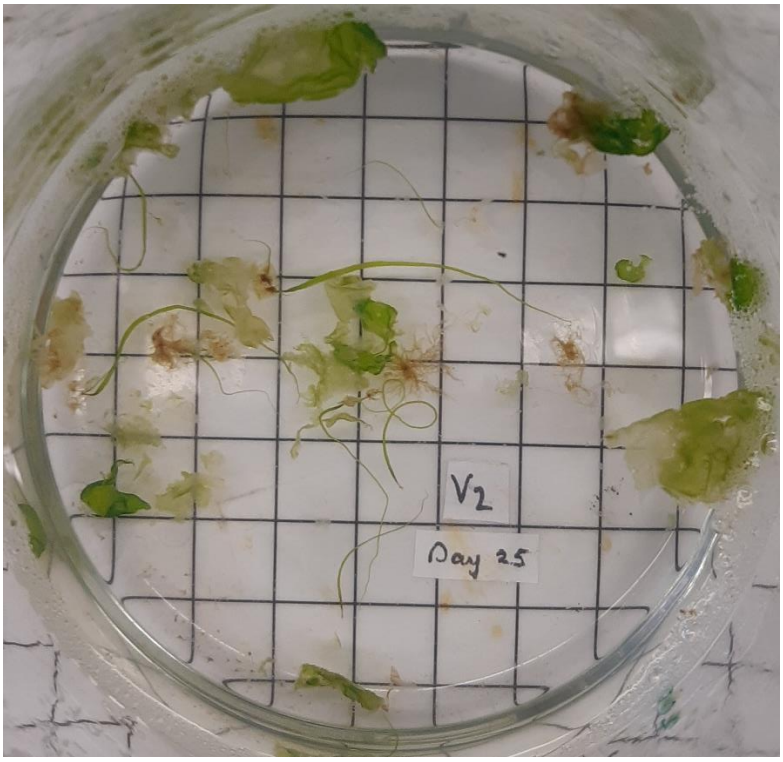
V2 21-04-15 (day 14) overview



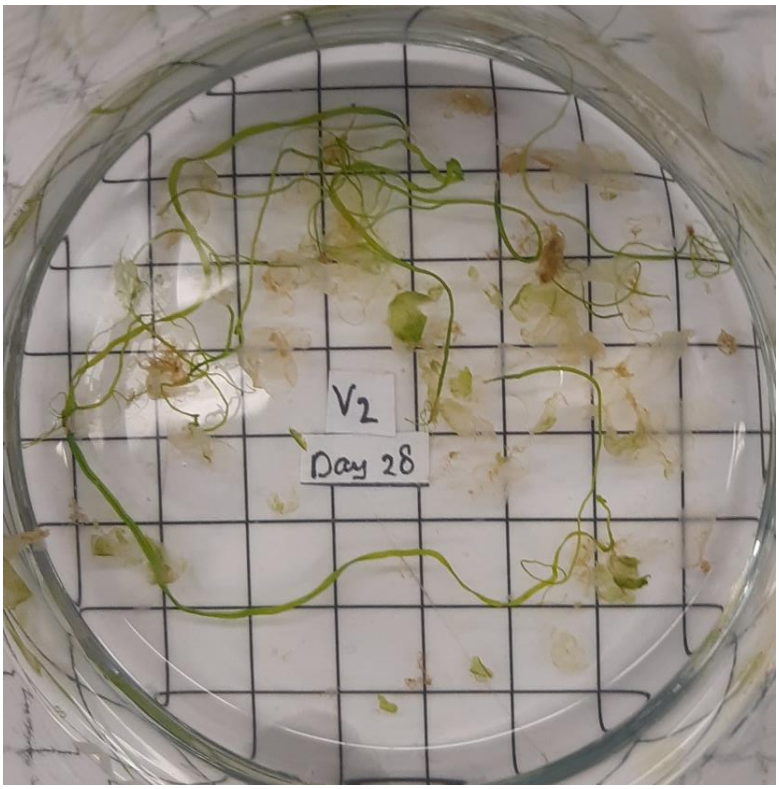
V2 21-04-19 (day 18) overview



V2 21-04-22 (day 21) overview

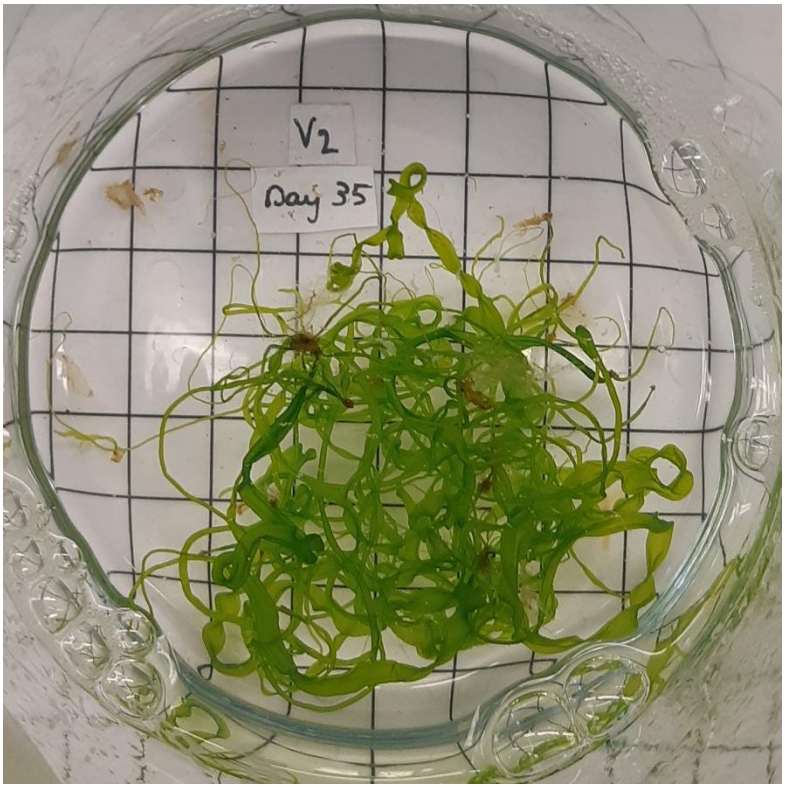


V2 21-04-26 (day 25) overview, outgrow of gametes



V2 21-04-29 (day 28) overview, outgrow of gametes

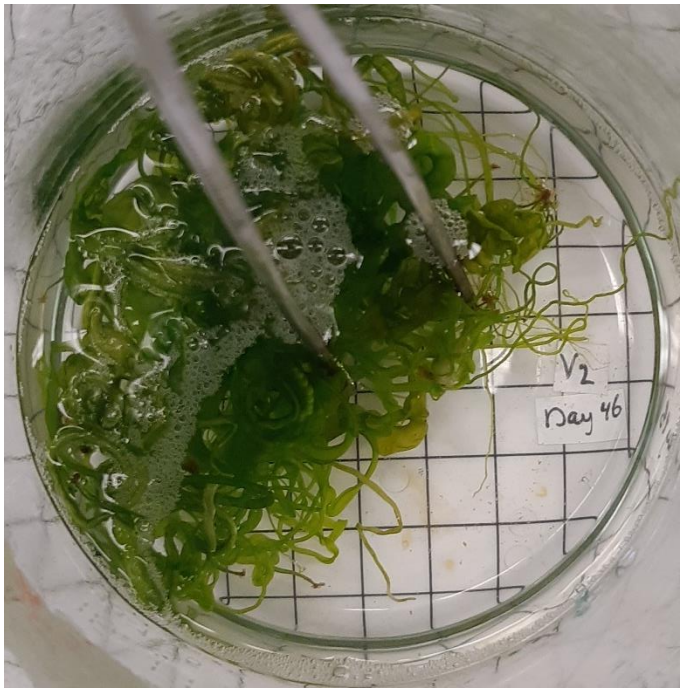
V2 21-05-03 (day 32) overview, outgrow of gametes



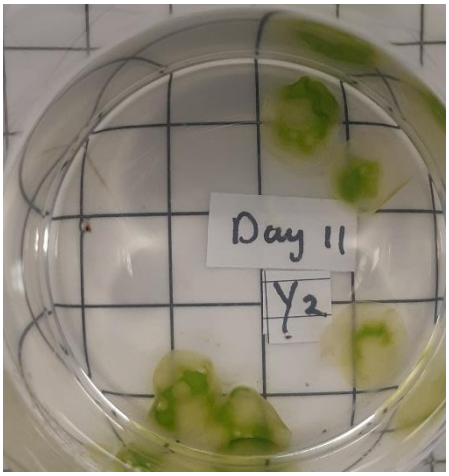
V2 21-05-06 (day 35) overview, outgrow of gametes

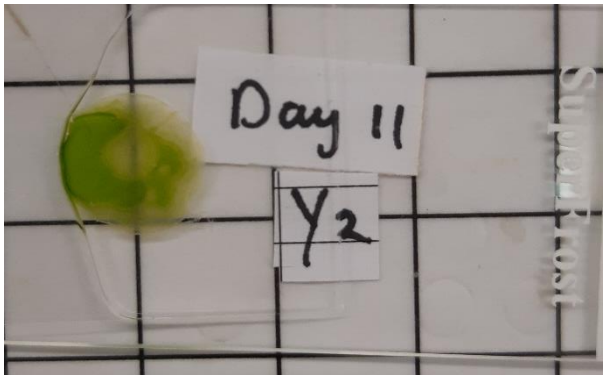
V2 21-05-10 (day 39) overview, outgrow of gametes

V2 21-05-13 (day 42) overview, outgrow of gametes

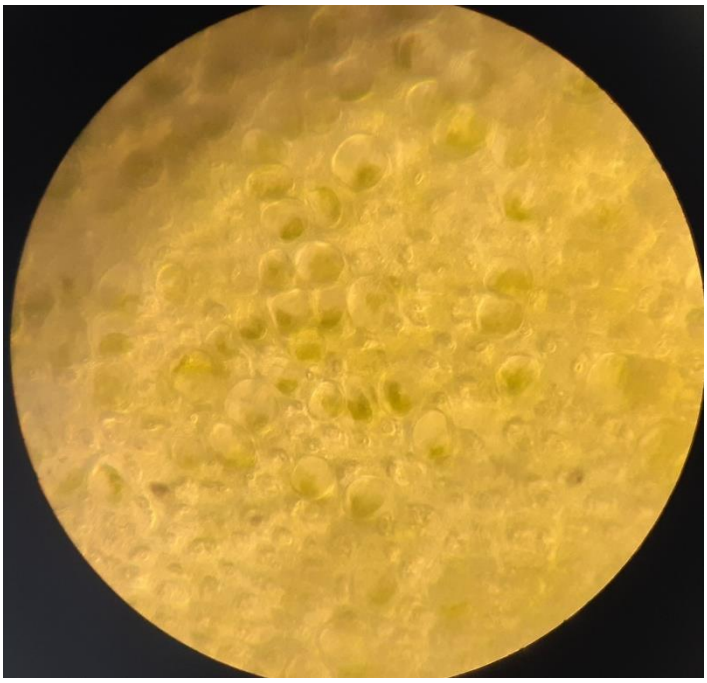


V2 21-05-17 (day 46) overview, outgrow of gametes



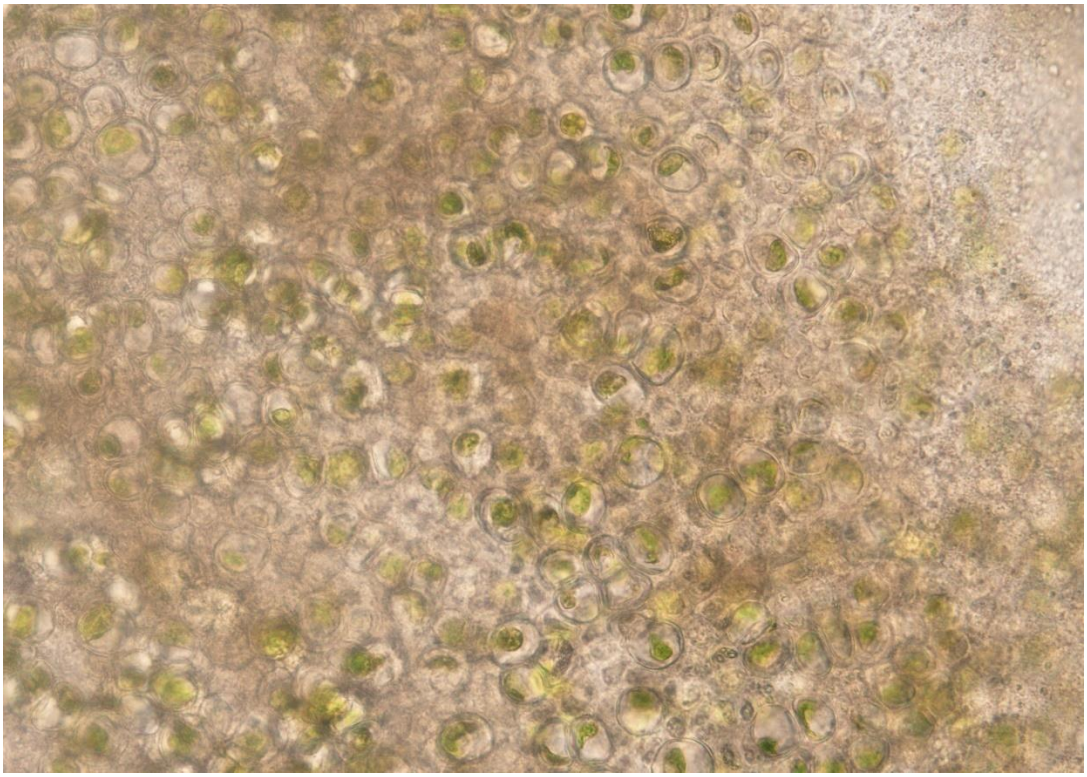


Y2 21-04-12 (day 11) overview and one detail thallus, used for microscope

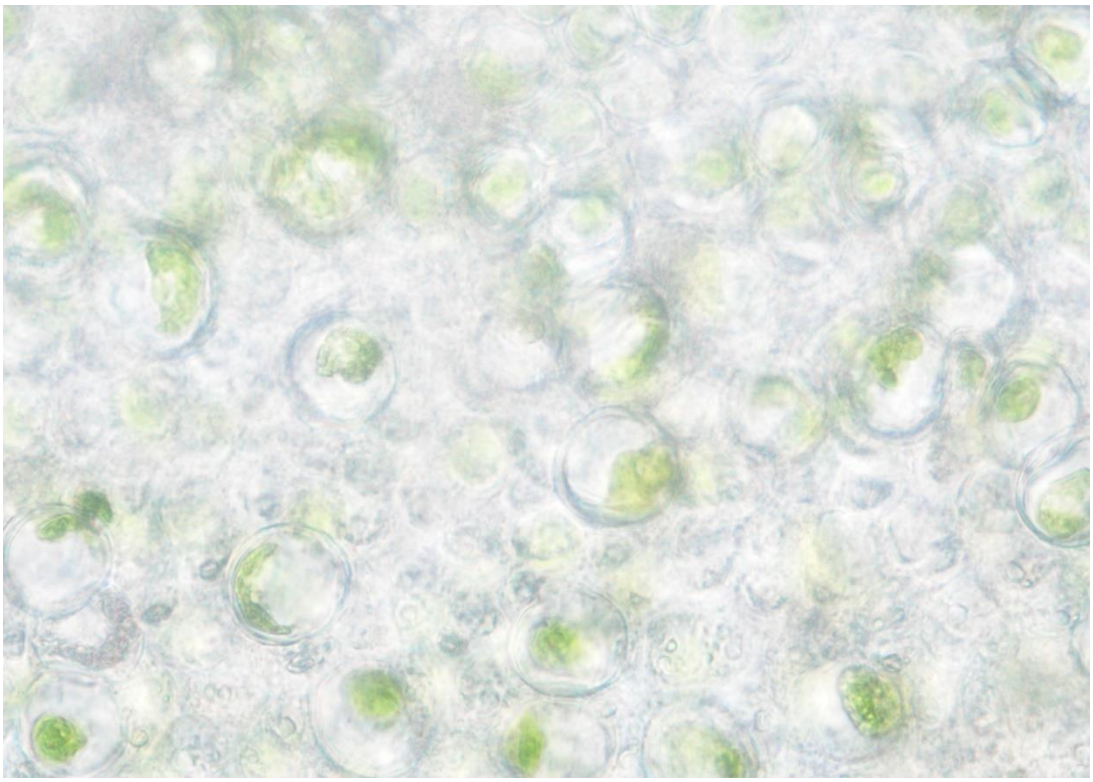


Y2 21-04-12 (day 11) Thallus tissue 400x

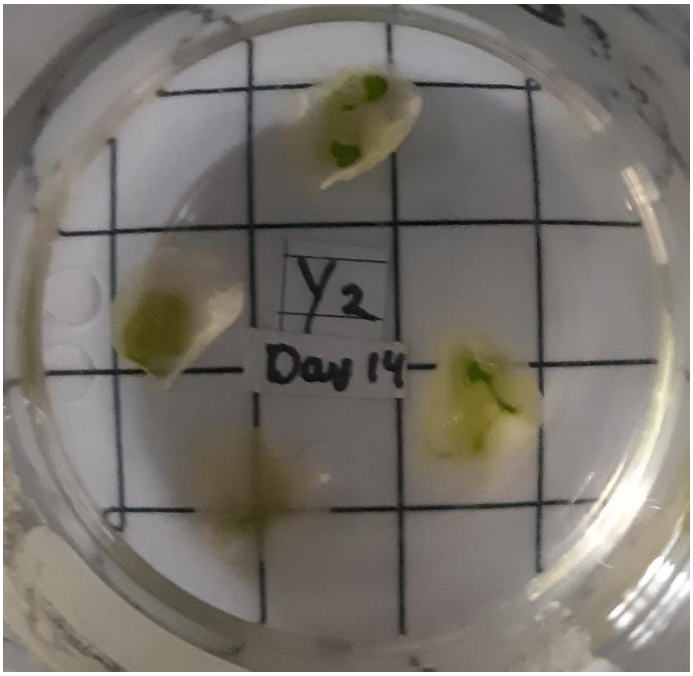
Y2 21-04-13 (day 12) One detail thallus, used for microscope



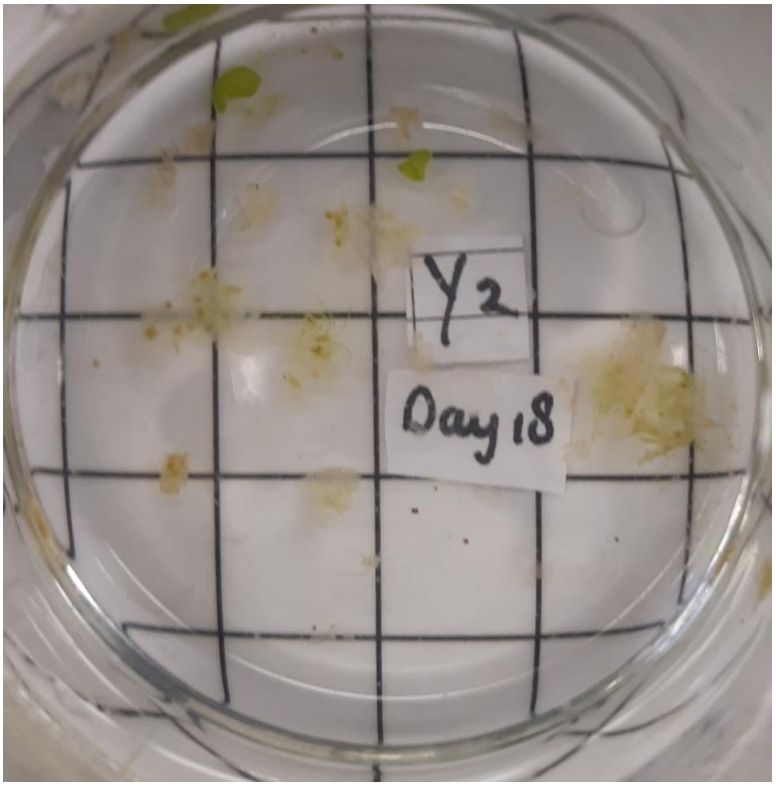
Y2 21-04-13 (day 12) Thallus tissue 200x Nikon 80i, no moving gametes or spores



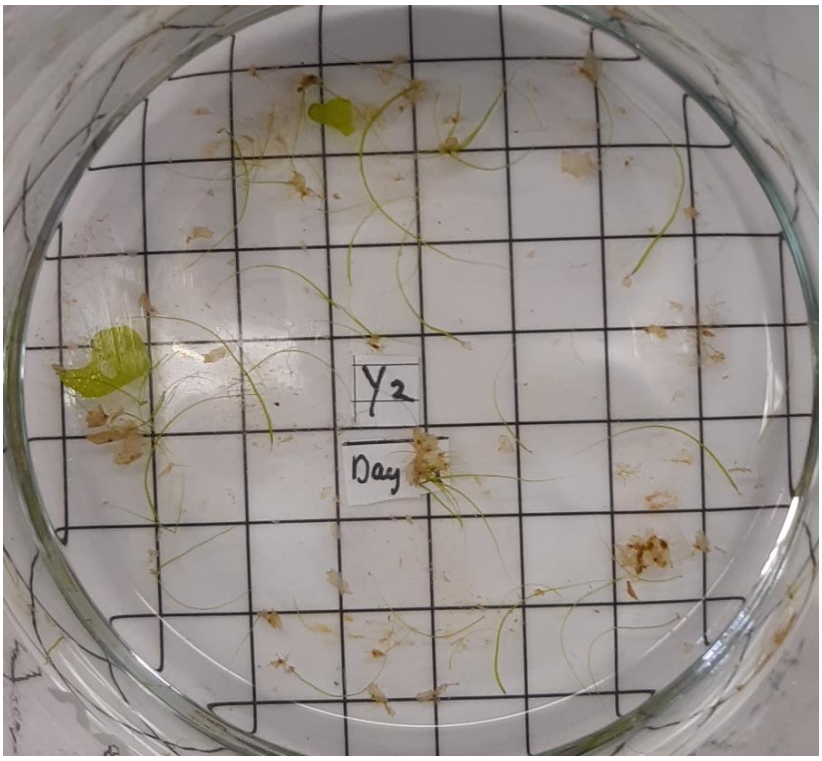
Y2 21-04-13 (day 12) Thallus tissue 400x Nikon 80i, no moving gametes or spores



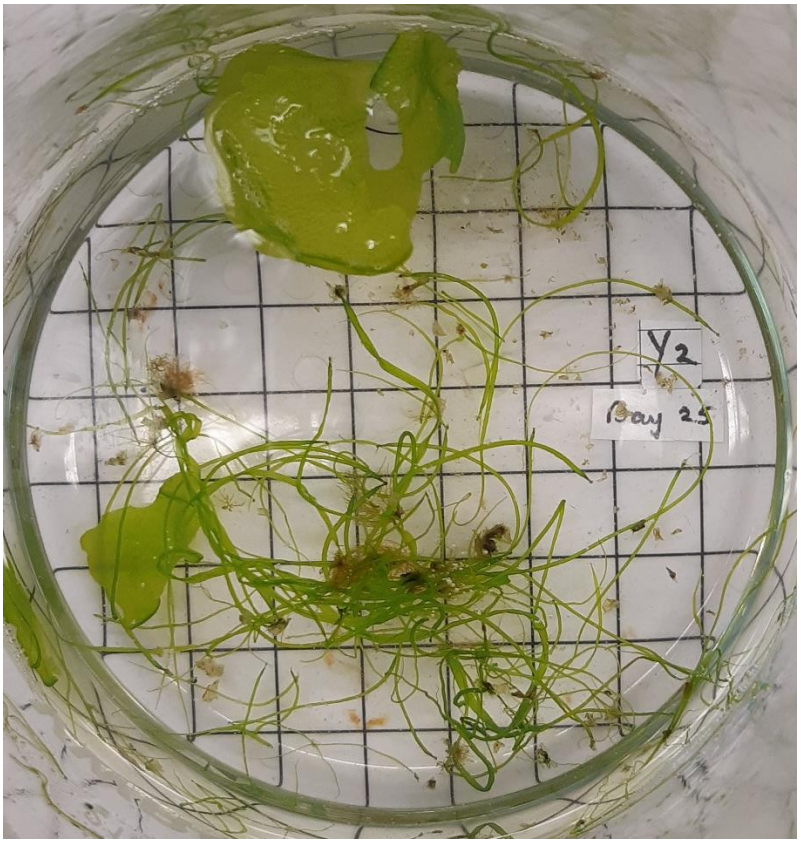
Y2 21-04-15 (day 14) overview



Y2 21-04-19 (day 18) overview

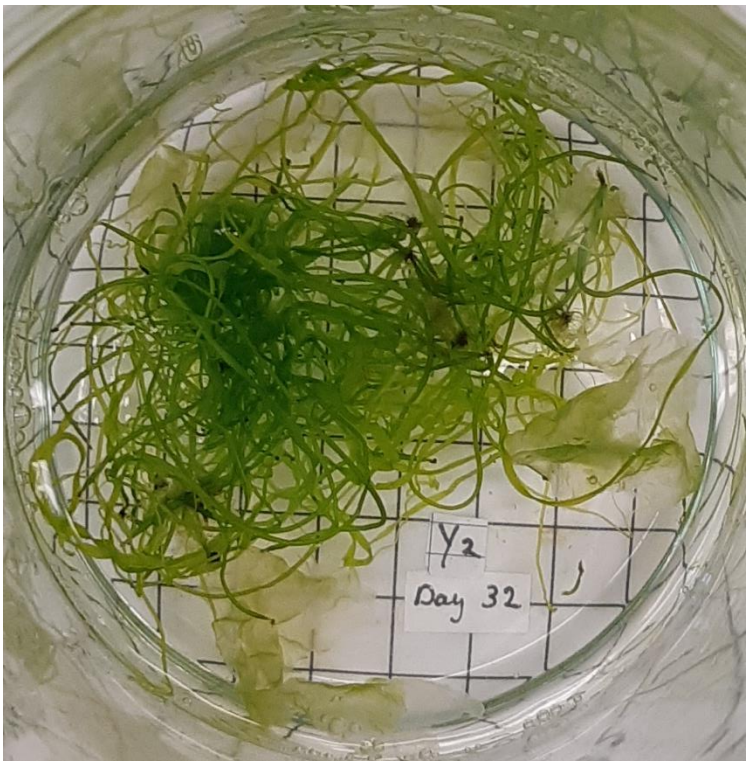


Y2 21-04-22 (day 21) overview, outgrow of gametes or spores

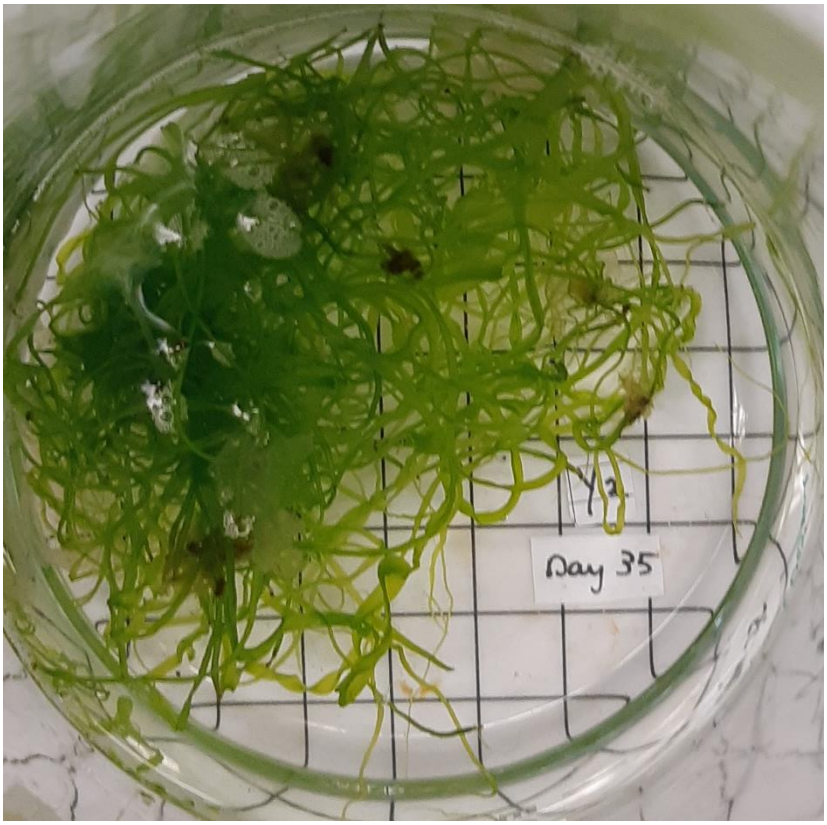


Y2 21-04-26 (day 25) overview, outgrow of gametes or spores

Y2 21-04-29 (day 28) overview, outgrow of gametes or spores



Y2 21-05-03 (day 32) overview, outgrow of gametes or spores

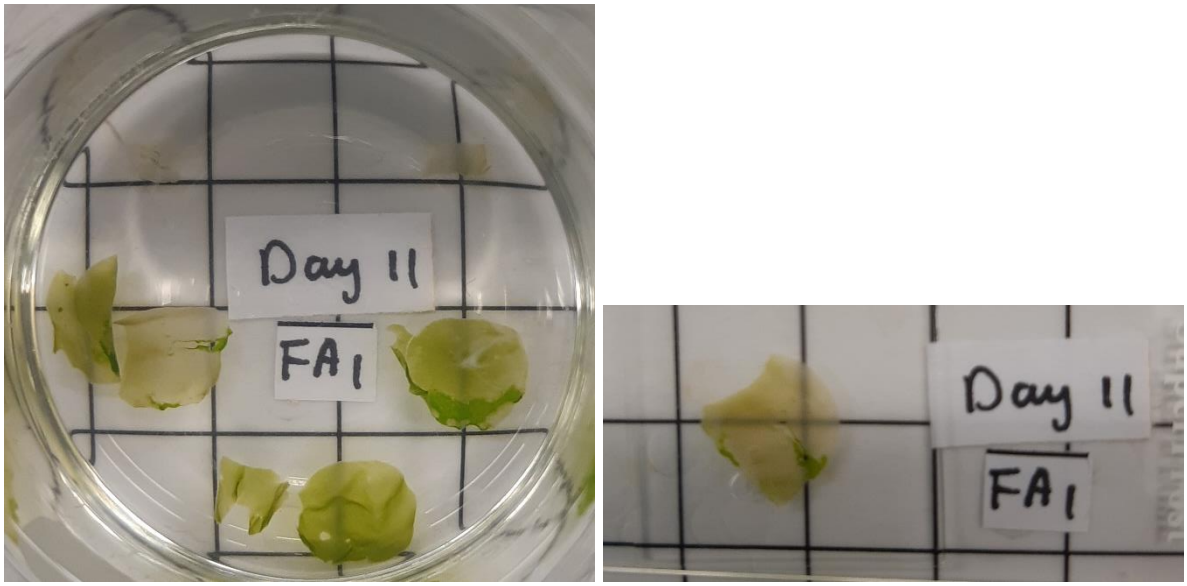


Y2 21-05-06 (day 35) overview, outgrow of gametes or spores

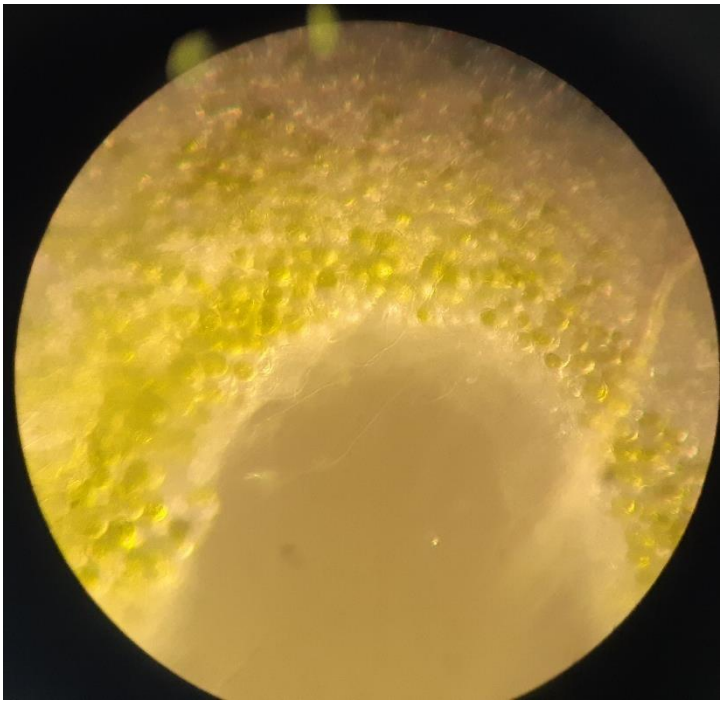
Y2 21-05-10 (day 39) overview, outgrow of gametes or spores

Y2 21-05-13 (day 42) overview, outgrow of gametes or spores

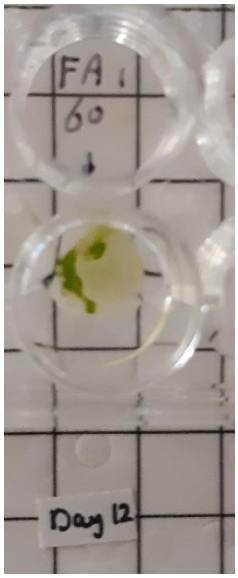
Y2 21-05-17 (day 46) overview, outgrow of gametes or spores



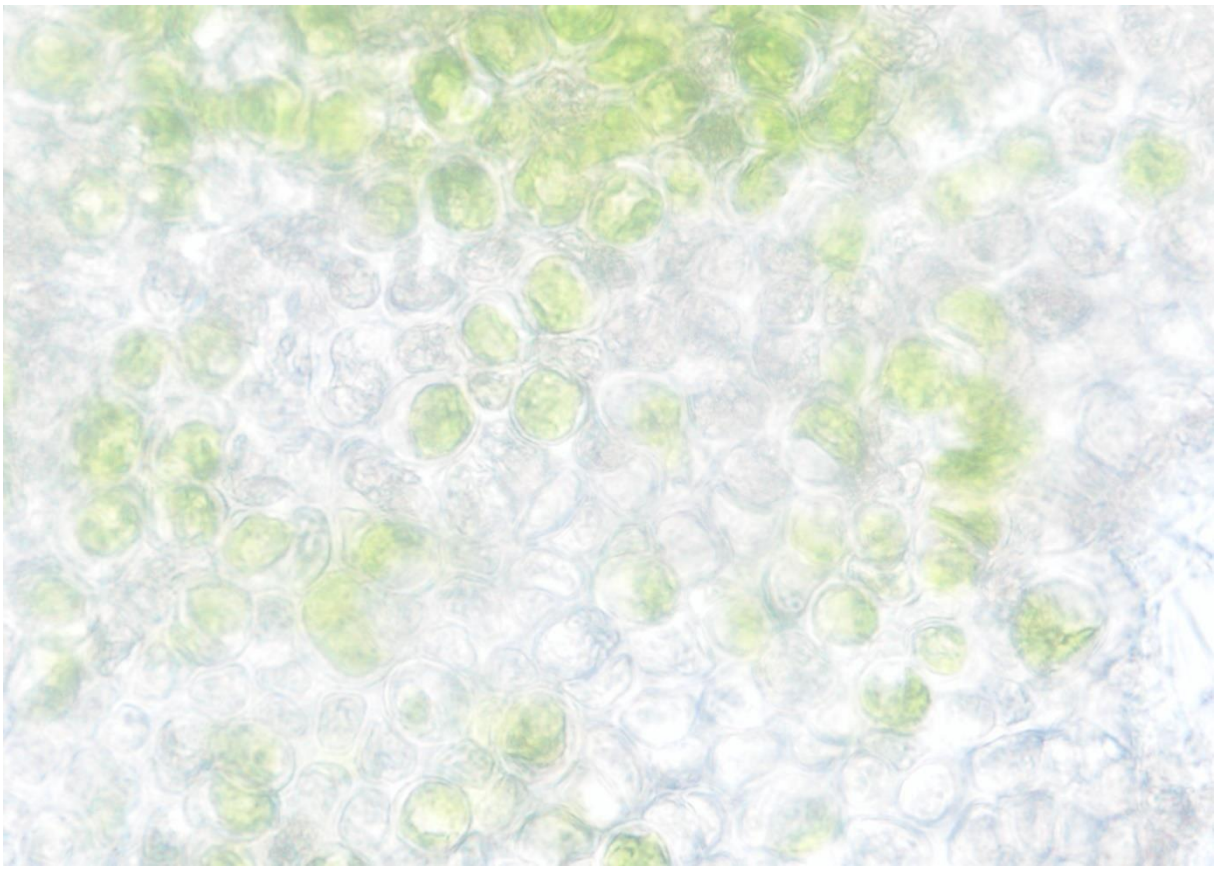
FA1 21-04-12 (day 11) overview and one detail thallus, used for microscope



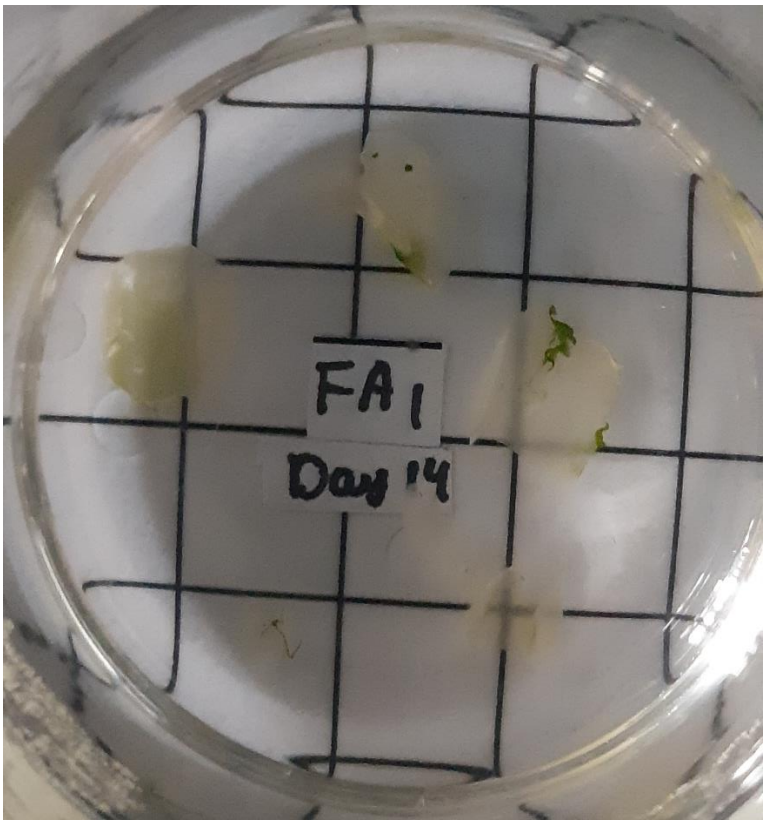
FA1 21-04-12 (day 11) Thallus tissue 200x



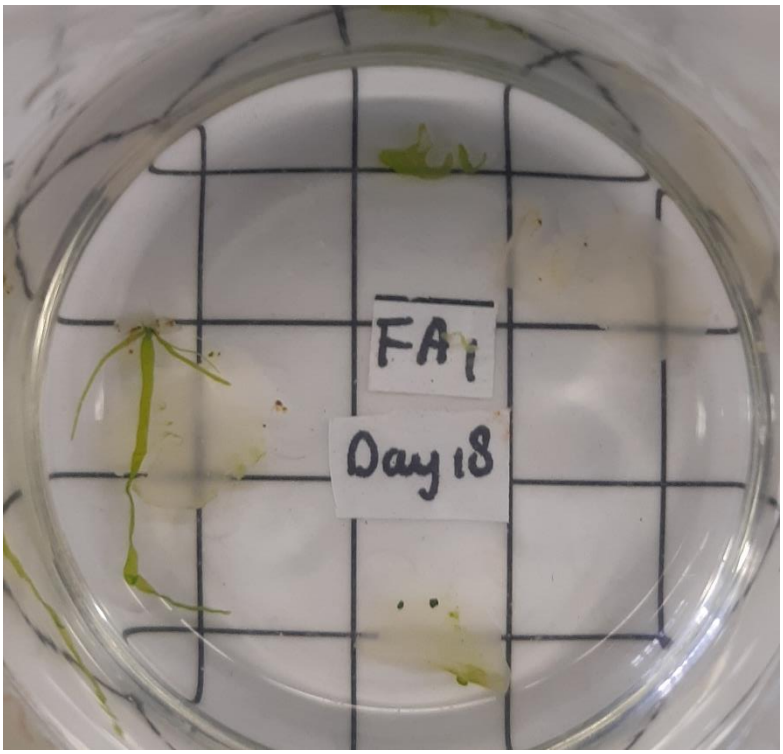
FA1 21-04-13 (day 12) One detail thallus, used for microscope



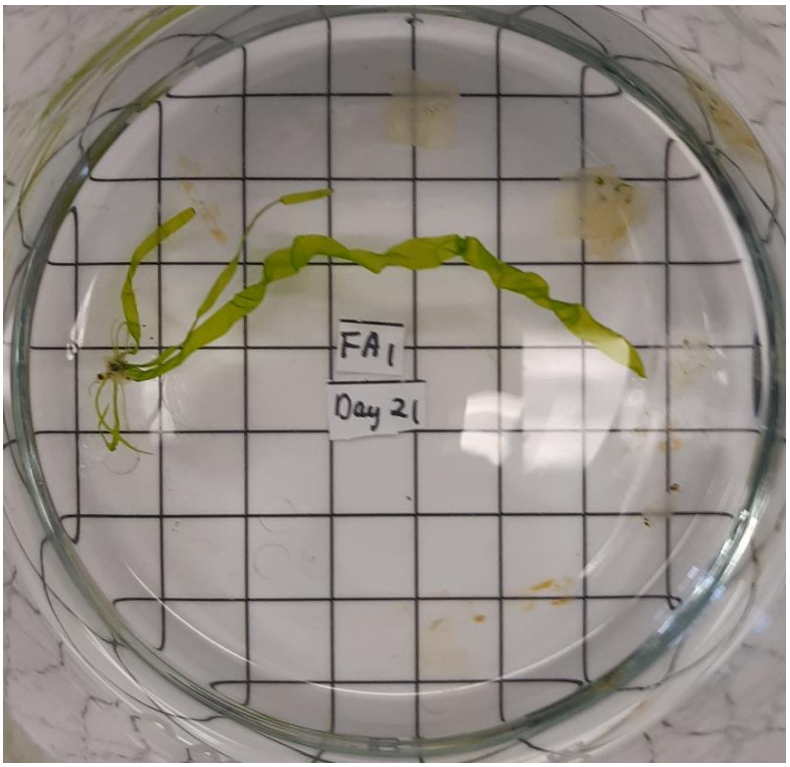
FA1 21-04-13 (day 12) Thallus tissue 400x Nikon 80i, no moving gametes or spores



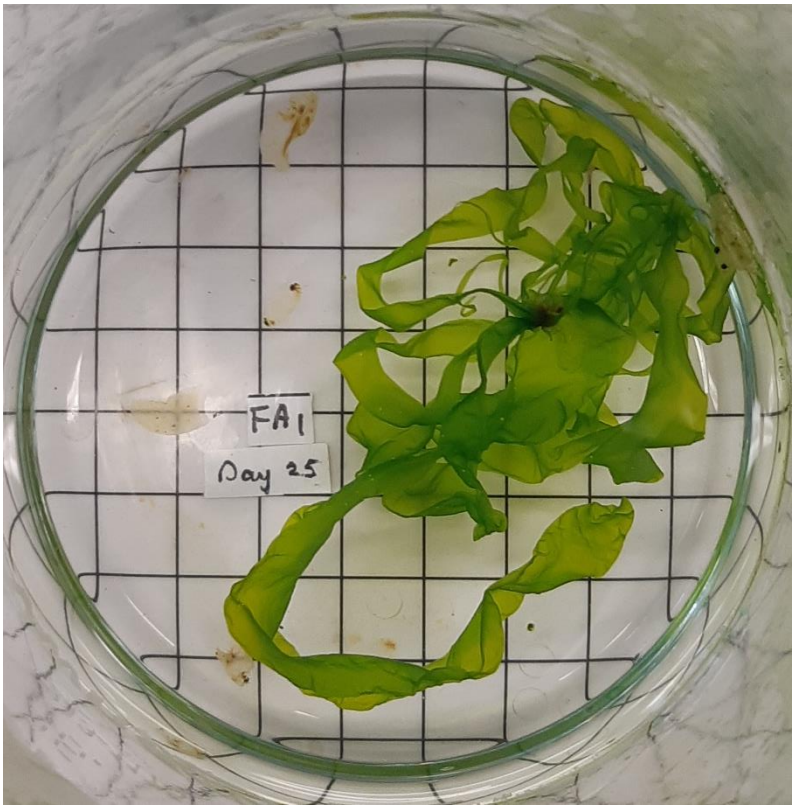
FA1 21-04-15 (day 14) overview



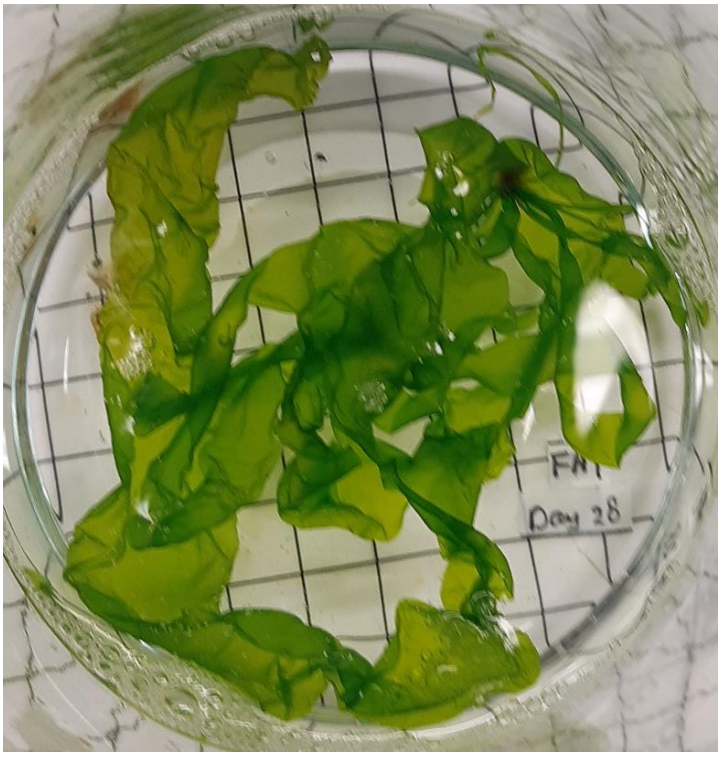
FA1 21-04-19 (day 18) overview



FA1 21-04-22 (day 21) overview



FA1 21-04-26 (day 25) overview

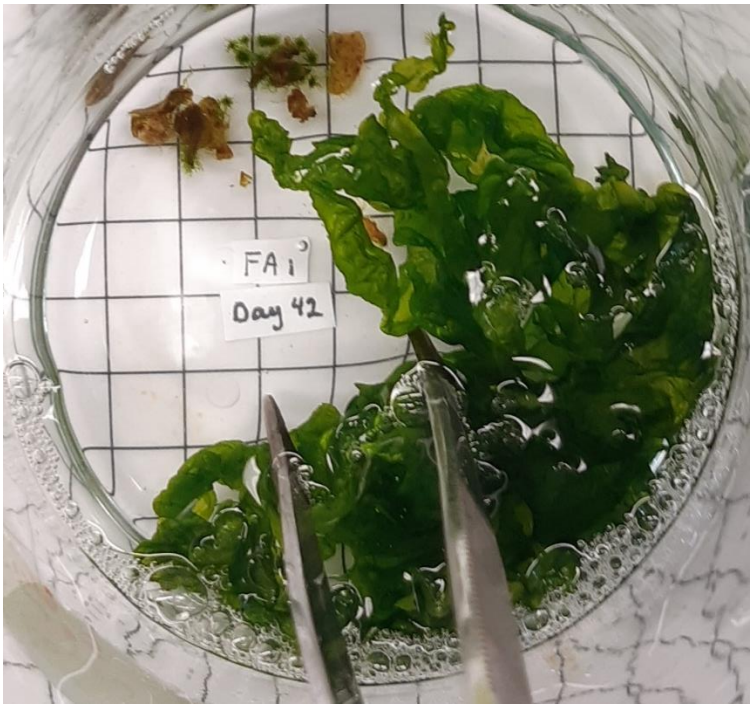


FA1 21-04-29 (day 28) overview, outgrow of gametes or spores

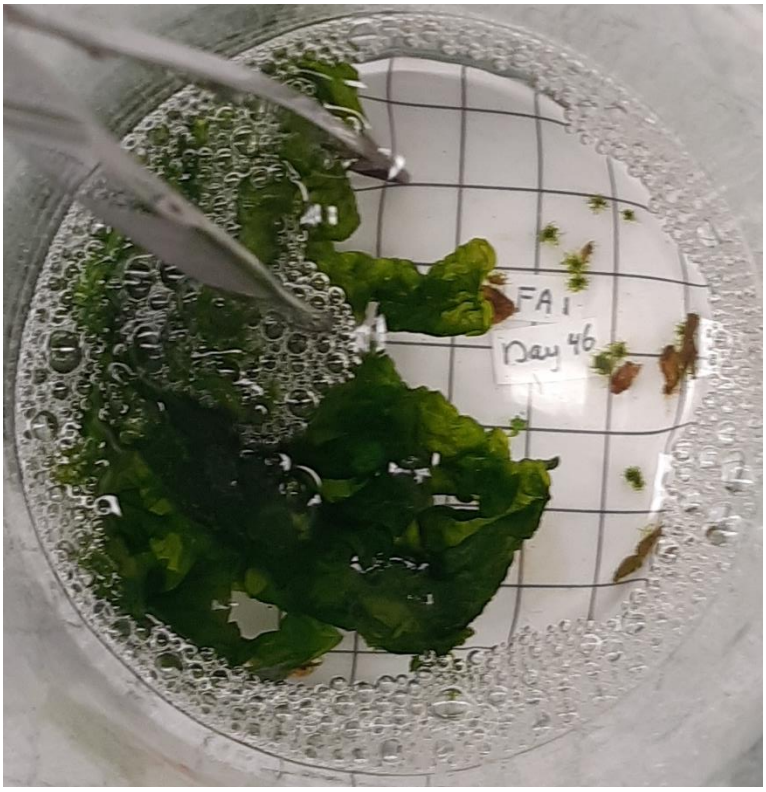
FA1 21-05-03 (day 32) overview, outgrow of gametes or spores

FA1 21-05-06 (day 35) overview, outgrow of gametes or spores

FA1 21-05-10 (day 39) overview, outgrow of gametes or spores



FA1 21-05-13 (day 42) overview, outgrow of gametes or spores



FA1 21-05-17 (day 46) overview, outgrow of gametes or spores

Annex 5 Data *Ulva* experiment 6

Table 5.1 Fresh weights on day 0 and day 21 of experiment 6.

Code	Pop.	Replicate	Stock_CellHIF2P (ml)	Location	1-11-2021 FW (g) day0	22-11-2021 FW (g) day21
A	FA160	1	0.2	w2	0.280	0.467
B	FA160	2	0.2	w2	0.452	0.766
C	FA160	3	0.2	w2	0.174	0.465
D	FA160	4	0.2	w2	0.306	1.424
E	FA160	1	0.4	w2	0.340	0.968
F	FA160	2	0.4	w2	0.361	0.510
G	FA160	3	0.4	w2	0.163	0.676
H	FA160	4	0.4	w2	0.162	1.310
I	V272	1	0.2	w2	0.755	1.881
J	V272	2	0.2	w2	0.141	1.979
K	V272	3	0.2	w2	0.107	1.604
L	V272	4	0.2	w2	0.114	1.057
M	V272	1	0.4	w2	0.281	2.653
N	V272	2	0.4	w2	0.325	1.175
O	V272	3	0.4	w2	0.189	1.389
P	V272	4	0.4	w2	0.070	0.449
Q	FA160	1	0.2	lk	0.151	0.262
R	FA160	2	0.2	lk	0.540	0.570
S	FA160	3	0.2	lk	0.126	0.324
T	FA160	4	0.2	lk	0.421	0.539
U	FA160	1	0.4	lk	0.150	0.218
V	FA160	2	0.4	lk	0.724	0.641
W	FA160	3	0.4	lk	0.203	0.262
X	FA160	4	0.4	lk	0.223	0.607
Y	V272	1	0.2	lk	0.532	0.519
Z	V272	2	0.2	lk	0.351	0.600
AA	V272	3	0.2	lk	0.222	0.483
AB	V272	4	0.2	lk	0.063	0.204
AC	V272	1	0.4	lk	0.286	0.270
AD	V272	2	0.4	lk	0.586	0.544
AE	V272	3	0.4	lk	0.102	0.290
AF	V272	4	0.4	lk	0.148	0.215

Table 5.2 Assessment sporulation or gamete formation had occurred, on day 3, 7 and 10 (experiment 6)

			4-11-2021	8-11-2021	11-11-2021
Code	**Pop.**	**Replicate**	**day 3**	**day 7**	**day 10**
A	FA160	1	not	not	not
B	FA160	2	not	not	very little bit green on bottom beaker
C	FA160	3	not	not	green on bottom beaker
D	FA160	4	not	not	very little bit green on bottom beaker
E	FA160	1	not	not	very little bit green on bottom beaker
F	FA160	2	not	little bit green on bottom beaker	very little bit green on bottom beaker
G	FA160	3	little bit green on bottom beaker	much green on bottom beaker	green on bottom beaker
H	FA160	4	not	not	some green on bottom beaker
I	V272	1	not	much green on bottom beaker	not
J	V272	2	not	not	very little bit green on bottom beaker
K	V272	3	not	not	very little bit green on bottom beaker
L	V272	4	little bit green on bottom beaker	not	very little bit green on bottom beaker
M	V272	1	not	much green on bottom beaker	very little bit green on bottom beaker
N	V272	2	not	not	not
O	V272	3	not	not	little bit green on bottom beaker
P	V272	4	not	not	not
Q	FA160	1	not	not	not
R	FA160	2	not	not	little bit green on bottom beaker
S	FA160	3	not	not	little bit green on bottom beaker
T	FA160	4	not	not	some green on bottom beaker
U	FA160	1	not	not	not
V	FA160	2	not	little bit green on bottom beaker	some green on bottom beaker
W	FA160	3	not	not	some green on bottom beaker
X	FA160	4	not	not	little bit green on bottom beaker
Y	V272	1	not	much green on bottom beaker	not
Z	V272	2	not	not	not
AA	V272	3	not	not	some green on bottom beaker
AB	V272	4	not	not	not
AC	V272	1	not	much green on bottom beaker	not
AD	V272	2	not	not	not
AE	V272	3	little bit green on bottom beaker	not	not
AF	V272	4	not	little bit green on bottom beaker	not

Table 5.3 Assessment sporulation or gamete formation and outgrow had occurred, on day 14 and 17 (experiment 6)

			15-11-2021	18-11-2021
Code	**Pop.**	**Replicate**	**day 14**	**day 17**
A	FA160	1	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
B	FA160	2	little bit of outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
C	FA160	3	not	little bit of outgrow spores or gametes on thallus
D	FA160	4	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
E	FA160	1	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
F	FA160	2	little bit of outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
G	FA160	3	little bit of outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
H	FA160	4	some green on bottom beaker	little bit of outgrow spores or gametes on thallus
I	V272	1	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
J	V272	2	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
K	V272	3	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
L	V272	4	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
M	V272	1	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
N	V272	2	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
O	V272	3	outgrow spores or gametes on thallus plus thallus outgrow	outgrow spores or gametes on thallus plus thallus outgrow
P	V272	4	outgrow spores or gametes on thallus	outgrow spores or gametes on thallus
Q	FA160	1	not	**no** outgrow spores or gametes on thallus
R	FA160	2	not	**no** outgrow spores or gametes on thallus
S	FA160	3	little bit green on bottom beaker	**no** outgrow spores or gametes on thallus
T	FA160	4	not	**no** outgrow spores or gametes on thallus
U	FA160	1	not	**no** outgrow spores or gametes on thallus
V	FA160	2	some green on bottom beaker	**no** outgrow spores or gametes on thallus
W	FA160	3	very little bit green on bottom beaker	**no** outgrow spores or gametes on thallus
X	FA160	4	some green on bottom beaker	**no** outgrow spores or gametes on thallus, piece of nice thallus visible
Y	V272	1	very little bit green on bottom beaker	very little bit of outgrow spores or gametes on thallus
Z	V272	2	very little bit of outgrow spores or gametes on thallus	very little bit of outgrow spores or gametes on thallus
AA	V272	3	little bit green on bottom beaker	little bit green on bottom beaker plus little bit outgrow spores or gametes
AB	V272	4	very little bit of outgrow spores or gametes on thallus	little bit of outgrow spores or gametes on thallus
AC	V272	1	very little bit of outgrow spores or gametes on thallus	very little bit of outgrow spores or gametes on thallus
AD	V272	2	little bit green on bottom beaker	very little bit of outgrow spores or gametes on thallus
AE	V272	3	green on bottom beaker	green on bottom beaker, **no** outgrow spores or gametes
AF	V272	4	very little bit of outgrow spores or gametes on thallus	little bit of outgrow spores or gametes on thallus

Table 5.4 Assessment sporulation or gamete formation and outgrow had occurred, on day 21 (experiment 6)

			22-11-2021
Code	**Pop.**	**Replicate**	**day 21**
A	FA160	1	outgrow spores or gametes on thallus
B	FA160	2	outgrow spores or gametes on thallus
C	FA160	3	outgrow spores or gametes on thallus
D	FA160	4	outgrow spores or gametes on thallus
E	FA160	1	outgrow spores or gametes on thallus
F	FA160	2	outgrow spores or gametes on thallus
G	FA160	3	outgrow spores or gametes on thallus
H	FA160	4	little bit green on bottom beaker plus outgrow of spores or gametes
I	V272	1	outgrow spores or gametes on thallus
J	V272	2	outgrow spores or gametes on thallus
K	V272	3	outgrow of spores or gametes plus growth of thallus visible
L	V272	4	outgrow spores or gametes on thallus
M	V272	1	outgrow spores or gametes on thallus
N	V272	2	outgrow spores or gametes on thallus
O	V272	3	green on bottom beaker plus growth of thallus visible plus outgrow spores or gametes
P	V272	4	outgrow spores or gametes on thallus
Q	FA160	1	**no** outgrow spores or gametes on thallus
R	FA160	2	**no** outgrow spores or gametes on thallus
S	FA160	3	**no** outgrow spores or gametes on thallus
T	FA160	4	**no** outgrow spores or gametes on thallus
U	FA160	1	**no** outgrow spores or gametes on thallus
V	FA160	2	**no** outgrow spores or gametes on thallus
W	FA160	3	**no** outgrow spores or gametes on thallus
X	FA160	4	**no** outgrow of spores or gametes plus growth of thallus visible
Y	V272	1	very little bit of outgrow spores or gametes on thallus
Z	V272	2	very little bit of outgrow spores or gametes on thallus
AA	V272	3	little bit outgrow spores or gametes
AB	V272	4	little bit of outgrow spores or gametes on thallus
AC	V272	1	very little bit of outgrow spores or gametes on thallus
AD	V272	2	very little bit of outgrow spores or gametes on thallus
AE	V272	3	green on bottom beaker, **no** outgrow spores or gametes
AF	V272	4	little bit of outgrow spores or gametes on thallus

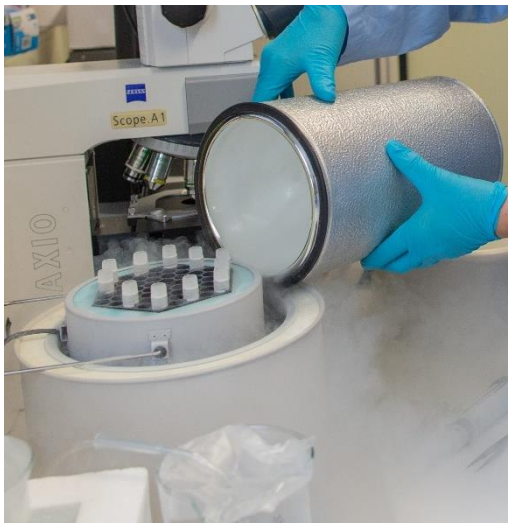
Annex 6 Data *Ulva* experiment 7

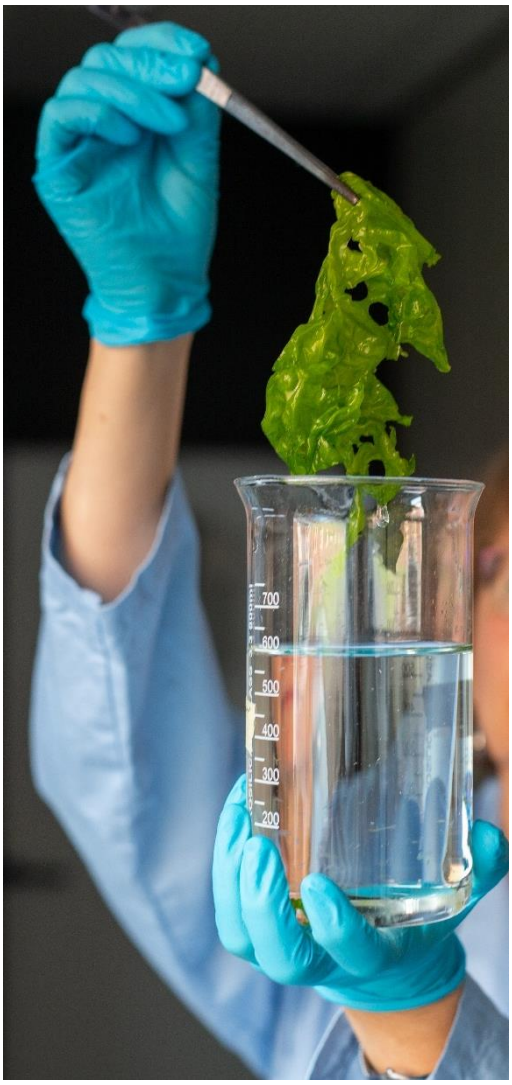
Table 6.1 Fresh weights on day 0 and day 21 of experiment 7.

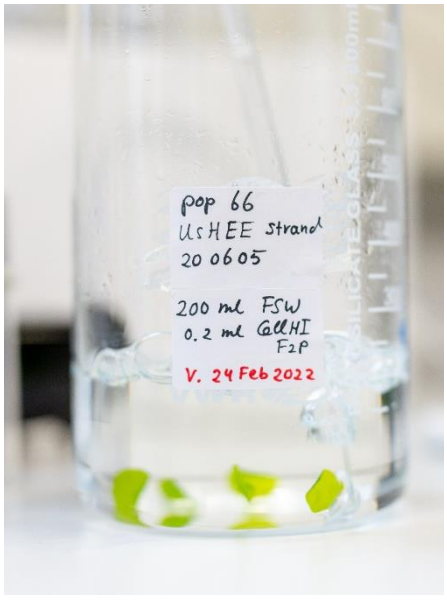
							Day45	Day66
Code-letter	**Code**	**Pop.**	**Replicate**	**FSW (ml)**	**Stock_CellHIF2P (ml)**	**Location**	13-1-2022 **FW (g) day0**	3-2-2022 **FW (g) day21**
A	AB6-1	Us-M-Slender211001	1	200	0.2	w2	0.004	0.581
B	AB6-2	Us-M-Slender211001	2	200	0.2	w2	0.092	1.433
C	AB6-3	Us-M-Slender211001	3	200	0.2	w2	0.095	0.710
D	AB6-4	Us-M-Slender211001	4	200	0.2	w2	0.146	0.240
E	AB6-5	Us-M-Slender211001	5	200	0.2	w2	0.054	0.104
F	AB6-6	Us-M-Slender211001	6	200	0.2	w2	0.067	1.249
G	AB6-10	Us-Zeebrug211014	1	200	0.2	w2	0.592	1.519
H	AB6-11	Us-Zeebrug211014	2	200	0.2	w2	0.553	1.709
I	AB6-12	Us-Zeebrug211014	3	200	0.2	w2	0.506	3.917
J	AB6-13	Us-Zeebrug211014	4	200	0.2	w2	0.153	0.793
K	AB6-14	Us-Zeebrug211014	5	200	0.2	w2	0.021	1.273
L	AB6-15	Us-Zeebrug211014	6	200	0.2	w2	0.262	0.485
M	AB6-18	Us-AM211011	1	200	0.2	w2	0.023	0.692
N	AB6-19	Us-AM211011	2	200	0.2	w2	0.416	1.609
O	AB6-20	Us-AM211011	3	200	0.2	w2	0.170	0.498
P	AB6-21	Us-AM211011	4	200	0.2	w2	0.181	0.363
Q	AB6-22	Us-AM211011	5	200	0.2	w2	0.085	0.201
R	AB6-23	Us-AM211011	6	200	0.2	w2	0.112	0.535
AK	AB6-7	Us-M-Slender211001	7	200	0.2	w2	0.220	1.836
AL	AB6-16	Us-Zeebrug211014	7	200	0.2	w2	0.160	0.557
AM	AB6-24	Us-AM211011	7	200	0.2	w2	0.081	0.126

Annex 7 *Ulva* cryopreservation photos

Photos Loek Buter







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