BIM Ireland's Seafood Development Agency

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Knowledge Gateway Scheme

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Bord Iascaigh Mhara Service Project

Report on BIM Seaweed Development Programme 2016

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BIM Seaweed Development Programme 2016 Final Report

BIM put the proposed 2016 BIM seaweed development programme to tender in late 2015 and Cartron Point Shellfish Ltd. (CPS) were awarded the contract to carry out a programme of work on both brown and red seaweeds. This work was carried out by Freddie O' Mahony CPS at the duly licensed Daithi O'Murchu Marine Research Station (DOMMRS) located at Gearhies, Bantry, Co. Cork.

This work is funded by the European Maritime and Fisheries Fund 2014-2020 (EMFF).

Specifically BIM wanted CPS to (1) cultivate 20,000 metres of seeded algal string collectors to meet the needs of the growing number of licensed seaweed farmers in Ireland (2) undertake a macroalgal training course and (3) include an investigation of the Irish coastline for the presence of asexual *Porphyra umbilicalis plants* and the cultivation of same under laboratory conditions.

Cultivation of *Alaria esculenta*

Alaria esculenta is a brown seaweed with a long non-digitate blade belonging to the order of Laminariales. It can grow up to 3 metres in length but much longer plants have been reported. The stipe is short with a narrow holdfast and the blade has a distinctive midrib. *Alaria esculenta* is found on rocky coastlines and is only exposed during good Spring tides. Reproductive spores are generally available from October to December but spores have been found in the South West of Ireland in March and April. The plant generally occurs in cooler waters and its distribution is controlled by an upper limit of approximately 16°C. The plants die back in summer and regeneration occurs in winter months.

CPS have been cultivating *Alaria esculenta* in a licensed seaweed hatchery at the Daithi O' Murchu Marine Research Station since 2004. Initial trials produced 1,000 metres of seeded string and this production has increased to a potential output of 20,000 metres in 2016. Traditionally mature sporophylls were collected in late September and October for sporulation and subsequent ongrowing. This system worked well when small amounts of seeded string were required. As demand for string increased it became imperative to start Spring sporulations in order to have adequate gametophyte cultures for spraying on to collectors in early Autumn. These sporulations were conducted during March and April 2016. Ripe sporophylls were collected adjacent to the hatchery during spring tides. These sporophylls were meticulously cleaned in the lab and partially dehydrated overnight at 10°C.

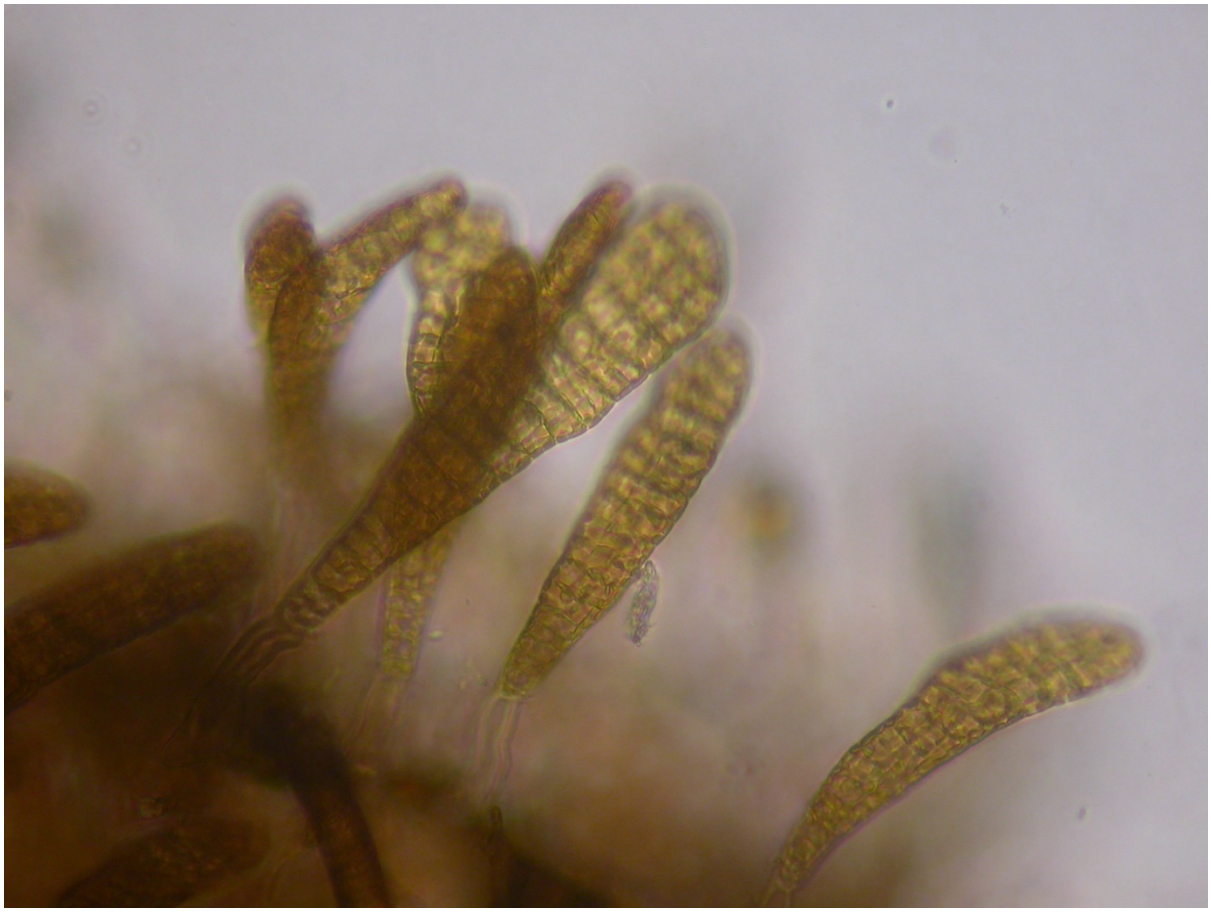
Photo. 1 Ripe sporophylls with darkened tips collected at Gearhies, Bantry in March 2016;

The following day the sporophylls were rehydrated with sterile seawater and spore release was instantaneous. This spore release normally lasted one hour. The spore solution was then strained on a 40 µm sieve and placed in a 3 litre flask containing sterile seawater and medium. This procedure was repeated multiple times in the spring to ensure that an adequate supply of culture was being held in the hatchery. Gametophyte cultures were maintained at 14°C under 24 hour artificial daylight. No ripe spring sporophylls were available after April 7th 2016.

Photo. 2 Inhibited gametophyte collection of *Alaria* cultures held under controlled conditions at DOMMRS in 2016

Careful culture monitoring and surveillance was essential. Post sporulation, the gradual development from spore to gametophyte was observed. This took about 28 days and regular monitoring was required to ensure that inhibition of fertilisation was achieved. Culture flasks were renewed every 8 days and transferred to larger vessels as the cultures grew. The cultures were also checked microscopically at each renewal stage to ensure there was no contamination. By September 2016 there were 65 litres of gametophyte culture in the lab. The first fertilization of an *Alaria* culture commenced on 12/09/2016. To induce fertilization, the temperature was gradually reduced to 10°C and the light regime was changed to 12: 12 light dark. After 9 days under these conditions the culture was then ready for spraying on to Kuralon string collectors. The first batch of collectors (5000 m) were sprayed on 20/09/2016. This process of fertilization and collector spraying was repeated 5 times at the DOMMRS. The sprayed collectors were held in 500 litre bins with UV treated filtered seawater at 10°C. The bins were renewed and cleaned every 6 days. Collectors were held for approximately 28 days in the lab and then transported to the sea sites for deployment. The string was sampled and checked microscopically to ensure that the ovoid zygotes and subsequent young plantlets had developed properly on the collector.

Photo. 3 Early stage *Alaria esculenta* sporophytes (magnification x 200) sampled from a collector string 20 days post spraying.



This staggered production of seeded collectors permitted the licensed farm sites to deploy the string in an orderly fashion from mid-October to December 2016. In earlier years the seeded string was only available for deployment in December when stormy weather quite often hampered the task of deployment. Almost 16,000 metres of seeded string was produced in DOMMRS. This string was distributed to six licensed sea sites in the West and South West of Ireland. Good weather conditions resulted in all the seeded string being deployed by mid-December. The string deployed in late October had attained an average length of 15 mm at sea by the end of December . The gametophyte culture remaining in the lab was used in a training programme which is described next in this report.

Further details on cultivating *Alaria esculenta* can be found in the BIM Aquaculture Explained Series No.21 Cultivation of Brown Seaweed *Alaria esculenta* written by John F. Arbona and Magali Molla

Macroalgal Training Course

A macroalgal training course was delivered at the DOMMRS station in Gearhies. It was specifically designed for people interested in cultivating macroalgal gametophyte cultures and producing seeded collectors for deployment at sea. The training was primarily practical in nature. The course commenced on a low spring tide where participants collected ripe *Alaria esculenta* sporophylls at a site adjacent to the lab. Participants then followed the protocol for preparation of sporophylls and conducted sporulations the next day.

These new cultures were maintained at the lab and subsequently fertilized and sprayed on to collectors. Two new method of spraying cultures on to collectors were demonstrated. The first method used a pressurized weed killer container. The process worked well and the overall cover on the collector looked good. The second process involved a commercial low pressure venturi operated sprayer. This was highly effective and has the advantage of needing only one operator to complete the task. Growth at sea using different collector spraying techniques will be assessed on farm sites.

All developmental stages were observed by participants and culture samples were made available to those who were in a position to cultivate them at their facility. Emphasis was placed on the necessity for the trainees to source ripe sporophylls in their own areas and to repeat the life cycle. Competency will only be acquired through practice. An outline of the training programme is shown below;

Macroalgal Training timetable 2016

Day 1 Oct. 16th

Start 10.30am sharp at DOMMRS, Gearhies, Bantry, Co Cork
General introduction to the seaweed hatchery.
Biology and life history of *Alaria*

Shore safety guidelines
Collection of ripe sporophylls on local shoreline and preparation in the lab

Day 2 Oct. 17th

Start 9.30 am at DOMMRS
Sporulation of Sporophylls
Maintenance of cultures
Microscopic inspection of cultures

Day 3 Nov. 2nd

Fertilization of culture
Collector production
Collector spraying
Collector maintenance

The feedback from the course was very positive and some participants are now preparing to conduct spring sporulations at their own facilities.

Asexual Porphyra umbilicalis Study

In Asia, *Porphyra* is an important part of the normal diet. China, Japan and Korea have a very extensive and lucrative *Porphyra* production industry. Growing demand and rising prices have encouraged many algologists around the world to try and develop cultivation protocols for this complex species. This current work concentrated on investigating the potential to cultivate asexual *Porphyra umbilicalis* in Ireland. The majority of *P.umbilcalis* plants in Ireland are sexual but asexual plants have been previously reported in Ireland (Professor Juliet Brodie pers comm). In general, the asexual plants are smaller and bushier in shape and have a slighter tougher texture. This may be due to the longer time period spent out of water and exposure to the elements. They tend to be found higher up on the shore and quite often are the primary colonisers on man-made structures such as piers and storm protection walls. However asexual and sexual plants cannot be differentiated by eye. The blade must be examined microscopically by cross section.

Beginning January 2016 numerous sites along the coast of Ireland were surveyed for the presence of *P.umbilicalis* populations. Searches in counties Clare, Kerry, Wicklow and Dublin were negative. Interestingly a site at DunLaoghaire which had a good population in 2015 was devoid of *P.umbilicalis* in 2016. The most detailed survey was conducted in West Cork and numerous populations of *P.umbilicalis* were found in an area stretching from Sheep's Head peninsula in West Cork and East to Clonakilty.

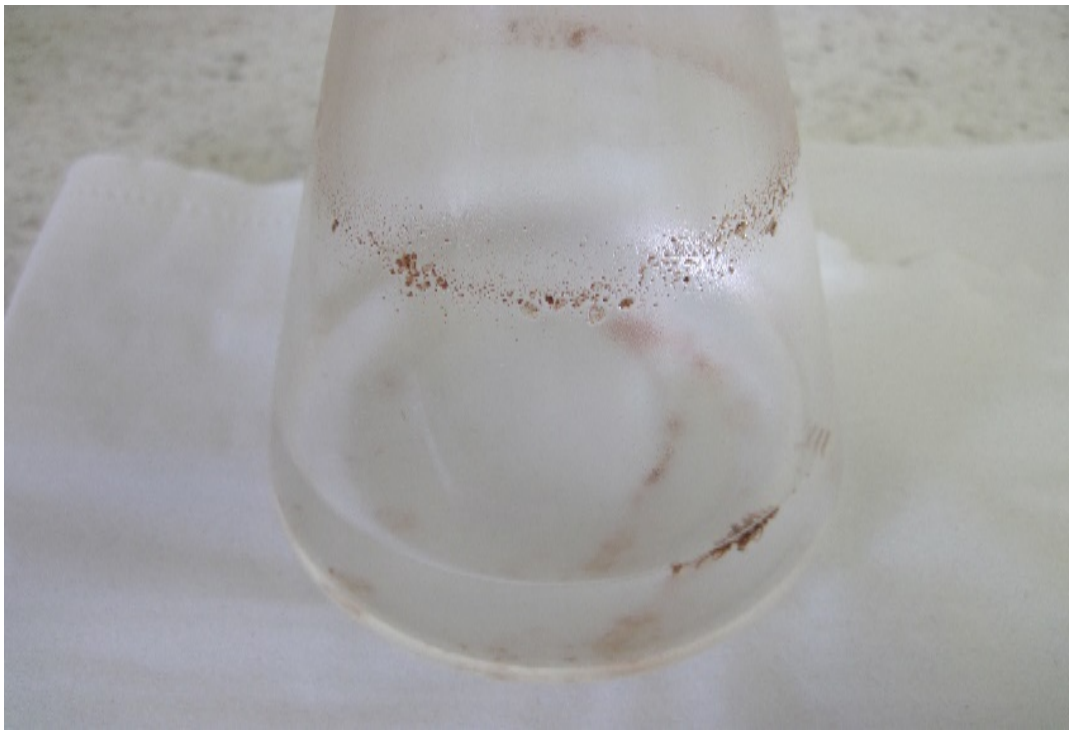
Photo. 4 Sexual *P.umbilicalis* West Cork, January 2016.

Once these populations were discovered a *Porphyra* workshop was held at the DOMMRS in February 2016 to train individuals in the identification of *P.umbilicalis* and differentiation between asexual and sexual plants. Sites in West Cork were assessed and sampled in the mornings and lab work conducted in the afternoons. Putative asexuals were held in flasks in the temperature control cabinets.

The most promising results were obtained from one very small isolated population in West Cork. Other sites with much larger *Porphyra* populations and plants very high up on the shore were not asexual. A small number of plants from two other locations looked asexual and fitted into the typical habitat of asexuals, being found at the top of the shore on new concrete and shore defences however they were finally confirmed to be sexual. We did experience difficulty in differentiating some plants. However by the end of the workshop we had identified 11 Irish asexual *P.umbilicalis* plants.

Following the workshop, the asexual plants were held in the temperature control cabinets at 10°C and 35 µmol light for 12 hours per day. The plants were vigorously aerated and kept in a West McBrides medium. The flasks were monitored daily and any changes observed. Some spore releases and matrix detachment occurred over the ensuing weeks. This material, where possible, was collected and settled on glass beads, slides or petri dishes where possible.

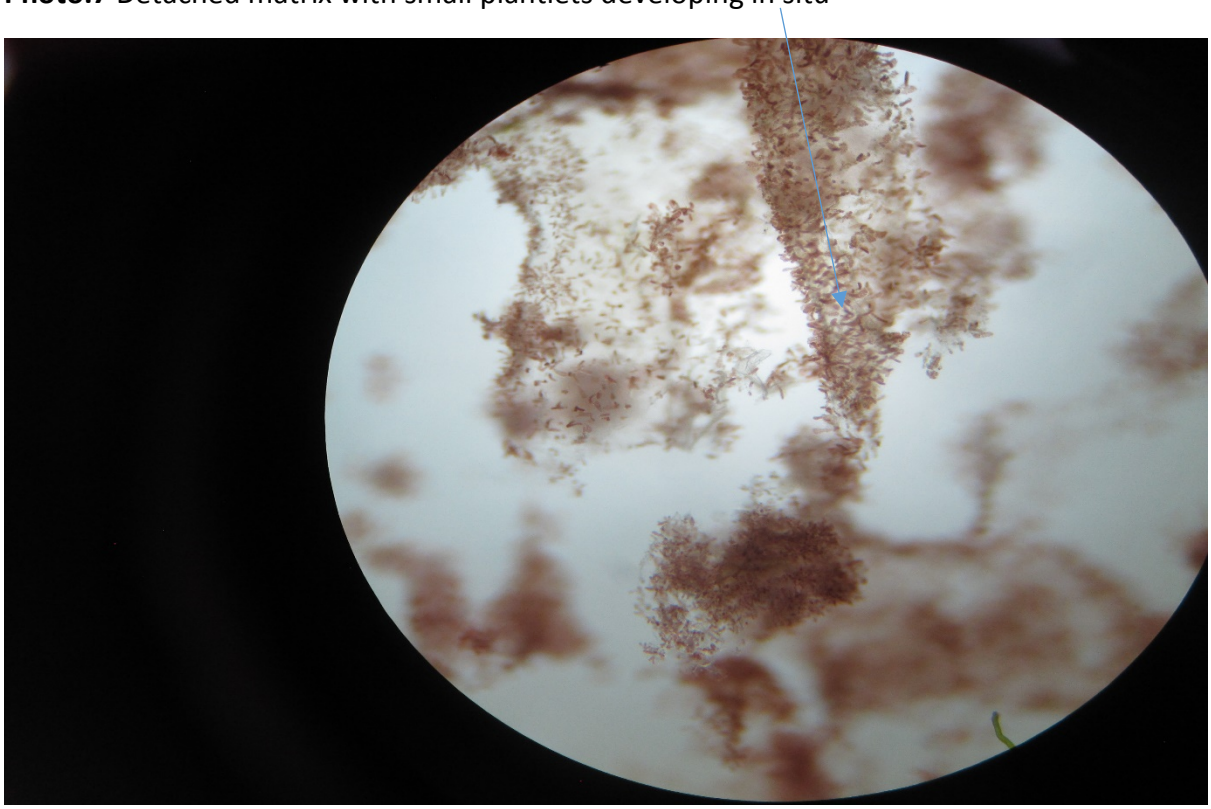
Photo. 5 Asexual spores on base and side of conical flask



The flasks were renewed on a weekly basis and plant margins checked microscopically. Plant margins, when suitable, were trimmed and dehydrated overnight for sporulation the following day. After 2 weeks in the lab the plant margins were more ragged and detached pieces of matrix and other decaying material were observed in the water column. The aeration was reduced and larger plants were moved to 1 litre flasks.

Photo. 6 Trimming *P.umbilicalis* margins

Photo.7 Detached matrix with small plantlets developing in situ



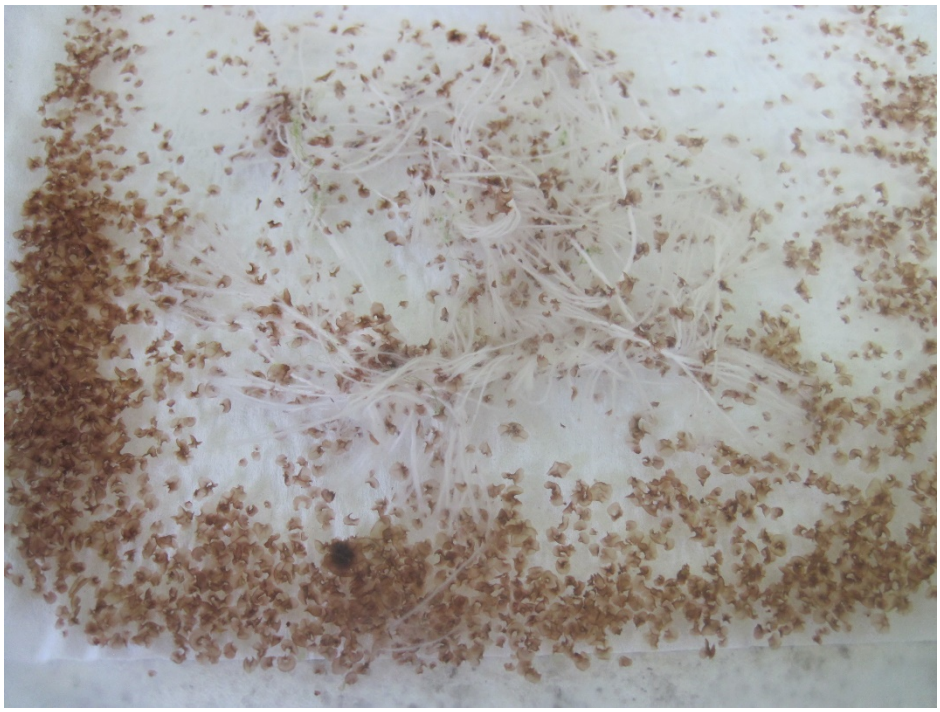
More asexual plants were collected and put into the cabinets on 9/03/2016. At present 7 asexual *P.umbilicalis* plants are being maintained at the lab in DOMMRS. Three plants are

from the first collection on 23/02/2016 and 4 plants are from a later collection on 9/03/2016. No definitive asexual plants have been found at any other sites to date. Gradually a methodology for holding these plants was developed. The plants are inspected microscopically once a week and transferred to a clean flask with West McBrides medium. Ragged margins and epiphytes are removed where necessary. Depending on plant growth the margins are trimmed and these cuttings are used for sporulations. Initially plant performance in the temperature control chambers was somewhat erratic and some plants just deteriorated slowly over time. Now we seem to have a reasonably stable broodstock population which are the source of spores for ongoing growth trials.

A series of sporulations have taken place from April to September. In general the best spore release appears to come from plants that have only been in the lab for a short period. For example, a lot of spores were released spontaneously from plants on Feb 18th after only 6 days in the lab. These spores have provided a large number of small plants which we have used in growth trials. A controlled sporulation of broodstock collected in the wild on 9/03/2016 was performed on 1/04/2016. This produced a good supply of spores which were then placed in petri dishes with sterile beads or Kuralon string. No plants developed on the beads. However good plant growth was observed on the Kuralon string. Two types of string were used in this experiment. String A was more hairy than string B which was more coarse to touch. The Kuralon string was moved to conical flasks on 10/05/2016 and monitored for 4 weeks. Detachment occurred in both flasks and eventually the Kuralon was removed on 8/06/2016. At this stage the majority of the plants had detached and some *Enteromorpha* contamination was visible on the string. Photo 8 shows plants attached to Kuralon and Photo 9 shows detached plantlets at the base of the flask.

Photo. 8 Plants attached to Kuralon

Photo. 9 Plants detached from Kuralon



Detachment occurred from both types of Kuralon string which does not bode so well for seeding nets, however the high density of settled spores originally on the Kuralon may also have contributed to the level of detachment observed in this experiment.

The original West Cork *P.umbilicalis* survey was conducted by Freddie O Mahony from Dec 2015-Mar 2016. During this period only one of the sites provided asexual plants. Every opportunity was taken to investigate other sites for *P.umbilicalis*. As the summer approached all the *Porphya* plants in the wild were very bleached and stressed which is quite normal for the time of year. It was not possible to differentiate between asexual and sexual plants in the summer.

Photo. 10 Bleached *P.umbilicalis* August 2016, West Cork.

New sites for *Porhyra umbilicalis* populations were identified in counties Donegal, Sligo and Galway over the summer of 2016. These sites will have to be revisited early 2017 to determine if any asexual plants occur at these locations. Many more sites require investigation as sites with asexual plants in 2016 may not provide asexual plants in subsequent years. This also proves the importance of keeping the asexual plants that we do have in safe conditions. In total, we have 6 of the original asexual broodstock plants in DOMMRS. We also have several hundred smaller F1 plants originating as spores from asexual plants collected in February and March 2016. *Enteromorpha* contamination has been a problem particularly in the broodstock but also in some F1 plants. Efforts to excise the *Enteromorpha* were not always successful. Heavily fouled broodstock plants were discarded. In the F1 populations vigilant microscopic work was quite effective in removal of contaminated plants.

A fungal infection was identified in mid-summer. Repeated washings in sterile seawater and partial drying with tissue paper was not effective. It did temporarily reduce the amount of fungus but it gradually grew back. We now finally appear to have the infection under control. This was achieved by weekly washing each individual flask of plants in fresh water. The initial immersion treatment time was 10 minutes and this was gradually increased to 20 minutes without any damage to the plants.

F1 *Porphyra* plant populations cultivated in the lab have been used in indoor growth trial experiments using 200 litre bins with a surface area of 0.3 m^2. The plants were grown under 12 hours light, with vigorous aeration and the addition of nitrate and phosphate. Temperatures fluctuated between 10 and 14°C as other biological activities were ongoing in the same cold room. The first growth experiment lasted 1 month and biomass increased from 34.2 g to 241.8 g (Figure 1)

These plants were then trimmed harshly and the second growth experiment lasted over 6 weeks and the biomass grew from 36.8 g to 473 g (Figure 2). A massive spontaneous

sporulation took place near the end of September. It was not possible to save the spores due to the heavy benthic diatom load in the water. However, it was reassuring to see such a large sporulation from F1 plants. This experiment could be replicated in a more controlled manner in the future to allow for seeding Kuralon nets with asexual spores.

The plants were trimmed again and grown for a further month. The biomass increased from 92g to 601g. (Figure 3)

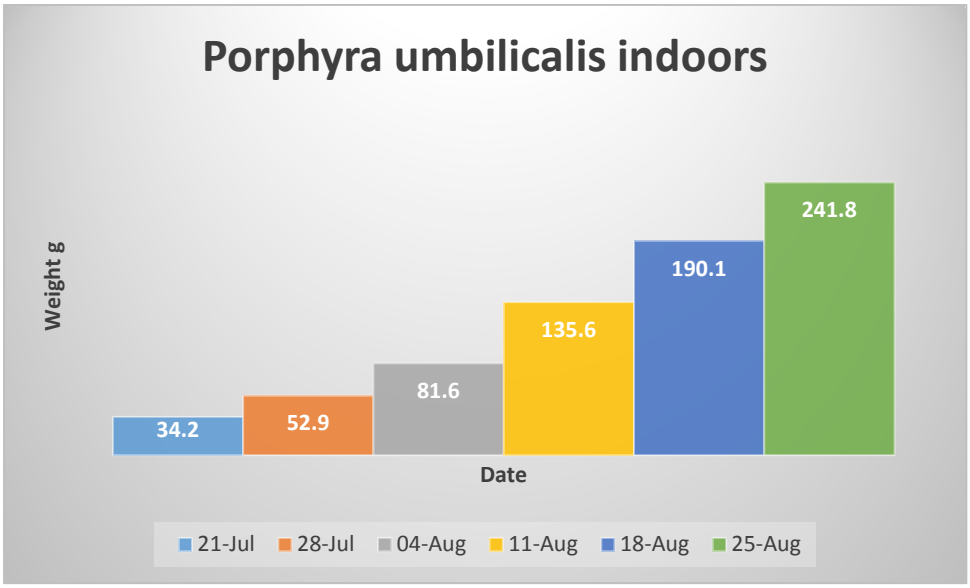


Figure 1. First growth experiment over one month in the lab using 200 litre bins with 12 hours light with vigorous aeration, addition of nitrate and phosphate and temperature in the range 10 to 14°C.

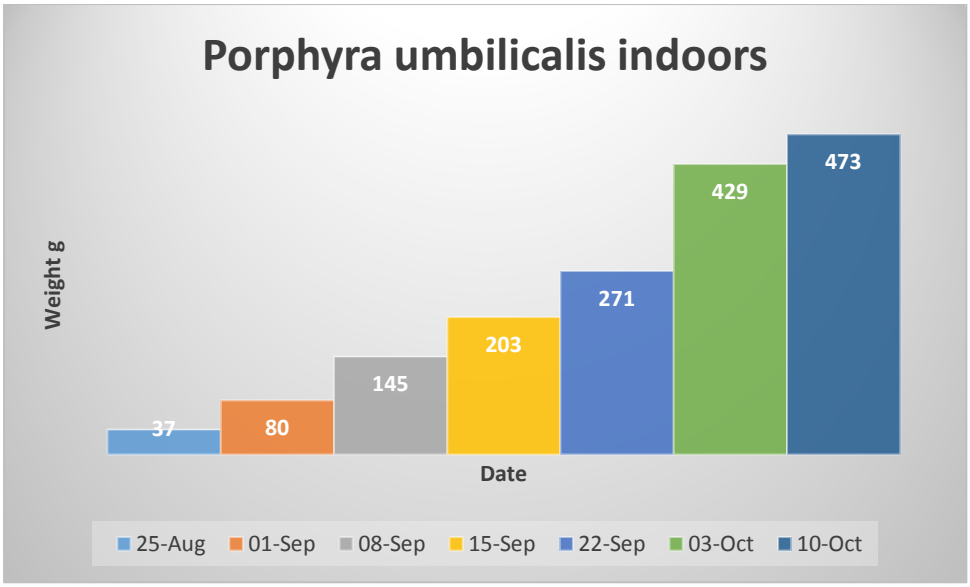


Figure 2. Second growth experiment of plants harshly trimmed over six weeks, a massive spontaneous sporulation took place at the end of September.

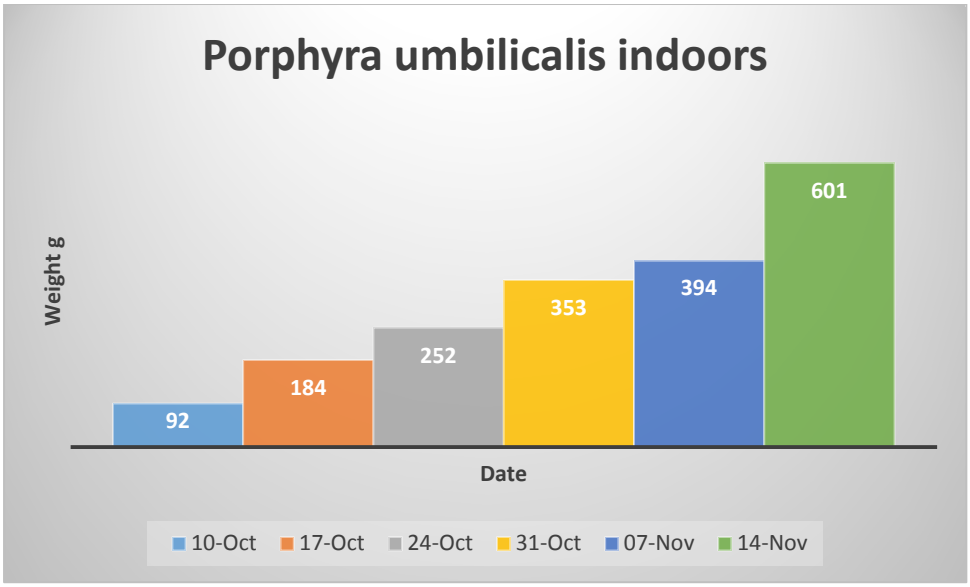


Figure 3. Third growth experiment of plants trimmed again over one month.

The indoor growth trials were terminated on 14/11/2016 to accommodate other macroalgal activities. Approximately 600 g of *P. umbilicalis* was transferred to an outdoor 500 litre bin with ambient flow through of approximately 3 litres per minute. Water temperatures varied from 5- 11°C. The plants adapted quite well to outdoor conditions and were severely trimmed on Dec 5th and returned to the bin (Figure 4). These trimmed plants had doubled in biomass in 9 days.

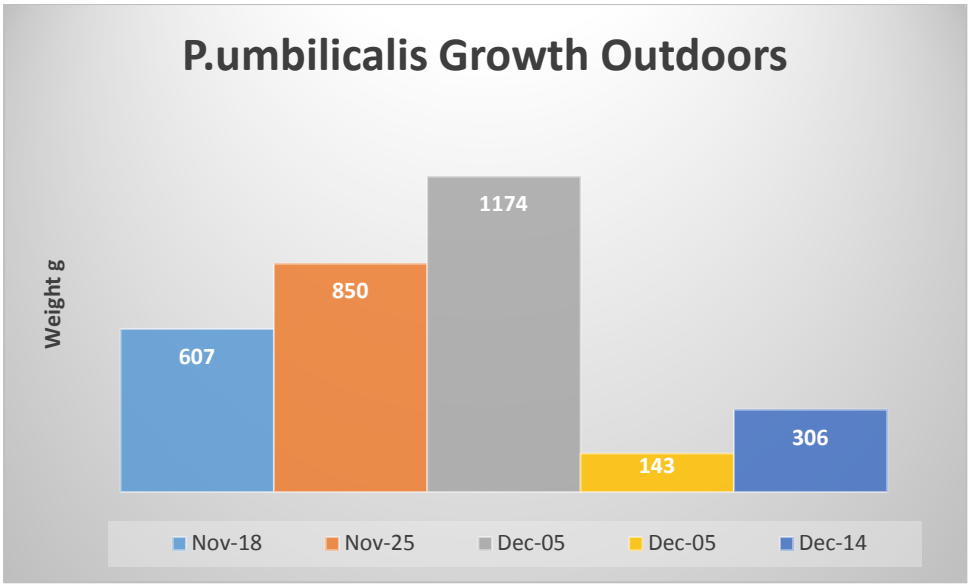


Figure 4. Fourth growth experiment in an out-door bin with ambient temperature ranging 5 to 11^{o}C.

Conclusion

Good progress has been made but more remains to be achieved.

The key areas for further research are;

- Sourcing more asexual plants in the wild.
- Performing controlled sporulations on to glass beads, string and nets.
- Conducting sea trials with seeded nets.
- Upscaling and improving indoor and outdoor growth trials.