Bord Iascaigh Mhara
Irish Sea Fisheries Board

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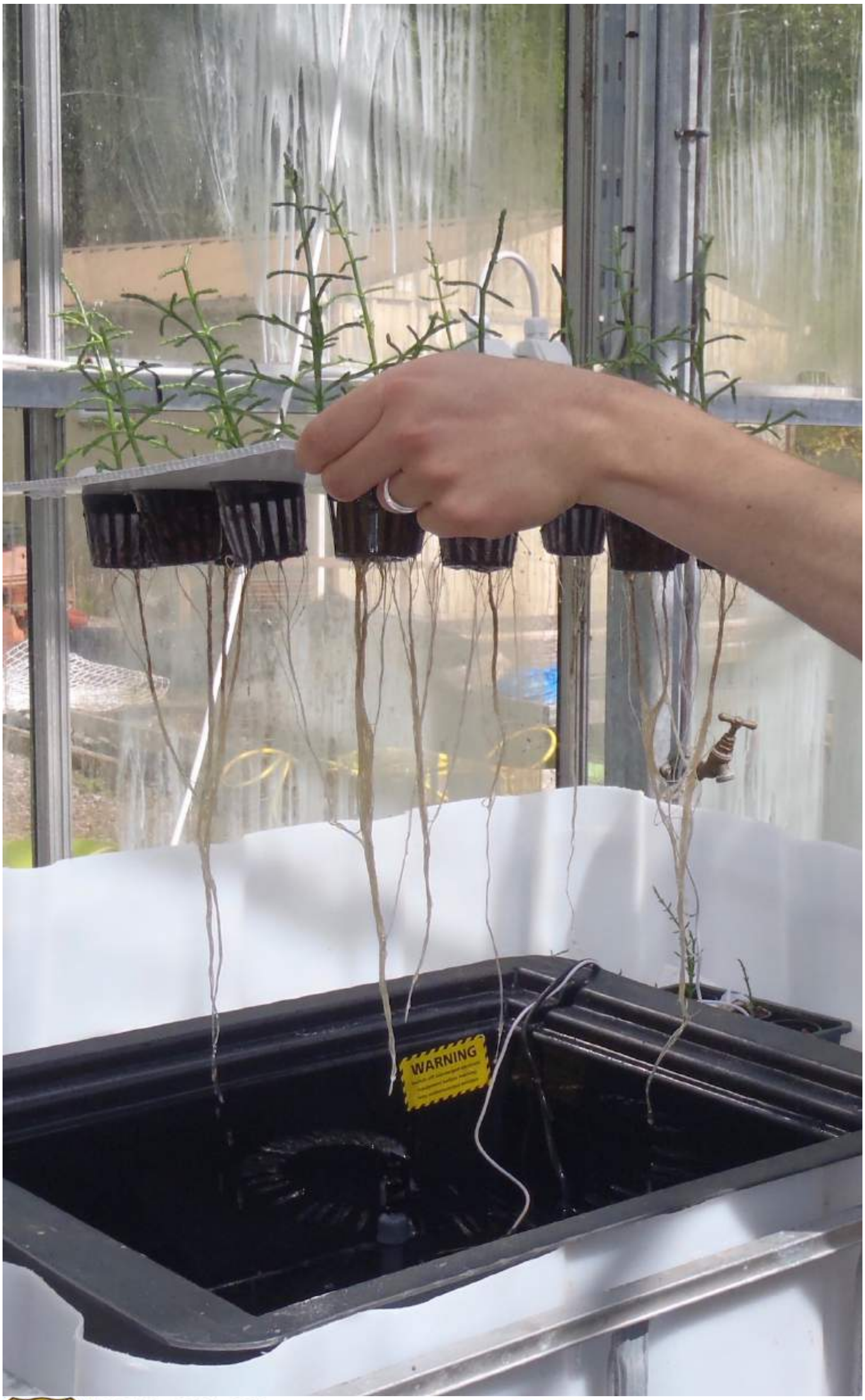
Cultivating *Salicornia europaea* (Marsh Samphire)



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Contents

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1. Introduction

1.1 Halophytes

Our planet is currently experiencing a crisis of dwindling fresh water supplies and salinization of soil and groundwater (Singh, *et al*. 2014 and Ventura and Sagi, 2012). This water shortage is expected to increase in the future due to a growing world population and rise in prosperity (De Vos *et al*. 2010). Already, almost one-third of the area farmed (380 million Ha) is affected by salinity (Ramani *et al*. 2006). With this in mind, it is essential that we develop new crops that have a greater salt tolerance than conventional agricultural crops (Ventura *et al*. 2011a). One option is to increase the salt-resistance of our salt sensitive conventional agricultural crops through conventional breeding programs or by developing genetically adapted plants. However, results from initial work on such techniques have been disappointing. Another option is to domesticate halophytes for commercial crop production (Ventura and Sagi, 2013). A halophyte is "a naturally evolved salt-tolerant plant that has adapted to grow in saline environments". In some cases halophytes require this exposure to salinity to survive (Singh *et al*. 2014 and Ramani, *et al*. 2006). Halophytes also have numerous commercial applications and potential, such as: raw material for vegetable or fodder, a source of oilseed with a high nutritional value, use as a biofuel precursor, and they can be used as secondary metabolites in pharmaceuticals, food additives, and nutraceuticals (Buhmann *et al*. 2015; Fan *et al*. 2013; and Liu *et al*. 2005).

Note: "Saltwort" is also a common name given to various genera of flowering plants that thrive in salty environments. Saltworts include plants from the following genera: *Salsola, Salicornia, Tecticornia, Sarcocornia, Suaeda,* and *Halogeton*

1.2 Marsh Samphire – Salicornia europaea

One such halophyte that has enormous potential in Ireland is Marsh Samphire (*Salicornia europaea* L.). Marsh samphire is common on the coastline of Ireland and the UK and has a long history of utilisation by humans. Over the past 5 years or so, it has gained huge popularity, especially in culinary circles, being a very popular addition to the menus of restaurants the length and breadth of the country.

1.2.1 Classification and description

S. europaea (Table 1) is a succulent annual (a plant that completes its life-cycle, from germination to production of seed, within one year, and then dies) halophyte with extremely reduced leaves (scale-like formations) and a spike-like terminal inflorescence (Singh *et al*. 2014). It stands erect, up to 35cm, and is fairly richly branched. It is dark green in colour, becoming yellowish green and ultimately flushed pink or red towards the end of its life-cycle (Figure 1) (Davy *et al*. 2001).

Table 1: Scientific Classification of *Salicornia europaea*

Kingdom	Plantae (Angiosperm)
Class	Eudicots
Order	Caryophyllales
Family	Amaranthaceae
Genus	*Salicornia*
Species	*Salicornia europaea* L.

Samphire plants produce minute flowers and under natural conditions usually produce them in August and September (Devlin, 2015d). There are usually 1-3 flowers per cyme (an arrangement of flowers in a plant inflorescence), with the lateral flowers one to two-thirds as large as the central flower. These flowers occur on the spikes (Figure 2) (Singh *et al*. 2014).

Although *Salicornia* spp. can grow successfully in highly saline environments, their germination is inhibited by high salinity and, generally, seed germination in European coastal halophytes occurs in early spring, when salinity is reduced by high freshwater soil moisture content and relatively low temperatures (Singh *et al*. 2014 and Khan and Weber, 1989).

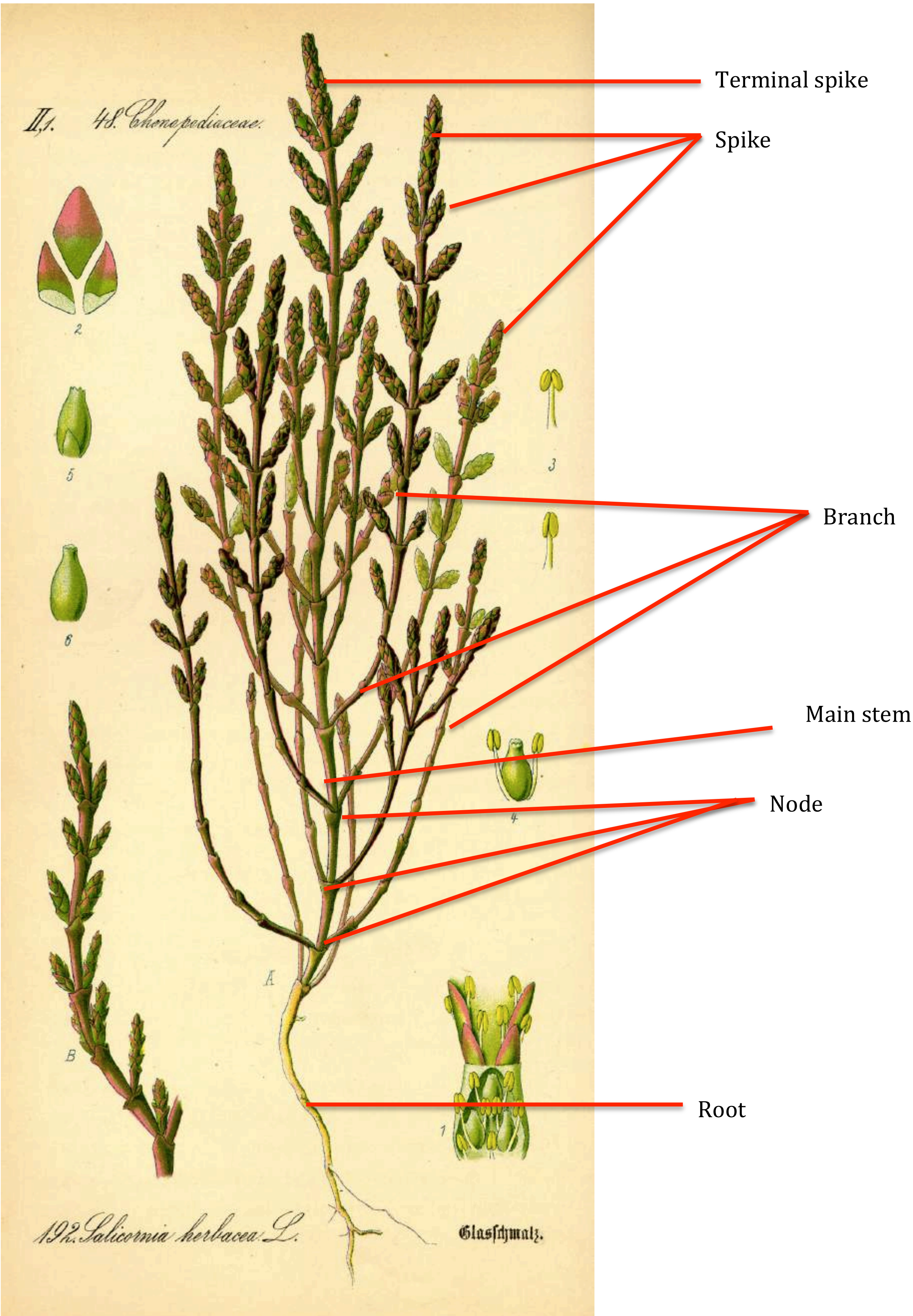

Figure 1: Morphology of marsh samphire (source: Biolib.de)

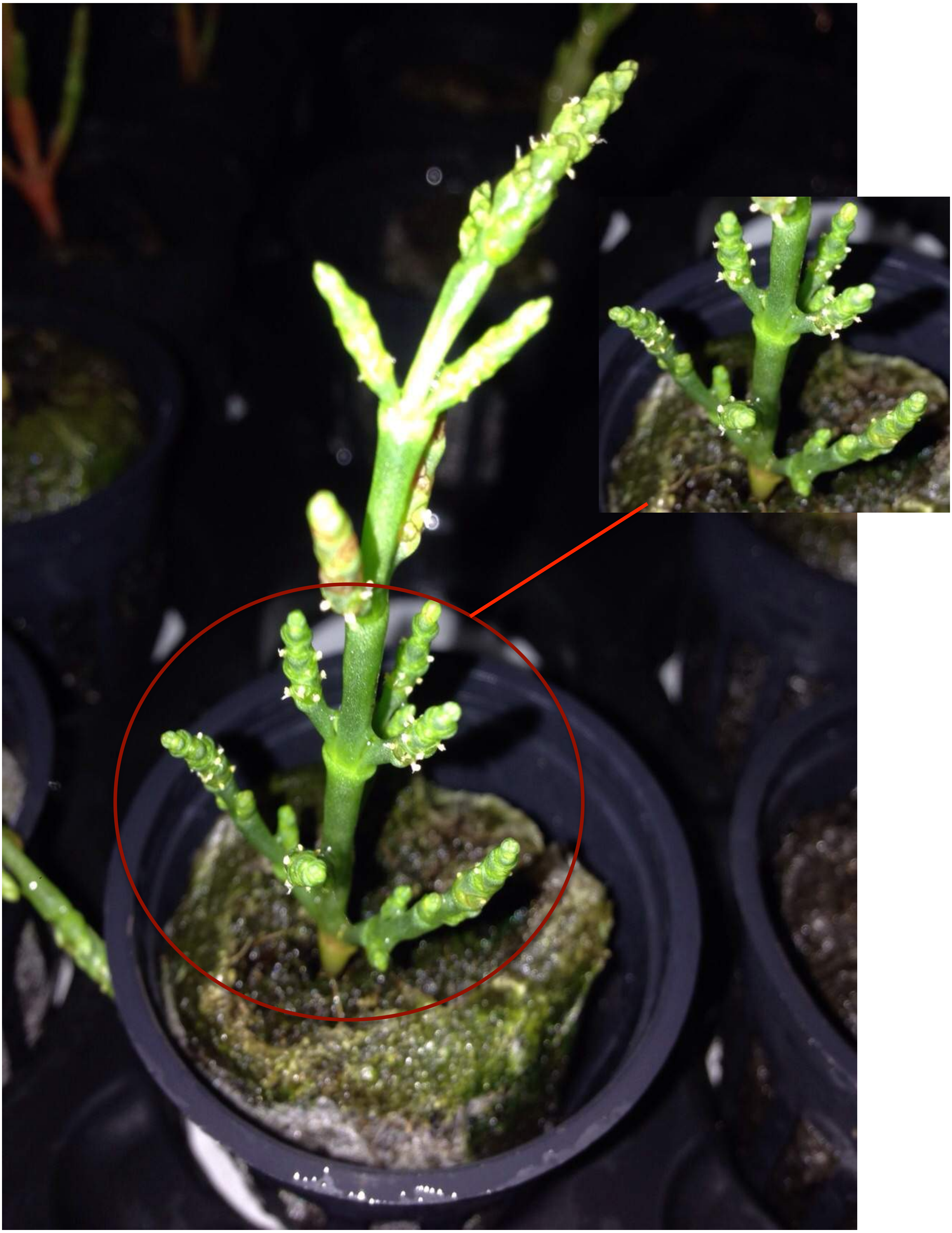

Figure 2: Flowers on the spikes of marsh samphire

1.2.2 Geographical range and habitat

The genus *Salicornia* is widely dispersed in Eurasia, North America, and South Africa (Singh *et al*. 2014). From a European context, *Salicornia* can be found on much of its coastline, from the Arctic to the Mediterranean and on the shores of the Black and Caspian Sea (Figure 3). It can also be found sporadically where inland saline waters occur across Europe (Davy *et al*. 2001). *Salicornia europaea* is the most widely distributed species in the *Salicornia* genus across the UK and Ireland (Figure 4).

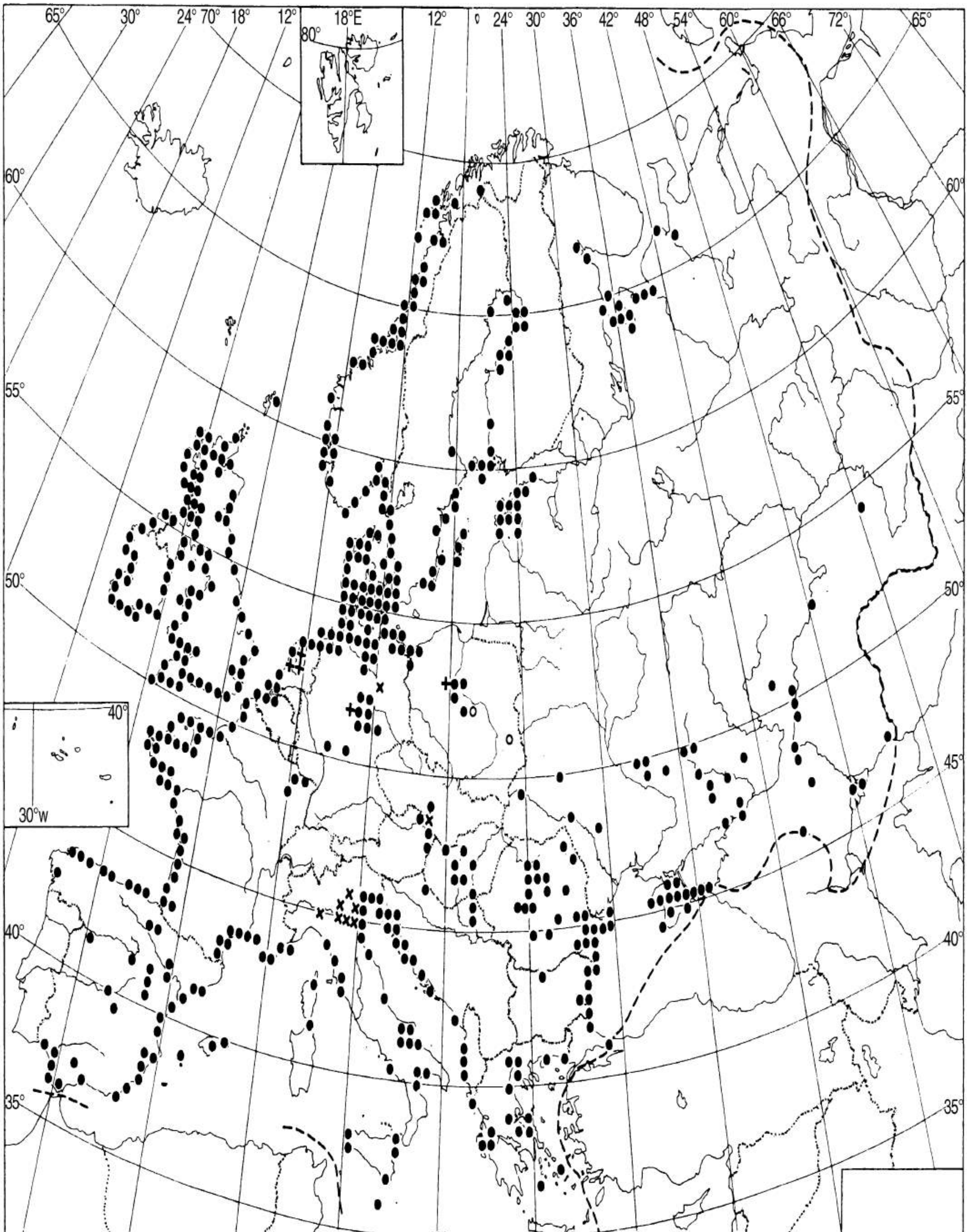

Figure 3: The distribution of *Salicornia europaea* aggregate in Europe. Each dot represents at least one record in a 50km square. (+) extinct; (x) probably extinct (Source: Davy *et al.* 2001)

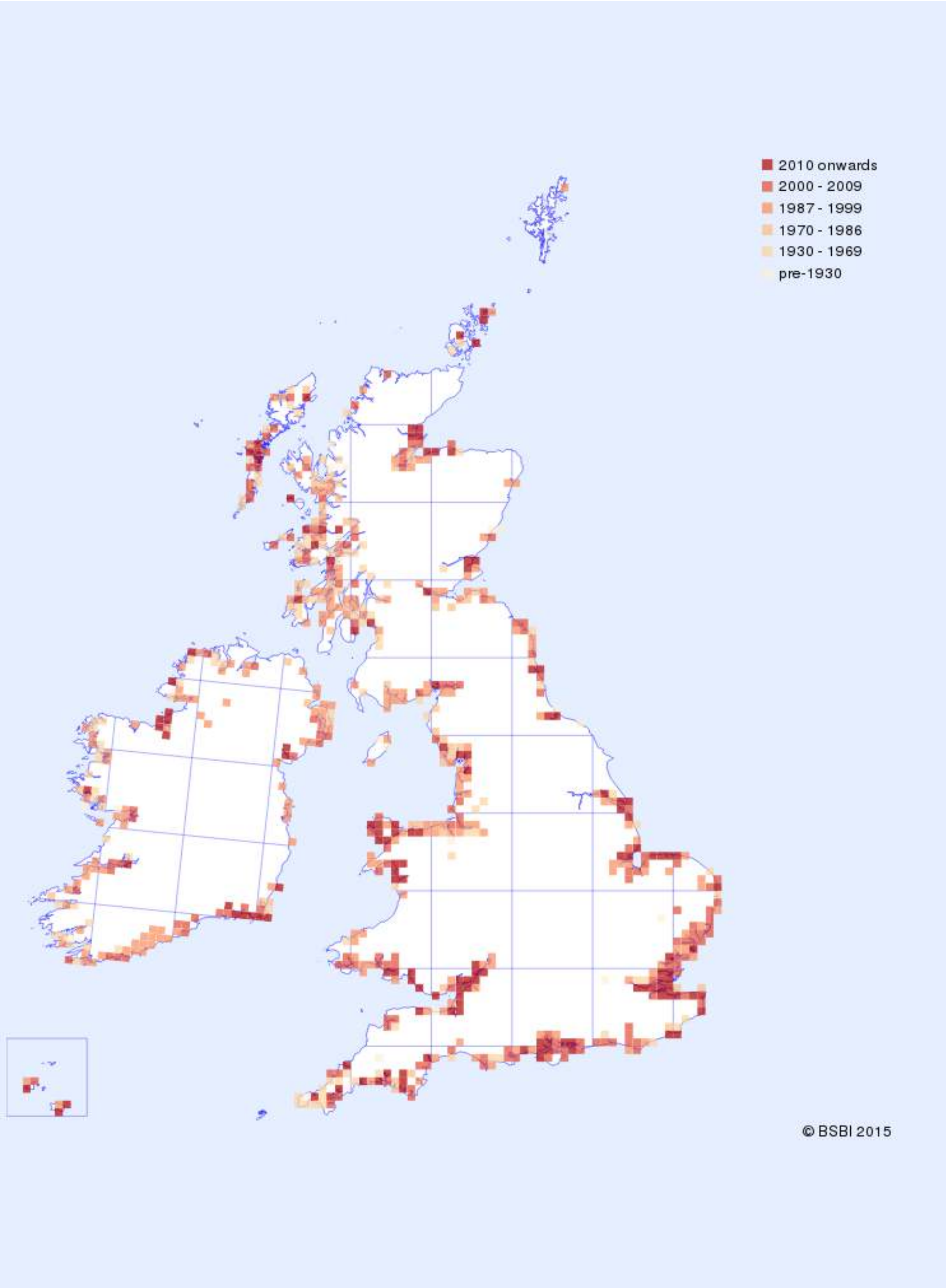

Figure 4: Distribution of *Salicornia europaea* across the UK and Ireland; cream squares represent recorded sightings from pre 1930; light orange/orange squares represent recorded sightings from 1930-2009; red squares represent recorded sightings from 2010 onwards (Source: BSBI 2015)

Marsh samphire can be found at all levels (low to high) of sandy and/or muddy saltmarshes, in the transitional area of saltmarsh to sand dunes, dune-slacks inundated with the tide, in channels and pans, mudflats, sandflats, and, on occasion, in open saline areas (e.g. behind sea-walls) (National Parks and Wildlife Service, 2014 & 2013, Davy *et al*. 2001 and Jefferies *et al*. 1981). Marsh samphire in intertidal habitats grows on a range of marine sediments; from silts to fine clays and in gravels and shelly-sand. On occasions, where marsh samphire is found in inland saline environments, the substrates can vary from fine clays to coarse sands. These substrates tend to be saline, brackish, or alkaline (Davy *et al*. 2001) (Figure 5).

Figure 5: Marsh samphire (outlined in red) in a saltmarsh; Fota Island, Co. Cork

1.3 Historical, current, and potential uses of Salicornia europaea

1.3.1 Glass and soap

In the 16th century, the word “glasswort” was coined to describe plants (wild or cultivated) growing in England that could be used for making soda-based glass (Oxford Dictionary, 1989). Marsh samphire is high in soda (sodium carbonate) and the burning of this plant releases sodium more easily than common salt. In medieval and early post-medieval centuries, marsh samphire was gathered and burned in heaps and the ash fused with sand to make glass. When a better quality glass was required, the ash was leached with limewater to make a solution of caustic soda, which was evaporated and added to the silica. Marsh samphire ash was also mixed with animal fats for its use in soap production. However, it is important to note that the term “Soapwort” does not refer to *Salicornia spp,* instead referring to the genus, *Saponaria* (Vernon, 2013). The appearance of “glasswort” as an English word in the 16th century coincided with the resurgence of English glassmaking, having experienced a decline after the Roman Empire era. Glassmakers who emigrated to England from Lorraine and Venice led this resurgence. These glassmakers, especially those from Venice whom were proficient in the technology of cristallo (producing immaculately clear glass by using soda ash as a flux), would have recognised marsh samphire as a source of soda ash (Kurinsky, 1991 and Haden, 1978). In 1790, the French chemist and surgeon, Nicolas Leblanc, developed an effective process for obtaining soda from sodium chloride (common salt). The Leblanc process dominated world production of soda ash for the remainder of the 19th century, bringing the era of extracting soda ash from saltwort plants to an end (Encyclopaedia of Britannica, 2015 and Clow and Clow, 1952).

1.3.2 Nutrition and culinary

Historically, many cultures across the globe have consumed marsh samphire for nutritional and culinary purposes; reflective in the multitude of common names it has been attributed: marsh samphire (English), glasört (Swedish), toongtoongmadi (Koren), zeekraal (Dutch), almyrides (Greek), and yan jiao cao and hamcho (Chinese) (Price, 2007). Marsh samphire has served as a functional food in colder coastal regions of Northern Europe, providing a high level of vitamin C in spring, following a winter in which few nutrient-rich vegetables would have been available (Price, 2007). In 1997, Guil *et al*. examined the nutritional components (carotenes, ascorbic acid {Vitamin C}, and dehydroascorbic acid {oxidised Vitamin C}) of various wild, edible plants, including marsh samphire. It was found that marsh samphire has very high levels of ascorbic and dehydroascorbic acid (over 100mg/100g) (Guil *et al*. 1997). The European Union's recommended daily allowance (RDA) of Vitamin C (80mg/day) could therefore be easily achieved by consuming approximately 80g of this vegetable (European Union, 2008). Marsh samphire is also a good source of carotenoids (c. 5mg/100g), which act as an antioxidant with strong cancer fighting properties (Guil *et al*. 1997).

The culinary interest in marsh samphire in the UK and Ireland has increased considerably over the past number of years. This tasty vegetable is commonly available at markets (e.g. the English Market in Cork City) and at various restaurants across the country, usually as an accompaniment to fish dishes. A testament to the popularity of this vegetable is the fact that famous Irish Executive Head Chef, Tom Walsh, named a restaurant after samphire, one of his favourite sea vegetables. Samphire at the Waterside, located in Donabate Co. Dublin, commonly has samphire on the menu. Marsh samphire has made its way onto the menu of many famous restaurants across Ireland, including but not limited to; Greenes and Elbow Lane in Cork City and Chapter One and Fade Street Social in Dublin.

1.3.3 Oil seed

When the fatty acid composition of marsh samphire seed oil was analysed by Liu *et al*. (2005), it was found that the oil was nutritive and the health value was high. There were five main components discovered: linoleic acid (75.62%), oleic acid (13.04%), palmitic acid (7.02%), linolenic acid (2.63%), and stearic acid (2.37%) (Liu *et al*. 2005). Austenfeld, (1986) also found that a high percentage of these fatty acids consisted of the unsaturated linoleic (77%) and oleic (13%) acid (Austenfeld, 1986). A study conducted by Glenn *et al*. (1991) on another species of *Salicornia*, *Salicornia bigelovii*, found that the yield of seed and biomass equalled or exceeded freshwater oilseed crops such as soybean and sunflower. The seeds were very low in fibre and ash (5-7%) and contained 26-33% oil and 31% protein. The oil was particularly high in polyunsaturated fatty acids, particularly linoleic acid, at 73-75% of the oil. Another benefit to using *Salicornia* seed as a source of oil seed is that the oil and meal can be extracted with standard milling equipment (Glenn, *et al*. 1991).

1.3.4 Forage/feed crop

Marsh samphire has the potential to be used as a livestock feed. Glenn *et al*. (1998) conducted a study in Arizona, USA and found that sheep and goats fed diets containing *S. bigelovii* gained as much weight as those fed a diet containing hay and the quality of their meat was unaffected by eating a diet rich in *S. bigelovii*. However, the feed conversion ratio (the amount of meat they produced per kilogramme of feed) was 10% lower than that of animals eating a traditional diet. They also found that *S. bigelovii* seed meal was a suitable alternative to conventional seed meals as a protein supplement in livestock diets (Glenn *et al*. 1998). Although this work was not conducted on *S. europaea* (marsh samphire), *S. bigelovii* is a very similar plant, within the same family as marsh samphire. Research conducted on *Salicornia* cultivation in Kuwait revealed that lambs had the best growth rate when fed a traditional alfalfa diet incorporated with 12.5% *Salicornia* (Abdal, 2009) (section 2.1).

1.3.5 Medicinal

Halophytes have developed several adaptive responses to living in extreme saline conditions; including the synthesis of several bioactive molecules (primary and secondary metabolites). A number of studies conducted on halophytes have indicated that many of these species have a high content of polyunsaturated fatty acids, carotenoids, vitamins, sterols, essential oils (terpenes), polysaccharides, glycosides, and phenolic compounds. These compounds have a number of medicinal properties, including: antioxidant, antimicrobial, anti-inflammatory, and anti-tumour activities, with application in the treatment of various diseases (e.g. cancer, chronic inflammation, atherosclerosis, and cardiovascular disorder) and ageing processes (Ksouri *et al*. 2011). There is growing interest amongst the medical-science community in the natural antioxidants that halophytes contain as they exhibit a strong biological activity. These antioxidants sometimes exceed the performance of many natural antioxidants from medicinal glycophytes (any plant that will grow healthily in soils with zero or low content of salt) or synthetic antioxidants, which have to be restricted due to their potential carcinogenicity (Suhaj, 2006). Halophytes have considerable potential in the areas of nutracueticals (the integration of nutrition and pharmaceutics - consumed as capsules, pills, and tablets) and functional foods (always consumed as ordinary food {when a phytochemical is included in food formulation, it is also considered a functional food}) (Ksouri, *et al*. 2011).

A study conducted by Rhee *et al*. (2009) showed that *Salicornia herbacea* (a synonym of *Salicornia europaea* {Asia}) contained the following compounds: tungtungmadic acid, quercetin 3-0-glucoside, and isorhamnetin 3-0-glucoside. These compounds show various pharmacological properties including: antioxidative, anti-inflammatory, and immunomodulatory activities (Rhee *et al*. 2009). In another study, Lee *et al*. (2006) isolated polysaccharides from *S. herbacea* and demonstrated their ability to activate macrophages. Macrophages are a type of white blood cell that engulf and digest cellular debris, foreign substances, microbes, and cancer cells, and play a significant role in the host's defence mechanism (Ksouri *et al*. 2011 and Lee *et al*. 2006). With these compounds, *S. herbacea* has a potential application in the treatment of constipation, obesity, diabetes, and cancer (Ksouri, *et al*. 2011).

1.4 Aquaculture - wastewater management

Although Ireland may not be experiencing a salinization crisis, the cultivation of halophytes as a commercial crop should be given serious consideration. Marine aquaculture farms can discharge large volumes of wastewater containing excreta, food waste, and dissolved metabolites such as organic matter, inorganic nitrogen, and phosphorous into surrounding waters (Webb *et al*. 2012). In 2014, the aquaculture industry accounted for 47% (51 million tonnes) of global human fish consumption. It has been predicted that this output will increase by 60-100% over the next 20-30 years as the world population grows and per capita fish consumption increases (Turcios and Papenbrock, 2014). Left untreated, aquaculture effluent has the potential to impact upon wildlife, tourism, and fisheries (Brown *et al*. 1999). High amounts of suspended organic solids can damage the gills of cultured and wild organisms. Also, when these organic solids become mineralised, ammonia is produced. Ammonia can be quite toxic to aquatic life and the level that animals can tolerate is species dependent (Buhmann and Papenbrock, 2013).

Although natural fish stocks and eligible coastal areas for aquaculture are decreasing, global seafood demand is increasing. In order to provide for this demand, while reducing subsequent environmental impacts, it is necessary to develop intensive inland fish cultures with efficient systems for wastewater treatment (Turcios and Papenbrock, 2014 and Buhmann and Papenbrock, 2013). In conventional inland aquaculture farms effluent is treated through mechanical methods. Although these methods are effective, they tend to be costly in terms of capital investment, energy consumption, and maintenance requirements (Webb *et al*. 2012).

Operating halophytes as a plant biofilter (use of living material to capture and biologically degrade pollutants) of marine aquaculture effluent is a low cost opportunity to mitigate potential negative impacts on the environment (Buhmann *et al*. 2015).

A recent study by Diaz *et al*. (2013) found that a number of halophytic species (*Salicornia bigelovii, Atriplex lentiformis, Distichlis spicata, Spartina gracilis, Allenrolfea occidentalis,* and *Bassia hyssopifolia*) grown under irrigation with saline drainage water over a 4-6 year period in the San Joaquin Valley of California grew very successfully and can effectively reduce saline drainage effluent.

Studies have also been conducted on the suitability of *Salicornia* spp. as a wastewater biofilter. Shpigel *et al*. (2013) demonstrated that a constructed wetland (CW) planted with *Salicornia persica* was effective in the removal of N, P, and total suspended solids (TSS) from a 1,000m^3 commercial, intensive, semi-recirculated aquaculture system growing 100 tonnes of gilt-head seabream (1-500g in size). It was estimated that about 10,000m^2 of wetland planted with with *S.persica* would be required to remove nitrogen and

TSS in wastewater during one year. This study also found that an average yield of 10,000m^2 of *S. persica* would be expected to produce about 28.8 tonnes (2.88 kg m^{-2} y^{-1}). The upper (edible) part constitutes approximately 80% of the yield, therefore, the marketable yield would be about 23 tonnes of fresh produce (Shipigel *et al*. 2013). Although using CWs for effluent treatment requires a relatively extensive area, a cost-effective analysis conducted by Cardoch *et al*. (2000) found that treatment by wetland costs approximately 75% less to the farmer than conventional onsite treatment (Cardoch *et al*. 2000). The use of a CW to treat aquaculture wastewater can be even more cost-effective if the wetland is planted with a crop that has market demand or potential market demand (Shipigel *et al*. 2013). The commercial application cost of CWs is estimated to be €0.20 per kg of fish produced. Therefore, the cost of the construction and operation of a CW for, for example, 500 tonnes of fish would be €100,000. With a conservative price of €6 kg^{-1} (fresh weight) the income from 23 tonnes of *S. persica* is expected to be €138,000 based on gross calculations. Another method for offsetting the cost of CW operations is to exploit it as a natural park or tourist attraction (eco-tourism) (Shipigel *et al*. 2013 and Sindilariu *et al*. 2008).

Marsh samphire (*S. europaea*) has also been shown to have significant potential in the treatment of aquaculture effluent. Webb *et al*. (2012) constructed a wetland filter bed planted with marsh samphire to evaluate its ability to treat the wastewater from a commercially operated marine fish and shrimp farm. The results demonstrated the effectiveness of a marsh samphire wetland in removing N and P from the wastewater, with 91-99% of influent dissolved inorganic nitrogen and 41-88% of influent dissolved inorganic phosphorus removed (Webb *et al*. 2012).

A marsh samphire growth trial was conducted from May to September 2015 using the wastewater from an Irish oyster hatchery to irrigate the plants (Section 5).

2. Commercial cultivation

The agricultural development of halophyte crops is still in its infancy, and due to its novelty, businesses involved in their culture are understandably wary about revealing too much information on their growth methods. Despite this, a number of examples of *Salicornia* spp. commercial production and/or trials are detailed in this section.

2.1 The Middle East

*I*n Israel, *Salicornia* is produced on a commercial scale for local and international markets (Ventura and Sagi, 2013), with a number of companies (e.g. Flora Export S.E Israel Ltd, Farmers Direct Ltd, Agrexco, and Bacto Sil Ltd) responsible for exporting Israeli-produced *Salicornia* around the globe (see Section 9.1 for information on these export companies). The cultivation of *Salicornia* is typically practised under simple nets or in greenhouses that occupy an area of approximately 0.5-1ha. In order to maintain the *Salicornia* at a high enough standard for market, the young shoots must be harvested manually, a labour intensive element that is critical to halophyte crop production. The most common and straightforward method for growing *Salicornia* in Israel is to cultivate it in native sand dunes watered with drip irrigation. This has been achieved successfully by some farmers in the Dead Sea and Ramat Hanegev regions (Figure 6a). When growing plants for vegetable production, only the fresh and tender parts of the plant are acceptable (Ventura and Sagi, 2013). Ventura *et al*. (2011b) developed a repeated harvesting regime (every 2, 3, or 4 weeks depending on the level of growth), by which the plants were cut (approximately 5cm above ground level – a “cutting table” height) for the first time when the shoots were approximately 10-15cm in height. After shoot re-growth, the plants undergo a number of repeated harvests, being cut back to the “cutting table” height (Figure 6b) (Ventura *et al*. 2011b).

Figure 6: a) *Salicornia* growing on native sand dunes watered via drip irrigation; b) sand dune culture after harvesting to 'cutting table' height (Source: Ventura and Sagi, 2013)

In areas of Israel where cultivation of *Salicornia* with native sand dune soil is not possible, growing systems detached from the native soil have been developed. Although these soilless systems which use inert (not chemically reactive) mediums (e.g. perlite or dune sand) are more labour intensive and costly, their irrigation regimes are more flexible in relation to the concentrations of salt that can be applied. Examples of soilless culture systems that have been utilised successfully in Israel for the culturing of *Salicornia* are: plastic sheet troughs containing an inert media (drip irrigation); raised beds containing an inert media (drip irrigation); floating units containing perlite; surface-flow constructed wetlands; and coconut-fibre-filled sleeves within a subsurface flow-through system (Figure 7) (Ventura and Sagi, 2013). Detailed information on these growth methods being utilised in Israel is not currently available.

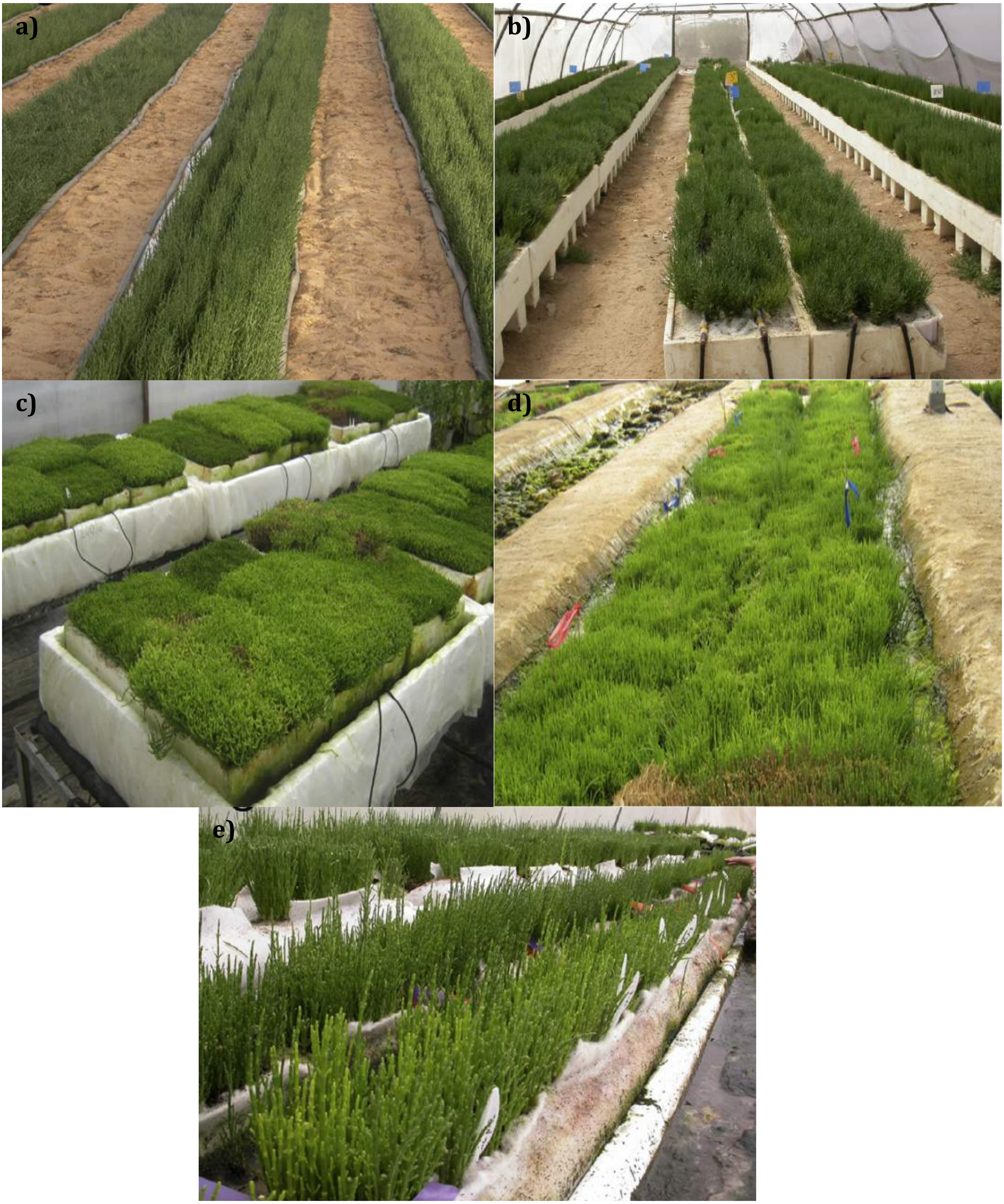

Figure 7: *Salicornia* growing in: a) drip-irrigated troughs built with plastic sheets (inert media); b) drip-irrigated raised beds (inert media); c) perlite filled floating units; d) a surface-flow constructed wetland; and e) a subsurface flow-through system (medium – coconut fibre sleeves) (Source: Ventura and Sagi, 2013).

The largest producer of *Salicornia* in Israel, "Ein Mor Crops Ltd" of Kadesh Barnea, has more than 15 years of experience culturing *Salicornia* in a greenhouse environment. They export over 150 tonnes of *Salicornia* every year to Europe through various export companies (http://www.tradekey.com/product-free/Salicornia-5032009.html).

The soil in Kuwait is sandy in texture and has a low nutrient content and water holding capacity. The availability of irrigation water for conventional crop production (glycophytes) is limited to desalinated seawater (very expensive to produce) and brackish water (too saline for conventional crop production). For agriculture to expand in Kuwait's difficult environment of salty waters, sandy soils, and harsh climates, the Kuwait Institute for Scientific Research believe plants that can tolerate the salinity and heat need to be researched for their potential as commercial crops. In cooperation with the University of Arizona, field trials on *Salicornia* spp. were conducted in Medairah, approximately 1km from the shore of Kuwait Bay. Fifty 20m X 8m plots were constructed for agronomic research trials. The salinity of the irrigation water for these trials was 25-33 g/L (close to the concentration of seawater). The biomass potential of *Salicornia* based upon different seeding dates and the potential for *Salicornia* as animal fodder was assessed. The total biomass (air-dried) achieved from all the plots was approximately 27 tonnes/ha (approximately 8 months after first seeding). These trials indicated that early October is the best seeding time for maximum biomass production, with plants seeded in October 1st or 15th having approximately 50% higher biomass than plants seeded on September 14th or November 1st. Plants seeded as late as January 1st had less than half the biomass of plants seeded in October (Note: The timing of *Salicornia* seeding for field production is dependent on local conditions and species of *Salicornia*). A 60-day feeding trial was also conducted on Australian lambs to assess how successful a diet incorporating *Salicornia* would be. They were fed 6 different treatments; ranging from 0-100% *Salicornia* "hay" incorporated into an alfalfa and/or feed concentrate diet. It was found that *Salicornia* can be successfully incorporated into the diet of lambs at a rate of up to 25%, however, the highest growth rate of lambs was achieved with 12.5% *Salicornia*. The lambs that were fed a 100% *Salicornia* diet had a net weight loss, indicating that it is not suitable as a lamb fodder unless it is mixed with other feed (e.g. alfalfa) (Table 2) (Abdal, 2009). Information on current *Salicornia* production and/or commercial trials in Kuwait was not available at the time of writing.

Table 2: Feed trial on Australian lambs (Abdal, 2009)

Treatment	***Salicornia* in diet (%)**	**Crude protein in diet (%)**	**Body weight gain (g/lamb/day)**	**Feed consumption (g/feed/day)**
1	0	10.6	93.7	1046
2	0	12.2	142.7	1040
3	12.5	11.7	154.7	1228
4	25	11.3	114.7	1076
5	50	10.4	45.4	895
6	100	7	-78.5	633

Early attempts at commercial cultivation of *S. bigelovii* was attempted by the Arabian Saline Water Technology Company (ASWTC) in 1993, when they established a c.300ha project at Ras al-Zawr, on Saudi Arabia's northeastern coast. The ASWTC were particularly interested in the high oilseed content of *S. bigelovii* (approximately 30% of the seed content). The *Salicornia* crop was irrigated by giant pivot-irrigation arms that sprayed seawater pumped from the Arabian Gulf. Although initial average output from the farm was not as high as expected, parts of the farm surpassed the goal of 10 tonnes of forage and one tonne of seed per hectare. Production at this site continued for several years, exporting *Salicornia* nationally and to Europe, however, it soon closed due to a lack of demand at the time (McGrath, 2010 and Clark 1994). As the need for governments and industries to seek low-carbon energy sources that do not compete with food crops for land and/or water resources continues to increase, the interest in halophytes as a source of agrofuel feedstock is at an all time high (McGrath, 2010). In 2012, the Abu Dhabi-based Masdar Institute of Science and Technology (MIST) established the Sustainable Bioenergy Research Consortium (SBRC), comprising of Boeing, Etihad, UOP Honeywell, General Electric, and Safran, to research sustainable aviation biofuels. Their flagship project, the Integrated Seawater and Agriculture system, couples the aquaculture of marine animals with the cultivation of *Salicornia*. Currently, a 2ha pilot facility (six aquaculture ponds, eight halophyte fields, and four mangrove swamps) is being constructed in the United Arab Emirates. For the next phase (commercial feasibility proof of concept) they plan to construct a 200ha demonstration facility in Abu Dhabi's western region. This site would have 140ha of *Salicornia*, 30ha of aquaculture, and 20ha of mangroves. It is hoped that this project will show that *Salicornia* could be a viable source of biofuel for aviation, with Lufthansa, KLM, and Etihad already trialling the biofuel (Hashem, 2015 and McGrath, 2010).

2.2 Mexico

Baja California Sur, Sonora, and Baja California are the most arid states of Mexico and are located in the northwest of the country. Baja California Sur, for example, has an annual precipitation of 80mm and some large areas can receive no rain for several years. Therefore, it is of considerable importance to introduce halophyte plants as an alternative crop into the local agriculture to ensure economic and social development and to conserve fresh water. The aridity of these regions in combination with the availability of saline water on the states' coastline creates an environment ideal for halophyte crop cultivation (Troyo-Diéguez *et al*. 1994). *S. bigelovii* occurs naturally in Mexico; the seeds of which have a potential as an alternative source of oil and flour, with a high oil and polyunsaturated fatty acid content (Troyo-Diéguez *et al*. 1994 and Glenn *et al*. 1991). Studies conducted by Troyo-Diéguez *et al*. (1994) in a natural salt marsh at the mouth of a seasonal stream in Baja California Sur found that *S. bigelovii* growth was positively correlated to soil organic content and negatively correlated with the percentage of sand as a component of texture and with the balance of sodium in the soil. The negative impact of high soil sodium levels on *S. bigelovii* was also shown in research conducted by Zerai *et al*. (2010) which found that biomass yields are reduced by about 40% when irrigated with seawater (35ppt) in comparison to 10ppt NaCl.

OceanDesertFood, the first commercial entity of the Organisation for Agriculture in Saline Environments (OASE) foundation (http://www.oasefoundation.eu/), has been importing Mexican *Salicornia* (*S. bigelovii*) into Europe since 1999. Careful breeding and selection has resulted in a plant that is large, sturdy, and has many branches. It is somewhat saltier than other varieties and contains less saponins and very little woody fibres, resulting in a better taste. OceanDesertFood cultivate this crop outdoors on a *Salicornia* plantation in Baja California without chemical pesticides and with minimum fertilisers. The crop is harvested by cutting off the tips, enabling 3-5 harvests of the same plant. The tips have an average length of 8-12cm. Importantly the Mexican climate allows for production when harvesting cannot take place in Europe (Figure 8) (http://www.oasefoundation.eu/project_sub/163; http://www.oasefoundation.eu/project/101).

Figure 8: Commercial *Salicornia* production in Baja California, Mexico (Source: http://www.oasefoundation.eu/)

2.3 Europe

In Europe *Salicornia* is either wild foraged, grown outdoors, or in greenhouses for supply, mainly, to local markets, greengrocers, restaurants etc. Therefore, information and data on the level of production achieved and methods of production employed are either scarce or non-existent. A brief overview of *Salicornia* production in Europe is given below:

There are 7-8 *Salicornia* growers in the Netherlands, mainly located in the Southwest. The "De Schorreblomme" company (the only producers in the Schouwen-Duiveland region) have been growing *Salicornia* outdoors for 5 years. Before De Schorreblomme occupied the property 6 years ago, *Salicornia* had been grown at the same site for 20 years. *Salicornia* grows wild in the Zeeland and Wadden Sea regions of the Netherlands. Samphire was historically foraged as a food source in the Netherlands, however, it is now a protected plant and the Dutch need a permit to cut *Salicornia* in the wild. De Schorreblomme sells to individuals, restaurants, and local supermarkets on the island region of Schouwen-Duiveland, with a (undisclosed) proportion designated for commercial trade. In August 2015, De Schorreblomme built a 2000 square foot greenhouse to expand their *Salicornia* production and growing season (http://www.schouwsezeekraal.nl/ and Fresh Plaza, 2015).

In France, 90% of *Salicornia* production occurs in the Bay of Somme, where professional "fishermen-by-foot" (a traditional method for gathering, for example, marine plants and shellfish on foot) have been collecting *Salicornia* from the region for generations. It is estimated that 400-500 tonnes is gathered per annum, being distributed nationwide and across Europe. Fishermen-by-foot require permits to collect *Salicornia* and the profession is framed by a system of co-management of professionals and the government to prevent over exploitation of the resource (Vlaams Institute, 2014 and http://www.ot-cayeuxsurmer.fr/en/discover/gastronomy/salicornia). The remaining 10% of production occurs in greenhouses, mainly on the western, Atlantic coast of France (Lucas Heitz, Alsagarden Nursery, personal communication, 2015). Data on France's *Salicornia* production levels was unavailable at the time of writing.

2.4 UK

Presently, the *Salicornia* market in the UK is primarily based on amateur gathering of branches from wild plants. As most natural wetlands are protected areas where harvesting is restricted or forbidden, the market supply is limited. The quality and quantity of wild collected *Salicornia* is also inconsistent and the product is neither clean nor uniform (Envirophyte, 2006). In England, marsh samphire (*S. europaea*) has been commercially harvested around the coasts of Essex, Norfolk, and Lincolnshire for generations. According to Sanderson and Prendergast (2002) only 5% of samphire supplied to the UK market is British, the rest being imported from France and the Middle East. Sanderson and Prendergast were not able to gauge the total annual harvest of samphire in England and Scotland, however, they estimated that there are over 100 pickers in Norfolk alone (Sanderson and Prendergast, 2002). In London approximately 50 fishmongers and specialist vegetable retailers sell samphire, though supplies tend to be quite erratic. Jefferson's seafoods, operating out of New Covent Garden Market, supplies many of these London shops, sourcing their wild foraged samphire mainly from Looe in Cornwall. They also source a lot of samphire from France, and, out of season, from Israel (Dimond, 2007).

In 2006, an EU funded project, Envirophyte, was initiated to assess "improvements to the cost effectiveness of marine land based aquaculture facilities through the use of constructed wetlands with *Salicornia* as an environmentally friendly biofilter and valuable by-product". As part of this project, Llyn aquaculture (based on the Llyn peninsula, North Wales) in collaboration with the University of Bangor (Wales), designed simple and effective constructed wetland biofilters to treat the discharge water from an on-land pilot turbot and shrimp aquaculture system. Through the use of horticultural polytunnels and specially designed culture beds, the project has developed techniques for propagation and production of high performing varieties of *S. europaea* over a summer season (April-October) (Figure 9). These constructed wetlands consist of layers of gravel and sand that allow for an effective circulation of water through the roots. During periods of low nitrogen flux, within each 24hr flood period, the wetlands removed almost 100% of the dissolved inorganic nitrogen in the wastewater used to irrigate the beds. Plants were trimmed every 3 weeks, generating a maximum of 4kg m^{-2} fresh weight yield per harvest (Envirophyte, 2011, and Llyn aquaculture, 2008). According to Llyn aquaculture, during the period of this project (2006-2009), they were the only UK producer of samphire (non wild foraged) (Llyn aquaculture, 2008). It is not clear if this is still the case or if they are still producing samphire on a commercial scale.

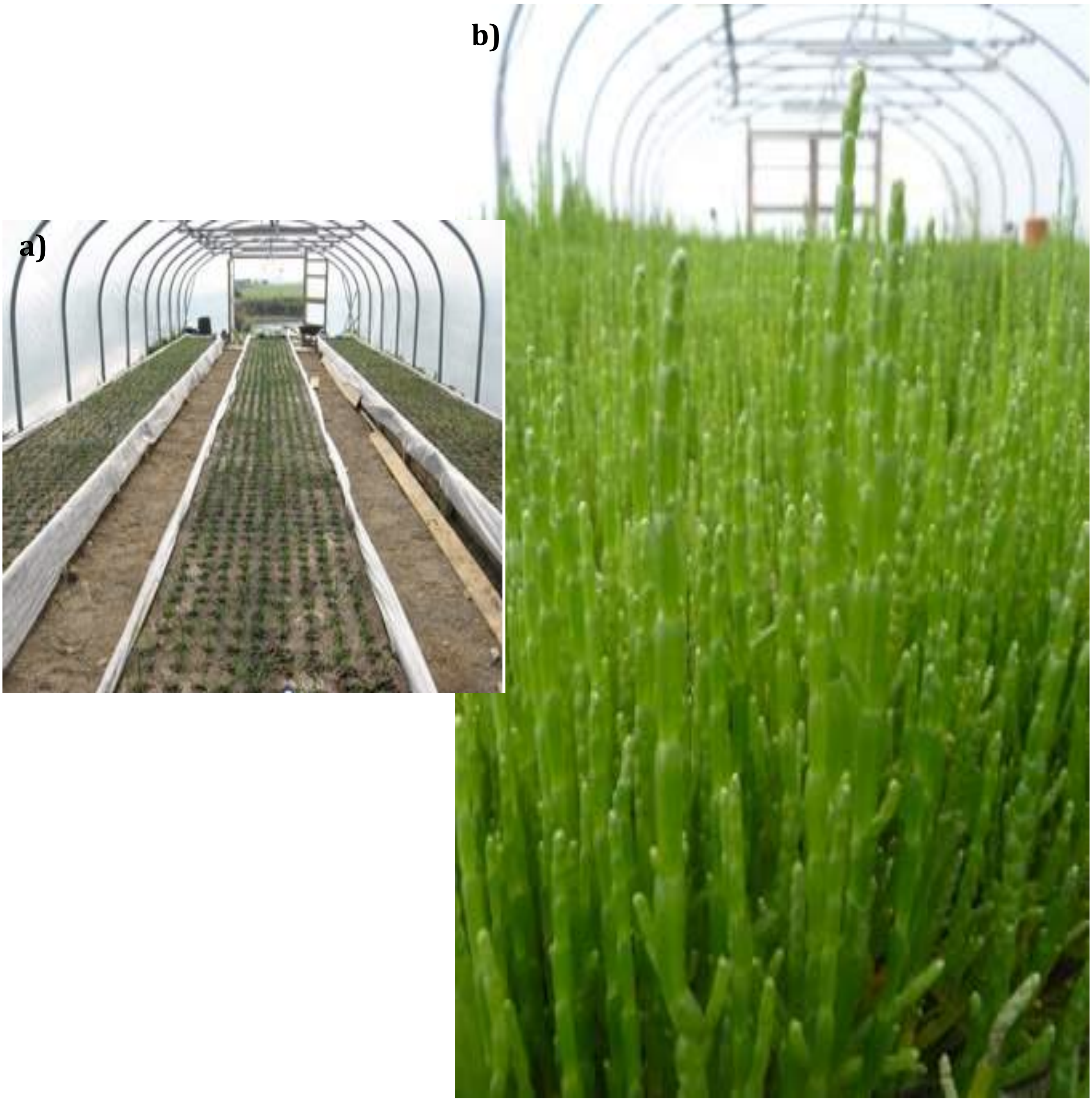

Figure 9: Llyn aquaculture's constructed wetland planted with *Salicornia*: a) at the beginning of the trial; and b) prior to harvest (Source: Llyn aquaculture 2008 and Envirophyte 2006)

2.5 Ireland

Although information on the commercial production and sale of samphire in Ireland is practically non-existent, personal communications (2015) with various Irish fishmongers and restaurants would indicate that a small proportion is sourced from wild foraging, with the majority imported from France, Holland, Mexico, and Israel. One Cork based restaurant sources approximately 20% of its annual biomass of samphire from wild foraging, while another used to source their samphire from wild collection until they switched to a fruit and vegetable wholesaler (Keelings - http://www.keelings.com/) a couple of years ago. Of all the fishmongers contacted, none reported sourcing samphire through wild foraging in Ireland, however, it is possible that other Irish fishmongers source samphire in this manner. Another potential source of samphire for the Irish consumer is small-scale locally foraged produce for sale by roadsides and at local markets during the summer season. The level at which this occurs was not measurable. Table 3 gives an overview of the commercial sale of samphire in Irish fishmongers. It must be noted that these figures are subject to change, with many retailers contacted indicating that "the prices vary greatly" and that the selling price to the consumer was "dependent on the cost price achieved at the time". One restaurant that was contacted stated that they are generally charge €23/kg for their imported samphire, however, this was the only business that quoted a cost price this high and they did not reveal their supplier (Personal communication with various Irish fishmongers and restaurants, 2015*).

The appearance of samphire in Irish retail outlets and restaurants seems to be restricted to summer months, despite the availability of imported *Salicornia* in the off season form the Middle East and Mexico. This would suggest that most retailers are sourcing their *Salicornia* from Europe, which is seasonal in nature, or from wild foraging in Ireland. There is no information available on whether or not commercial production (outdoors or in greenhouses) of samphire is occurring in Ireland, however, there is an indication that a grower may have begun commercial production in Co. Dublin. This has not been confirmed.

*Note: For business confidentiality reasons, the business names cannot be revealed in this report.

Table 3: Commercial sale of samphire in Irish Fishmongers**

Cost to Business (€/kg)	5 – 7
Biomass purchased per annum (kg)	60 - 80
Cost to Consumer (€/kg)	8.99 – 11.95
Time period sold	summer months
Source of samphire	Imported from: Holland, France, Mexico, Israel
Wholesaler links	http://cheflinkseafood.com/ http://www.rungismarket.com/

**Note: The information in this table is ONLY representative of those businesses contacted. Data, source of samphire, wholesalers, etc. may vary.

2.6 Selective breeding and bacterial assisted growth of Salicornia

The main impediment to large-scale cultivation of halophytes has been the prevalence of undesirable crop characteristics (e.g. non-uniform flowering and ripening) in wild germplasm. In order to develop high-salinity agriculture, including *Salicornia*, there is a need to improve upon these undesirable traits through selective breeding. The wild accessions of *S. bigelovii* differ significantly in plant size, biomass, seed yield, days to flowering, and days to harvesting. Hence the wild germplasm exhibits sufficient genotypic diversity and a favourable flowering system to support a breeding program (Zerai *et al*. 2010). Zerai *et al*. (2010) compared *S. bigelovii* lines produced in two breeding programs with wild germplasm in greenhouse trials irrigated with brackish water (10ppt NaCl). Lines produced from wild germplasm by mass selection and hybridization in Tucson, Arizona had a higher biomass yield than starting (wild) germplasm. Improvements to lines have resulted in 33-44% higher seed and biomass yields since breeding programmes on *S. bigelovii* began (Zerai, *et al*. 2010).

Inoculation of crop plants with plant-growth-promoting bacteria (PGPB) is a contemporary agricultural practise used to improve crop yields. A study by Bashan *et al*. (2000) found that *S. bigelovii* inoculated with *Azospirillum halopraeferens*, a mixture of two *A. brasilense* strains, a mixture of *Vibrio aestuarianus* and *V. proteolyticus*, or a mixture of *Bacillus licheniformis* and *Phyllobacterium* sp. significantly increased plant height and dry weight (Bashan *et al*. 2000).

Selective breeding and PGPB work has not yet been conducted on *S. europaea*, however, this work could greatly improve its commercial development.

3. Growing *Salicornia europaea*

3.1 Pre-germination treatment - Cold stratification

*[Begin in late March/early April]**

*Based on natural conditions (Jefferies *et al.* 1981); no controlled light and temperature. With controlled light and temperature there is the potential to grow *Salicornia* year round.

In many cases, seeds may be undergoing a period of dormancy. To ensure successful germination, first expose the seeds to a period of cold stratification. Stratification is the process of treating stored or collected seeds prior to sowing to simulate natural winter conditions that a seed must endure before germination. The method used to break the dormancy of *S. europaea* seeds is known as cold stratification.

The cold stratification period should be carried out for 30 days in a dark refrigerator (no light) at approximately 5°C. Place 25 seeds on damp 90mm (or 70mm) filter paper in a 90mm petri dish. At this stage, the filter paper should be made damp with freshwater. Place the lid over the petri dish and seal with tape (Figure 10). Check on the seeds daily to ensure the filter paper is damp. The filter paper should never be saturated or dry. If fungal growth has appeared on the seeds, a light paintbrush can be used to remove it. If a large amount of fungal growth appears, remove with a light paintbrush and replace the old filter paper with fresh filter paper. After 30 days, remove the petri dish(es) from the refrigerator. On some occasions, you may already see seedling emergence at this stage.

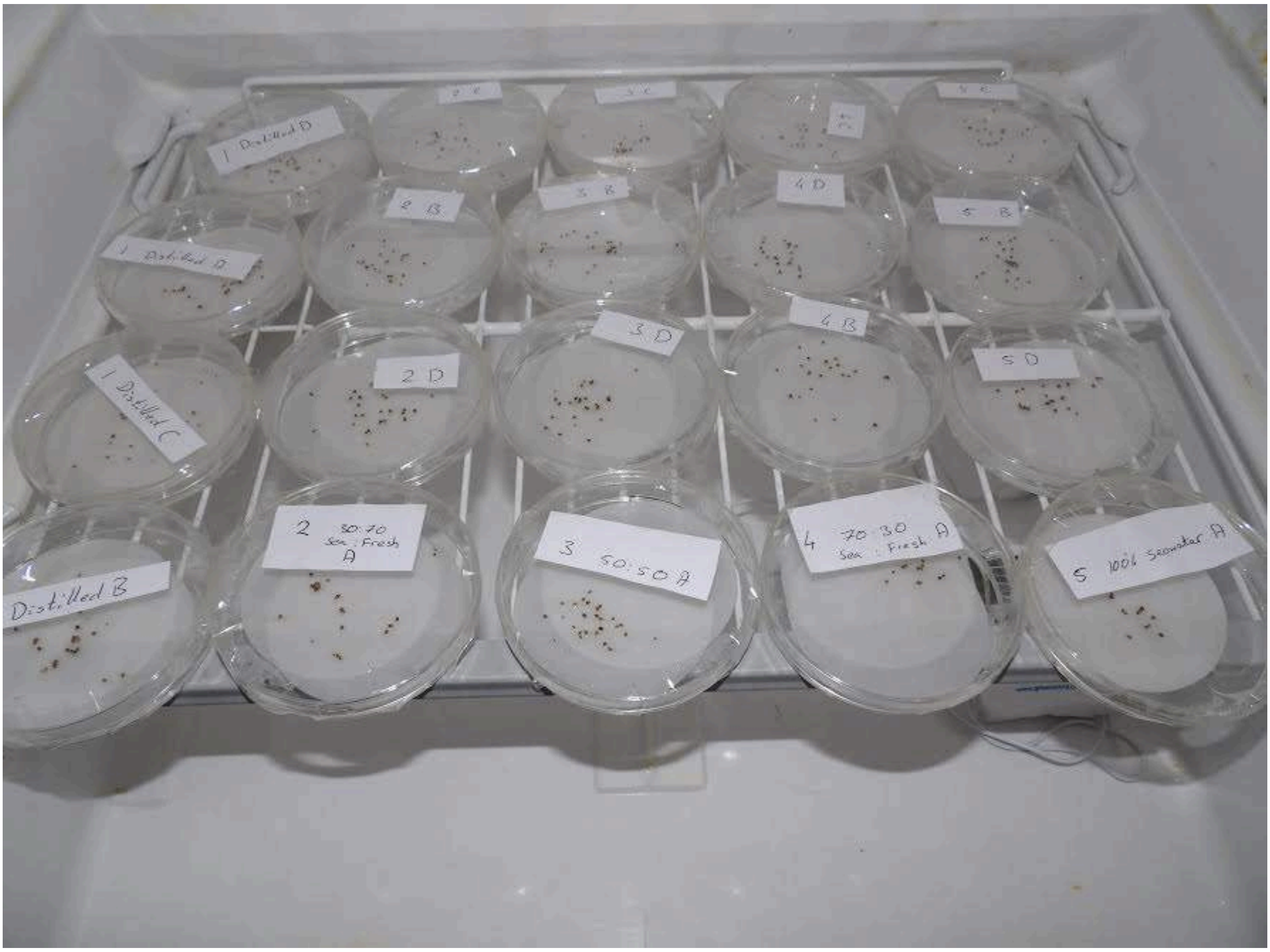

Figure 10: Marsh samphire seeds undergoing cold stratification

3.2 Early germination

[Begin in late April/early May]

Note: If you have the ability to control light and temperature, the recommended temperature and light cycle at this stage varies from 15°-25°C days and 5°-20°C nights and 12-16h days and 8-12h nights (Lv *et al*. 2012 and Keiffer *et al*. 1994). If you cannot control temperature, it would be recommended that the temperature in your chosen "early germination" area does not drop below 5°C or exceed 30°C.

Following removal from the refrigerator, the next step is to introduce the seeds to sunlight and salinity. Move the petri dishes to a location that has a full day of exposure to sunlight (e.g. glasshouse or windowsill). Remove any fungal growth with a light paintbrush and replace the filter paper if necessary. Like the previous step, the filter paper should be kept damp, however, this time the water should be a mix of between 30:70% to 50:50% seawater:freshwater solution (approximately 0.5ml is required to keep filter paper damp). This range of salinities was chosen as trials conducted for this manual and published literature (Lv *et al*. 2012 and Aghaleh *et al*. 2009) have indicated that yield and survival is negatively impacted at salinities (>400mM) approaching the strength of full seawater (seawater has a salinity of approximately 599mM). This step takes approximately 10-14 days and by the end most seeds should have evidence of seedling emergence (Figure 11).

Figure 11: Seedling emergence after 10 days exposed to sunlight and salinity

3.3 Seedling development

<u>Note (for seedling development and on-growing stages):</u> If you have the ability to control light and temperature, the recommended temperature and light cycle at this stage is c.25°C days and 15°-20°C nights and 15-16h days and 8-9h nights (Lv *et al*. 2012 and Keiffer *et al*. 1994). However trials conducted on *S. europaea* have shown that seedlings and adult plants can grow successfully in temperatures that peak above 40°C (aeroponic medium trial {Section 3.4.2} and oyster hatchery trial {Section 5}).

[Begin early/mid May]

The next step involves preparing the seedlings for on-growing to adult size. To increase the chances of successful development to adult plants, the seedlings must first develop a robust root and shoot system. To achieve this, the seedlings need to be transplanted to a 50:50 sand:soil mixture.

Mix commercial potting soil and horticultural sand together (50:50%) in a seed tray (a typical seed tray would be approximately 36.5x22.8x5.3cm) (Figure 12a). Carefully transfer the seedlings from the petri dishes into the sand:soil medium. This should be done with a small paintbrush. Lift the seedling by gently brushing against it with the bristles of the brush and transfer to the medium. As gently as possible, cover the roots of the seedling with the medium, being careful to not handle the seedlings excessively. Each seedling should be spaced approximately 2-3cm apart to limit competition for water and nutrients. Place the seed tray(s) on a standard garden tray (a typical garden tray would be approximately 79x41x4.6cm) (Figure 12b).

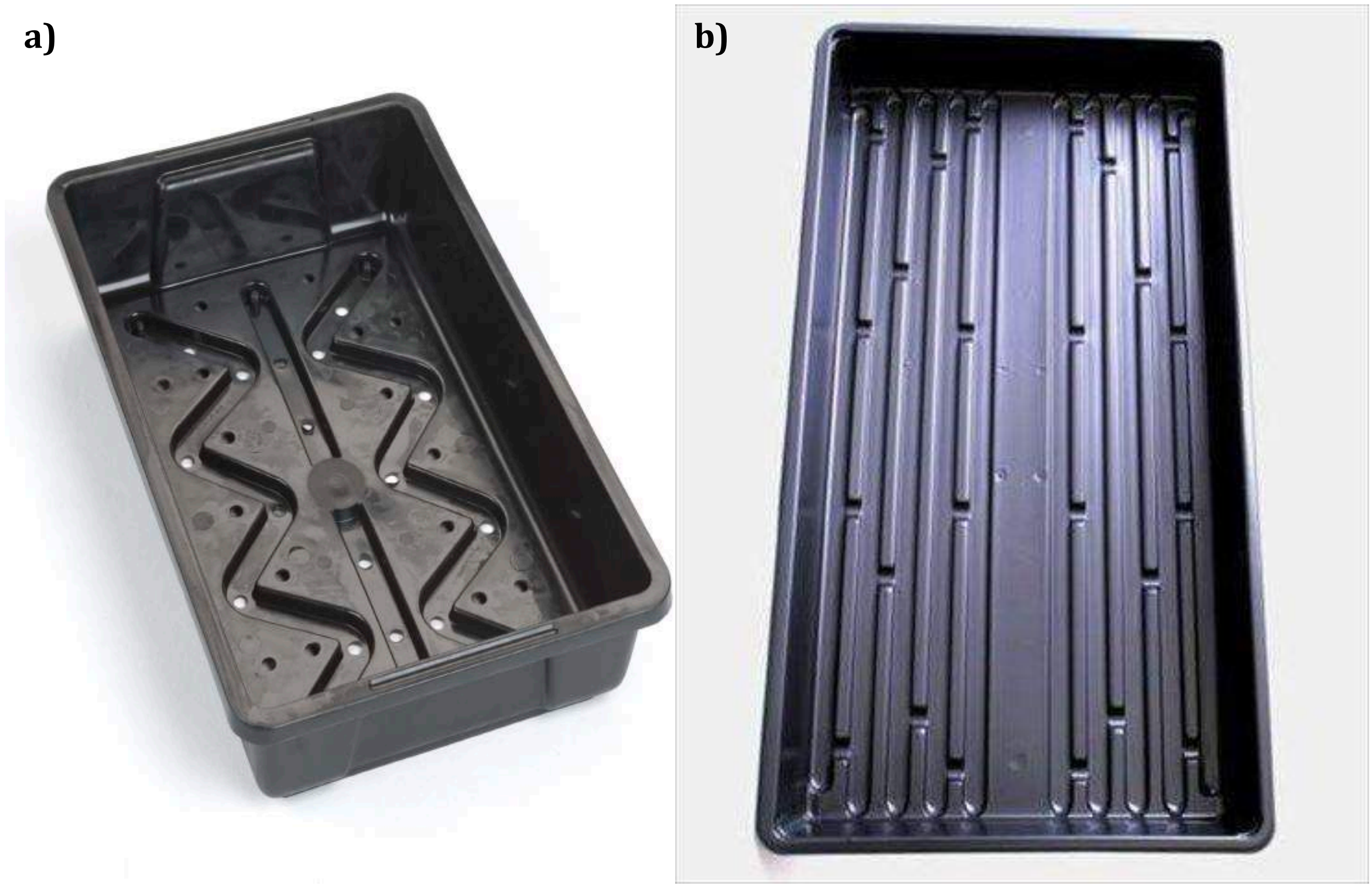

Figure 12: Example of a standard: a) seed tray and b) garden tray

The seedlings are watered with a 30:50%-50:50% seawater:freshwater and phostrogen plant feed (N:P:K 14:10:27) solution (add approximately 1/5 of a tablespoon {c. 1ml} of phostrogen per 1L of seawater:freshwater solution). Add this solution to the garden tray until the water is coming in contact with the bottom of the seed trays. These garden trays should be refilled when the water level has not been in contact with the bottom of the seed tray for a period of approximately 24 hours. This stage lasts between 4-6 weeks and specimens will range from approximately 3 to 70mm in size at its end (Figure 13).

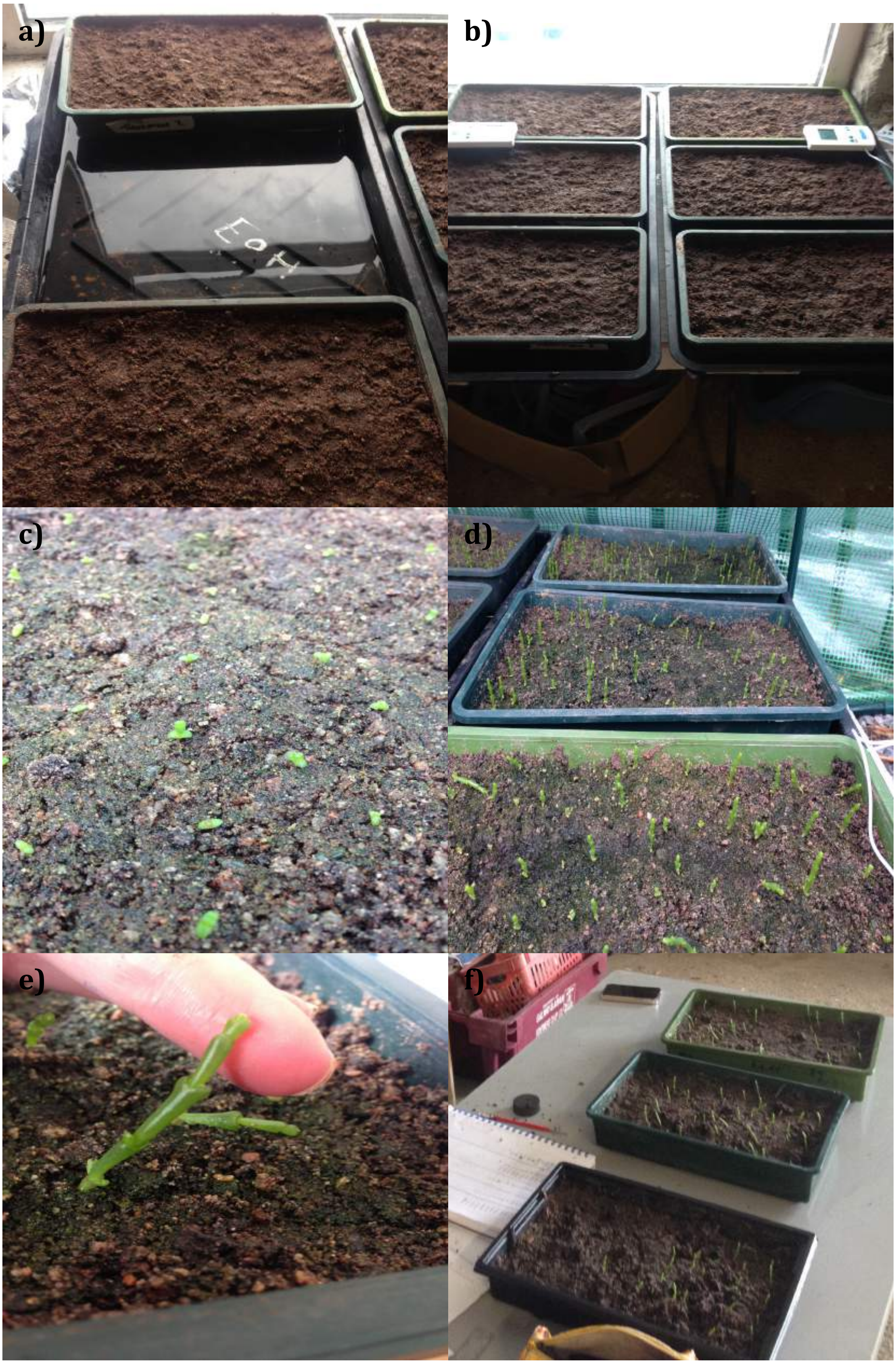

Figure 13: Seedlings - a+b) transferred to seed trays sitting in garden tray containing water treatment; c) 11 days in sand:soil; d) 26 days in sand:soil; e) 28 days in sand:soil and f) being transferred to the aeroponics units after 36 days in sand: soil

3.4 On-growing stage

3.4.1 Aeroponics

To develop *S. europaea* seedlings/young plants to adult size, we utilised the aeroponic method.

Aeroponics is a growth method where nutrients are intermittently or continuously supplied in a water mist directly to the root system, often without the use of soil or an aggregate medium; however, the addition of an organic medium can sometimes be beneficial (Christie and Nichols, 2004, Barak *et al*. 1996, and Nir, 1982). Oxygen and water are quite often a limiting factor in conventional soil and water media systems, however, as nutrients and water are applied directly to the roots in an aeroponic system, they are in adequate supply (Nir, 1982). From a commercial perspective it is very economical as the nutrient solution can be re-used (the length of time that the solution can be re-used will be dependent on the quantity of nutrients present in the solution and the biomass of plants being grown). This re-use of water and nutrients means that aeroponics is an ideal growth system in regions where the water quality is poor and/or supply is scarce. This method also results in higher yields and only requires minimal training for the grower (Nir, 1982). For example, Movahedi *et al*. (2012) conducted a study comparing aeroponic and conventional soil systems for potato minituber production. The plantlets were grown in both aeroponic and conventional soil systems at a density of 100 plants per m^{-2}. It was found that growing the minitubers with an aeroponic system led to an increase in stem length, root length, stem diameter, and yield. The end product was also of better quality when grown in an aeroponic system (Movahedi *et al*. 2012). These systems can also be run on a continuous basis, apart from some downtime for cleaning or changing the plants (Nir, 1982). Aeroponics can be utilised for both crop production and plant research. For example, Christie and Nichols, (2004) from Massey University (New Zealand) have developed aeroponic systems for growing vegetable crops (e.g. tomatoes, cucumbers, potatoes, and herbs) and flower crops (e.g. Zantedeschia and Lisianthus) and for researching crop nutrition, growth analysis, and the gas levels in the root zone (Christie and Nichols, 2004).

A brief description of aeroponic propagators is provided below before detailing the on-growing stage using these units.

3.4.2 Aeroponic propagators

Aeroponic propagators consist of a top tray, containing a varying number of plastic net pots in which the seedlings/young plants are placed. Commonly available aeroponic propagators contain 40-120 net baskets (slots) in the top tray, however smaller practise units are available. The top tray sits on top of a reservoir, which contains the water and nutrients. The volume of the reservoir will depend on the size of the aeroponic propagator. For example, a 20-slot propagator would have a capacity of approximately 20L and a 120-slot propagator a capacity of approximately 70L. The reservoir contains 1-2 spray assemblies (H-spray bar and pump) depending on the model size, which sprays the roots of the growing plants (Figure 14 and 15). This provides the root system with a constant supply of water, nutrients, and aeration, resulting in rapid and strong growth. Aeroponic propagators are readily available at many aquaponic stores across Ireland and online.

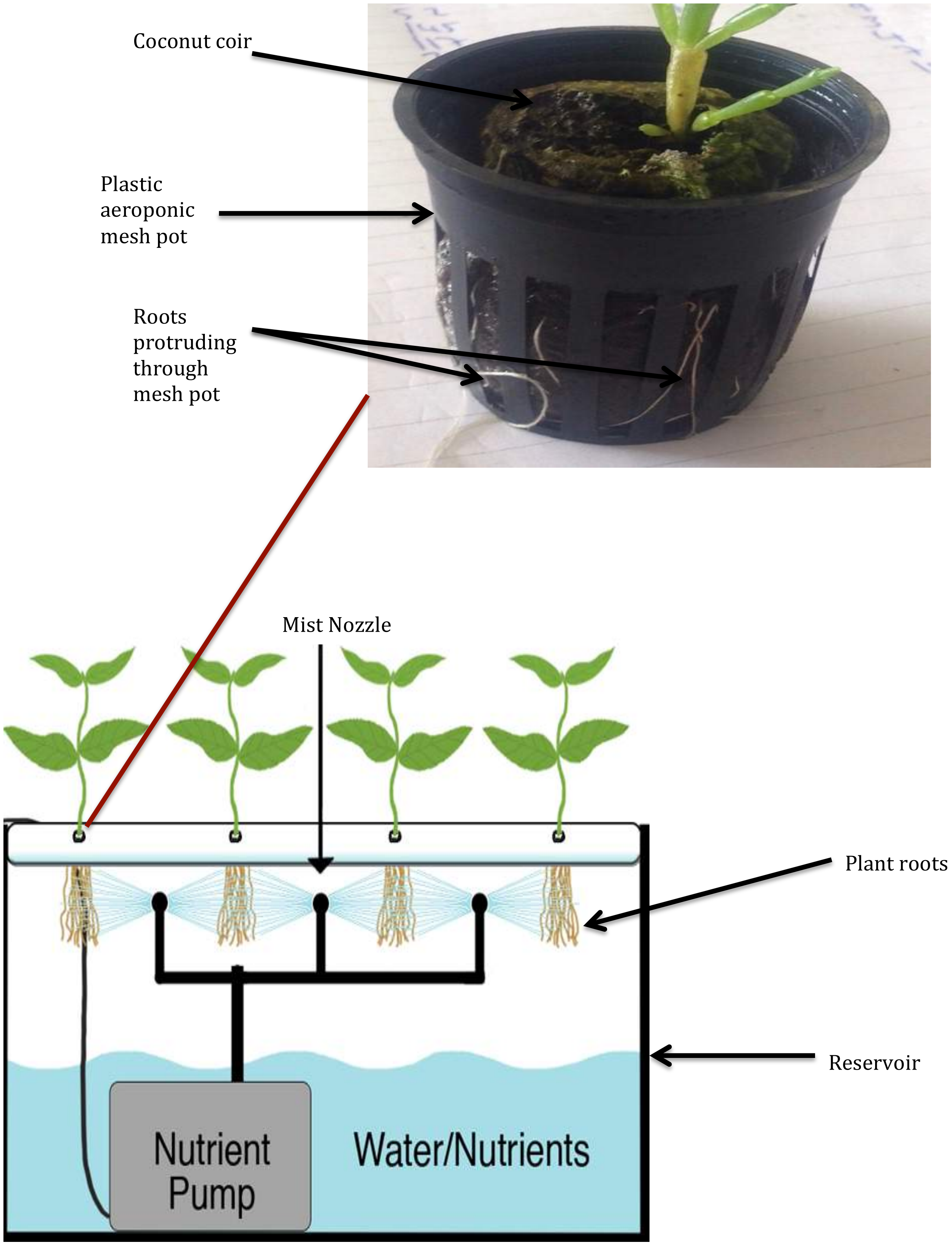

Figure 14: Schematic of an aeroponic propagator

Figure 15: Example of a) 40 slot aeroponics propagator (dimensions: 60x50x40cm); b) 120 slot aeroponic propagator (dimensions: 115x64.5x46cm) c) 40 slot top tray; and d) the spray-bar in the reservoir below

There are a number of growing medium options for use with aeroponic propagators, ranging from inert neoprene discs which hold the seedlings in place (Figure 16c), exposing the roots to the water without the need for an organic medium, to coconut coir (Figure 16a); a natural fibre extracted from the husk of coconuts.

A trial conducted from 25th July – 5th September 2014 at the School of Biological, Earth and Environmental Sciences, University College Cork tested the suitability of four different media within an aeroponic propagator: 1) Coconut coir; 2) 50:50 sand:soil mixture; 3) 100% soil; and 4) Hydrocorn (clay pebble substitute for soil). For each measurement of growth (number of nodes and branches, and height), the coconut coir showed the best growth (see Section 9.2 for detail on *Salicornia* growth measurement techniques) (Figure 17). Although the neoprene discs were not assessed during this trial, experiments conducted in Ireland (Section 5) showed that this medium is only suitable when the seedling has very well developed roots. The water in the reservoir was replaced once a week and was a solution of 30:70% seawater:freshwater and Phostrogen plant feed.

Figure 16: a) Dehydrated coconut coir discs; b) hydrocorn; and c) neoprene disc

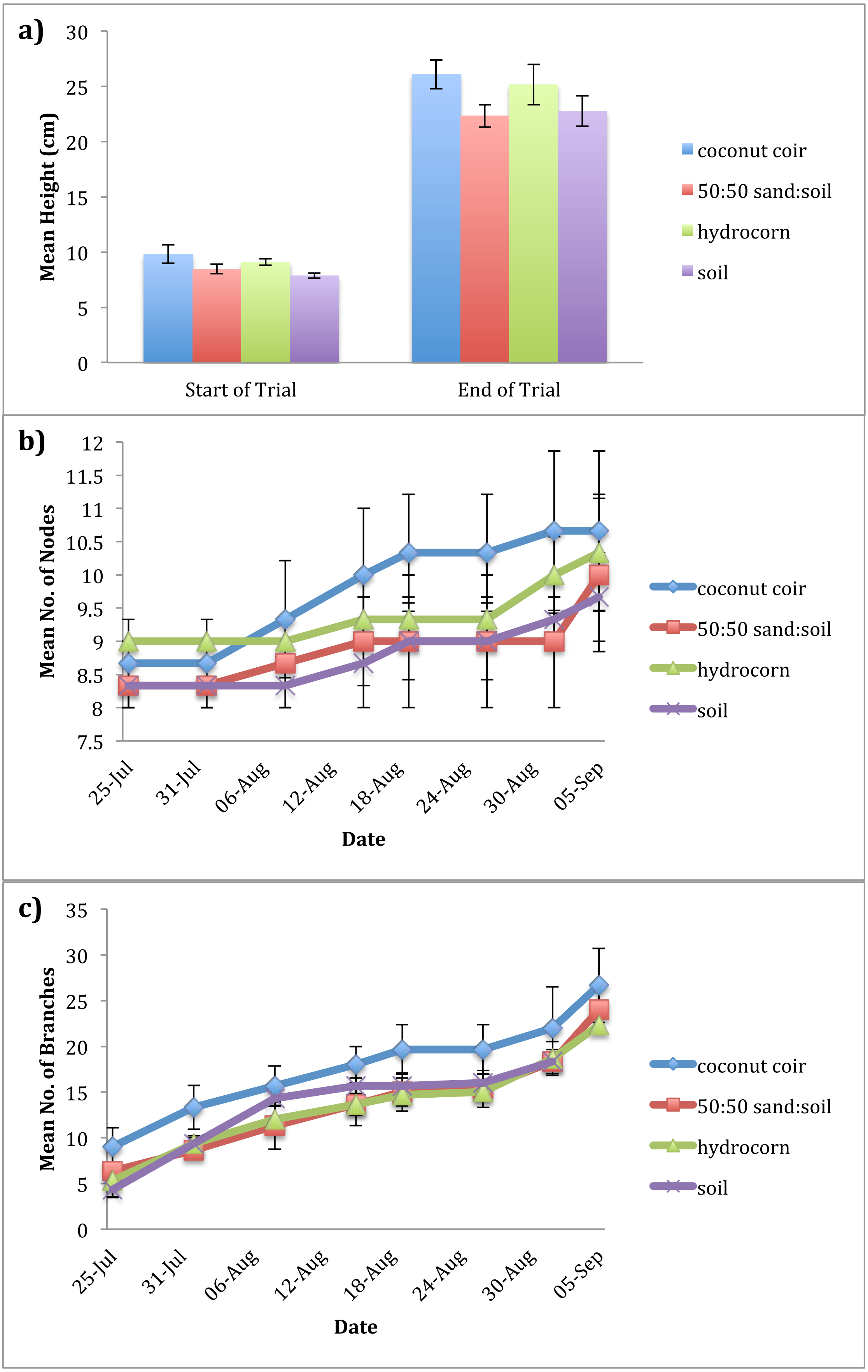

Figure 17: Mean a) height; b) number of nodes; and c) number of branches of marsh samphire plants grown in 4 different mediums

3.4.3 On-growing with aeroponics
[Begin early-mid June]

In light of the aeroponics medium trial conducted in the summer of 2014, coconut coirs are the recommended medium for transplanting seedlings to the aeroponic propagators. These coirs are available as dehydrated discs and are available in most aquaponic stores and online.

Before transplanting begins, the coconut coirs need to be hydrated. Submerge the coirs in 30:70% - 50:50% seawater:freshwater for approximately 20 minutes and they will expand to their full size (Figure 18).

Figure 18: Coconut coir discs prior to hydration (right) and fully hydrated (left)

The coconut coir material is held together by cloth netting, and when hydrated, the top has an opening to the medium inside. Using a blunt tool (e.g. a pen), push a hole in the middle of the opening, and push approximately ½-¾ of the way down. Using a fine paintbrush, gently remove the seedling from the sand:soil mixture, being careful to not damage any of the developed roots. Carefully lower the seedling, roots first, down into the hole that you created in the top of the coir. If the bottom of the coir is completely covered with the netting, rip the netting a little and create a gap. This will allow the developing roots to expand downwards towards the water reservoir. The final step is to place the coir with transplanted seedling into the plastic net pot of the aeroponic unit (Figure 19). Based on results achieved in the oyster hatchery trial (Section 5), we would recommend transferring seedlings when they have achieved a minimum height of >2mm.

Figure 19: Coconut coirs with recently transplanted seedlings in the aeroponic plastic net pots

3.5 Samphire flowering

As samphire is an annual (germination to production of seed in one year), unless you are controlling light and temperature at spring/summer levels, the plants will flower and produce seed in August/September. The plants tend to turn red in the winter, towards the end of their life-cycle, an indication that flowering will occur within days/weeks (Devlin 2015d, Deane, 2014a, and Davy *et al*. 2001). For the hatchery growth trials conducted from May-September 2015 (Section 5), flowering took place approximately 1 week after the plants started to turn red. The red parts of the plant are saltier (Deane, 2014a) and, depending on your preference, may be more appetising. From a visual aesthetic perspective, the fully green samphire is often preferred (Figure 20).

However, you should aim to crop your samphire before, or at the latest, as soon as flowering occurs. The texture and taste is negatively impacted by the flowering and seed production process, particularly due to the development of woody stems as the plant ages (Deane, 2014a).

Figure 20: a) Fully green samphire and b) showing red colouration (occurs in August/September – weather dependent)

4. Seed sourcing

4.1 Wild source

As detailed in the introduction, *Salicornia europaea* can be found in most coastline areas of Ireland and a history of wild collection exists in many areas. However, many of the habitats where one would find marsh samphire (e.g. saltmarshes) are often protected by "Special Area of Conservation" (SAC) status. SACs have legal protection under the EU Habitats Directive, which outlines the need for the conservation of best examples of natural and semi-natural habitats and species of flora and fauna throughout the EU. Each member state is required to designate a number of SACs to protect those habitats and species that are listed in the annexes of the Directive. SACs in Ireland cover an area of approximately 13,500 sq. km (National Parks and Wildlife Service, 2015a). A large number of SACs contain *Salicornia* spp., including *Salicornia europaea* (National Parks and Wildlife Service, 2015b). An SAC is selected based on the type of habitat and/or the presence of species listed in Annex I/II of the EU Habitats Directive. A number of SACs in Ireland list item 1310 [*Salicornia* and other annuals colonising mud and sand] as a reason for designating the area a SAC. Examples of such areas include; Dundalk bay (*Salicornia* spp.), Courtmacsherry estuary (*Salicornia* spp.), lower river Shannon (*S. europaea*), Galway bay complex (*S. europaea*), Blackwater river {Cork/Waterford} (*Salicornia* spp.), North Dublin bay (*S. europaea* & *S. dolichostachya*), Dunbeacon shingle (*S. europaea*), and Tramore dunes and Backstrand (*Salicornia* spp.). Many more SACs that have *Salicornia* spp. present exist in Ireland and a site synopsis of these sites, as well as all of Ireland's SACs, are available on the "protected sites" section of the National Parks and Wildlife's website (http://www.npws.ie/protected-sites) (National Parks and Wildlife Service, 2015b). As wild collection of flora is not permitted at a SAC, we would not recommend the wild collection of marsh samphire specimens or seed at any location in Ireland to ensure no law has been broken and to protect the natural flora of Ireland.

4.2 UK/European nursery sources

Salicornia europaea seeds can be found at a number of nurseries across Europe. Two nurseries that have supplied seed for the trials detailed in this report are given below.

UK source:

Nursery: Victoriana Nursery, Kent, UK.

Website: https://www.victoriananursery.co.uk/Samphire_Seed/

Retail Price: £2.50 for 50 seeds

Seed viability: >90% seedling emergence

European sources:

Nursery: Alsagarden, France.

Website: http://www.alsagarden.com/en/20-salicornia-europaea-salicorne-graines.html#sthash.HtncLXDr.dpbs

Retail Price: €8.25 for 1,000 seeds

Seed viability: >95% seedling emergence

4.3 Seed saving & storage

To collect samphire seed for use the following year, allow for a selection of plants to grow un-hindered from mid/late August. As autumn approaches the foliage reddens and the seed is formed. When the plants brown and die back, cut off all of the top growth before the plants collapse and lay on newspaper to dry. The seed will then be released.

Next, store all the top growth, and anything that has fallen onto the newspaper, in dry paper bags, shaking occasionally (e.g. 2-3 times per month). These bags should be stored in a cool dry place.

The bulk of the seed will have naturally fallen from the plants by the next spring. The unnecessary material (e.g. dirt, dried stems & shoots, etc.) can be winnowed off (Victoriana Nursery Gardens, personal communication, 2015).

5. Irish oyster hatchery wastewater – samphire growth trial

5.1 Aim

To assess the suitability of an oyster hatchery's wastewater (Figure 21) as a source of nutrients for the growth of *Salicornia europaea* and its subsequent ability to remove excess nutrients from the hatchery's wastewater.

Figure 21: Wastewater pond at an Irish oyster hatchery

5.2 Methods

5.2.1 Cold stratification

Approximately 700 *Salicornia europaea* seeds were purchased from a nursery in Kent, England. These seeds were distributed equally amongst 28 90mm petri dishes containing damp 90mm filter paper (i.e. 25 seeds per petri dish). The 90mm filter paper was made damp with freshwater prior to the addition of seeds. The lids of the petri dishes were taped shut and the petri dishes were then placed in a 5°C refrigerator on the 13th of May 2015. The seeds were checked on a daily basis. If mould was present on the seeds, it was gently removed with a fine paintbrush. If a large amount of mould had formed within the petri dish, the mould was removed and the filter paper replaced. The filter paper was kept damp throughout the 4 week cold-stratification period. It is important that the filter paper is never saturated or dry. The filter paper required dampening approximately every 3-4 days. The petri dishes were removed from the refrigerator on the 13th of June 2015.

5.2.2 Early germination

The petri dishes were kept indoors under natural light (17h days and 7h nights) and ambient temperature (mean 17°C) conditions. The filter paper from each petri dish was replaced and any mould formation removed. For a period of two weeks (until 26th of June 2015) the petri dishes were checked daily for mould and the filter papers were kept damp with a 50:50% seawater:freshwater solution (approximately 0.5ml is required to keep filter paper damp) (Figure 22).

<u>Treatment groups:</u>

On 26th June 2015, 675 seedlings (25 seeds did not successfully germinate) were evenly distributed amongst 3 different treatment groups (9 petri dishes per treatment, with 25 seeds per dish):

- Treatment 1: 33.33%:66.66% saline wastewater:freshwater
- Treatment 2: 66.66%:33.33% saline wastewater:freshwater
- Treatment 3: 100% saline wastewater:freshwater

For one week (until 3rd July 2015) each treatment was given their respective solution (0.5ml) at the beginning of the week and/or when the filter paper appeared to be dry.

Figure 22: a) Petri dishes exposed to natural light conditions, ambient temperature, and 50:50 sea:freshwater solution & b) seedling emergence after 14 days exposed to these conditions

5.2.3 Seedling development

On 3rd July 2015, the seedlings from each treatment were transferred evenly amongst 36.5x22.8x5.3cm seed trays (3 seed trays per treatment), with approximately 2-3cm between each seedling. The three seed trays from each treatment were placed in their own 79x41x4.6cm garden tray containing the respective treatment solution. All treatment trays were moved to a temporary greenhouse for this development stage (Figure 23).

The greenhouse was positioned outside, so received natural sunlight (c. 15-17h light and 7-9h dark). The mean daytime temperature inside the greenhouse was 21.8°C (mean daily maximum temperature: 36.18°C and mean daily minimum temperature: 13°C). (Note: the very high mean max temperature may be due to direct sunlight hitting the thermometer and may not be an accurate account of the air temperature surrounding the plants. If possible, do not place the thermometer in direct sunlight. Unfortunately for this trial, this was not possible).

The seedlings were checked on a daily basis, and the respective treatment solution added to the garden tray if required (when the water looses contact with the base of the seed tray). Measurements (height, number of nodes and branches) were monitored on 31st July 2015. This stage was ended after 36 days as there were enough seedlings of suitable size for the next stage.

Figure 23: a+b) Seedling trays in the greenhouse; c) seedlings on day 18 of seedling development stage; d) Seedlings on day 28 of seedling development stage

5.2.4 On-growing

NOTE: Due to a lack of a greenhouse onsite that was large enough to house the three aeroponic propagators, these units had to be positioned outside. Each propagator has a lid and therefore the seedlings were protected from the elements. Also, the wiring was very carefully insulated. However, if possible, it would be ideal to house these units inside a greenhouse from a safety perspective, to have easier access to electricity, and to have more control over temperature and possibly light.

On 8th August 2015, 120 seedlings from 3 different size brackets (small, medium, large) were measured and transferred to individual 120 slot aeroponic propagators (one aeroponic propagator per treatment) (Table 4) (Figure 24). Three different size classes were chosen to assess the impact of seedling size on survivability in the propagators. The criterion for each size class was unique to each treatment group, dependent on the level of growth achieved in each group by this stage.

Table 4: Size range of samphire seedlings added to each aeroponic treatment group

Treatment	**Size (height – mm)**		
	Small	**Medium**	**Large**
1	3-30	31-50	51-76
2	3-20	21-38	39-60
3	1-9	10-17	18-30

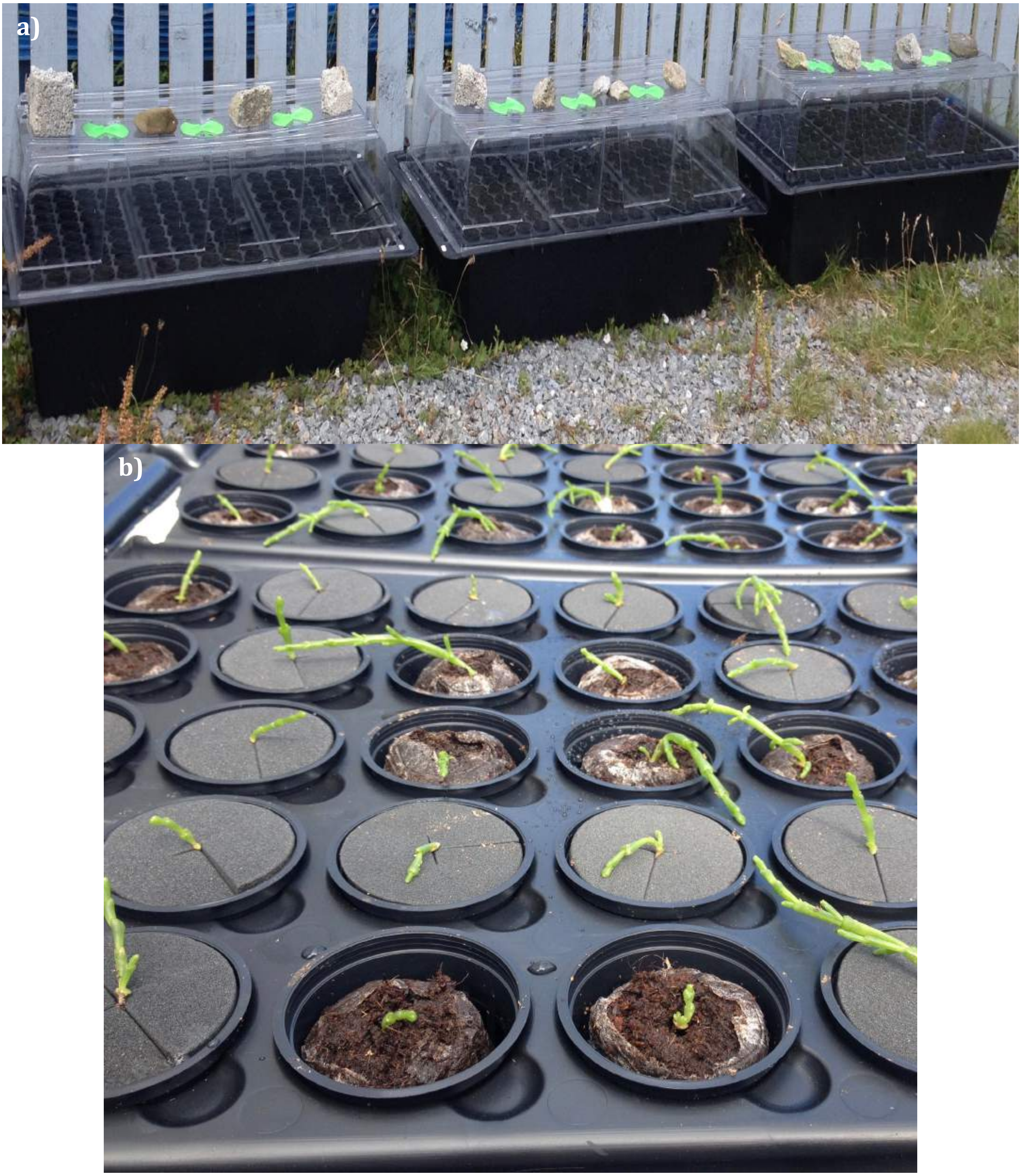

Figure 24: a) Location of aeroponics propagators and b) seedlings in aeroponic propagator at the beginning of the on-growing stage. Note: Grey neoprene discs were replaced with coconut coir early in the trial due to high mortalities

For each treatment group, each size group of seedling was divided evenly amongst two different medium types; neoprene discs and coconut coir (i.e. each size bracket had 20 seedlings transplanted to the neoprene discs and 20 transplanted to the coconut coir). The positioning of each seedling within the aeroponic propagator was completely randomised through Microsoft Excel (the positioning of each seedling was randomised to ensure that the results obtained were not solely influenced by the position within the unit. For example, a corner position may get less water spray than a centre position. See Section 9.3 for excel-randomisation methods). After 4 days, it became apparent that the neoprene discs were not a suitable medium for the seedlings and mortalities were replaced with seedlings from the same size class and transplanted to coconut coir.

The bottom reservoir of the aeroponic propagators contained 60L of the following:

- Treatment 1: 20L saline wastewater:40L freshwater
- Treatment 2: 40L saline wastewater:20L freshwater
- Treatment 3: 60L saline wastewater

The treatment solution in each propagator was replaced every 7 days. A sample of the solution from each propagator was taken from when it was first added to the units and again after 7 days, before the water was replaced. Four weeks of samples (start & end of the week for each treatment propagator) were sent to the water analysis laboratory at the Environmental Research Institute, University College Cork for ammonia, nitrite, nitrate, and phosphate analysis.

The aeroponic propagators were positioned outside, so received natural sunlight (c. 12.5-15h light and 9-11.5h dark). The average daytime temperature amongst the three treatment propagators was 24.32°C (measured inside the lid, amongst the plants) (mean daily maximum temperature: 42.75°C and mean daily minimum temperature: 12.57°C. Note: the very high mean max temperature may be due to direct sunlight hitting the thermometer and may not be an accurate account of the

air temperature surrounding the plants. If possible, do not place the thermometer in direct sunlight. Unfortunately for this trial, this was not possible).
Growth parameters (height {mm}, number of nodes, and number of branches) were measured for each seedling of each treatment on 27th August, 11th September, and on the final day of the trial, 18th September 2015. On the 18th of September, an overall biomass (g) for each treatment was also taken.

The lid remained on the units for the duration of the trial, only being removed when measuring the seedlings, checking on their condition, and replacing the water. The butterfly flap was left open during the day to allow for the circulation of air (closed when raining) and was closed at nighttime to limit the reduction in temperature. During periods of heavy rain, the gap between the lid and the unit was sealed with waterproof tape to ensure rainwater did not enter the reservoir.

The trial was ended on the 18th of September 2015 as the plants had begun to flower (see Section 3.5 for details on samphire flowering).

5.3 Results

For all aspects of growth monitored the plants grown in treatment 1 (33.33%:66.66% saline wastewater:freshwater) were the most successful, having the largest average height, average number of branches and nodes, and the highest overall final biomass (Figure 25 and 26) (note: average growth parameters excluded mortalities). By the end of the trial, treatment 2 had the lowest number of mortalities, at 11 out of 120 and treatment 3 had the highest, with 41 out of 120. Although treatment 1 had a higher level of mortality (23/120) than treatment 2, the overall biomass of the surviving plants at the end of the trial was higher in treatment 1.

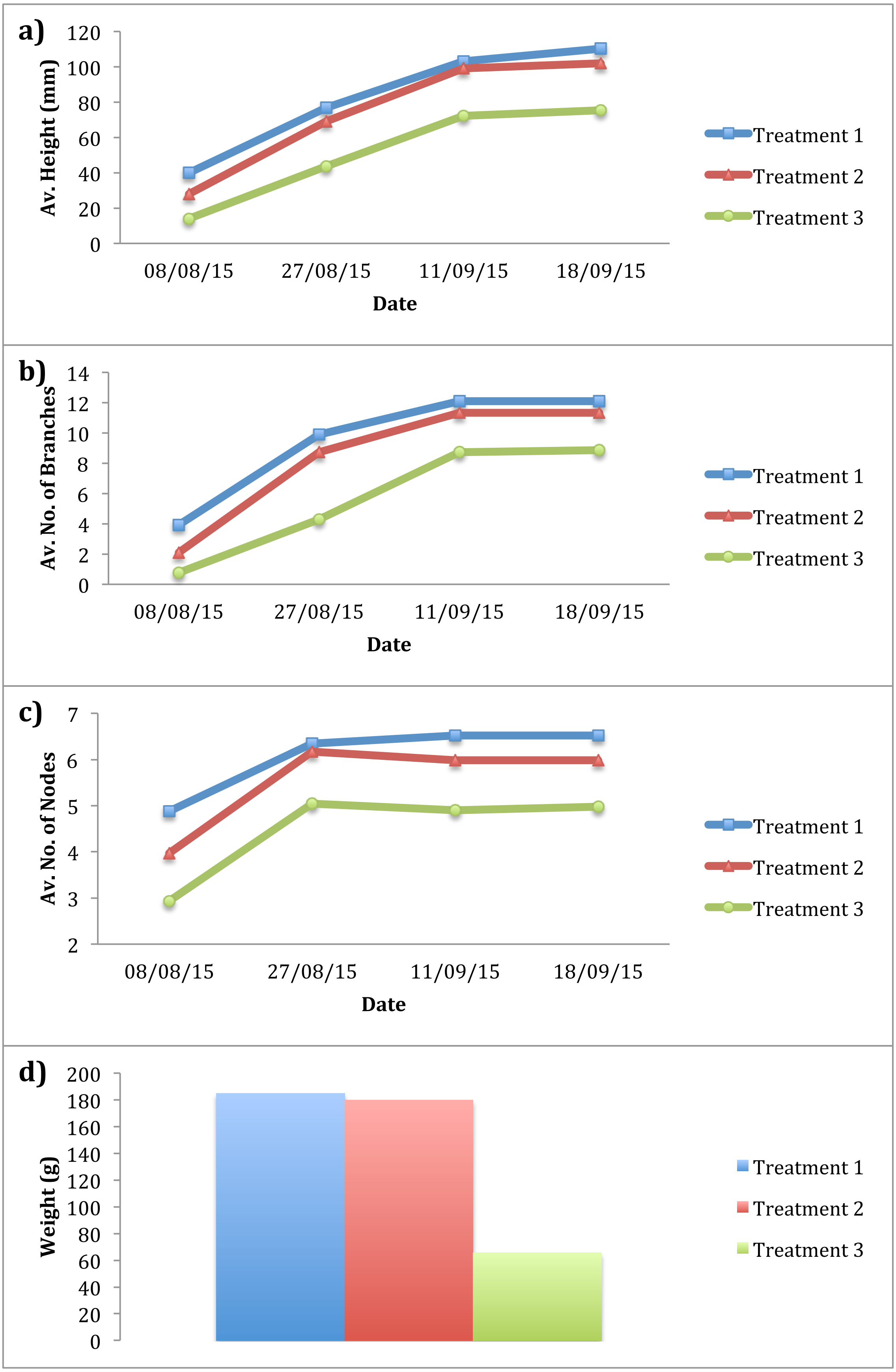

Figure 25: Average a) height; b) number of branches; and c) number of nodes for and each treatment. d) Final weights for each treatment

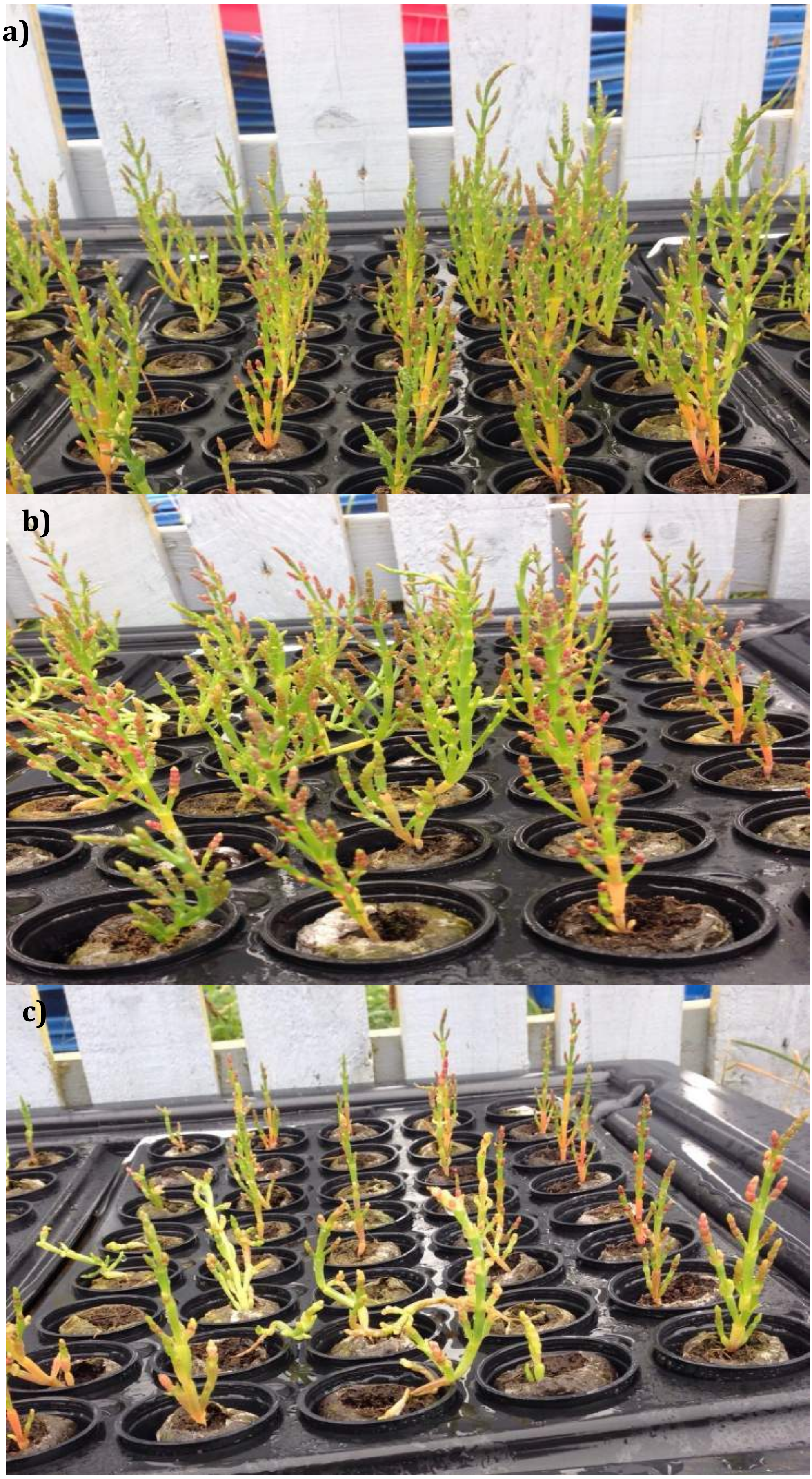

Figure 26: Samphire plants after 5 weeks in a) treatment 1; b) treatment 2; and c) treatment 3 aeroponic propagators

In the majority of cases, the level of ammonia, nitrite, and nitrate in each of the treatment waters was reduced after 1 week in the aeroponic propagators. As the level of nutrients in the wastewater was very low to begin with, the change is only minor. On the week beginning the 19/8/15 however, the nutrient levels in the hatchery wastewater was a lot higher than other weeks, reflecting the variation in levels found in the wastewater that the hatchery releases. However after one week in the aeroponic propagators ammonia, nitrite, and nitrate had reduced to levels that were similar to the 'end of the week' levels from other weeks. The level of phosphate present after one week was more variable, being reduced after one week in the aeroponic propagators on 50% of occasions for all treatments (Figures 27 – 30) (note: The wastewater added to the propagators on the 03/09/15 remained in the propagators for an extra week; two weeks as apposed to one week). Refer to Section 9.4 for full data set.

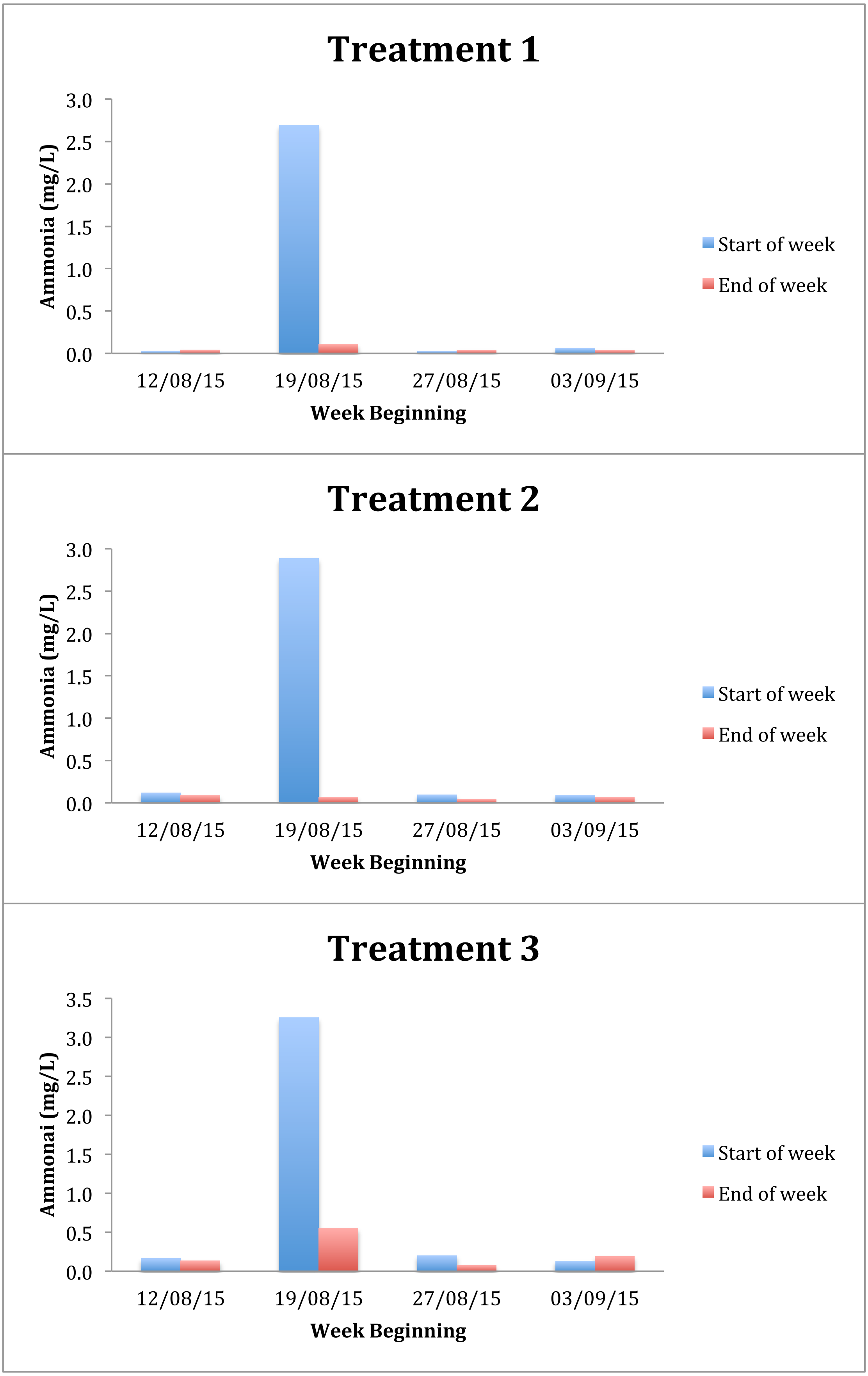

Figure 27: The change in wastewater ammonia levels at the beginning and end of four monitored weeks

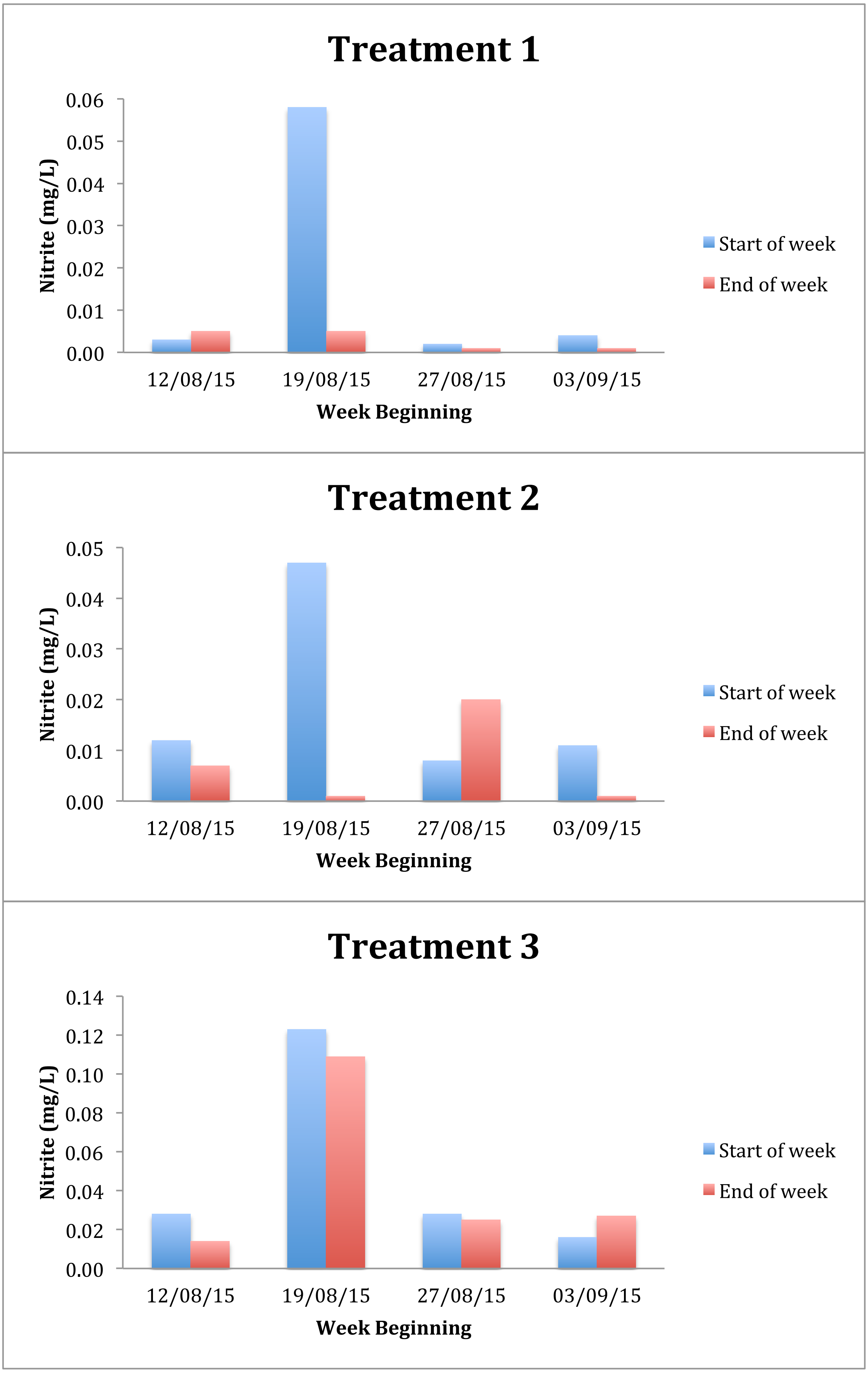

Figure 28: The change in wastewater nitrite levels at the beginning and end of four monitored weeks

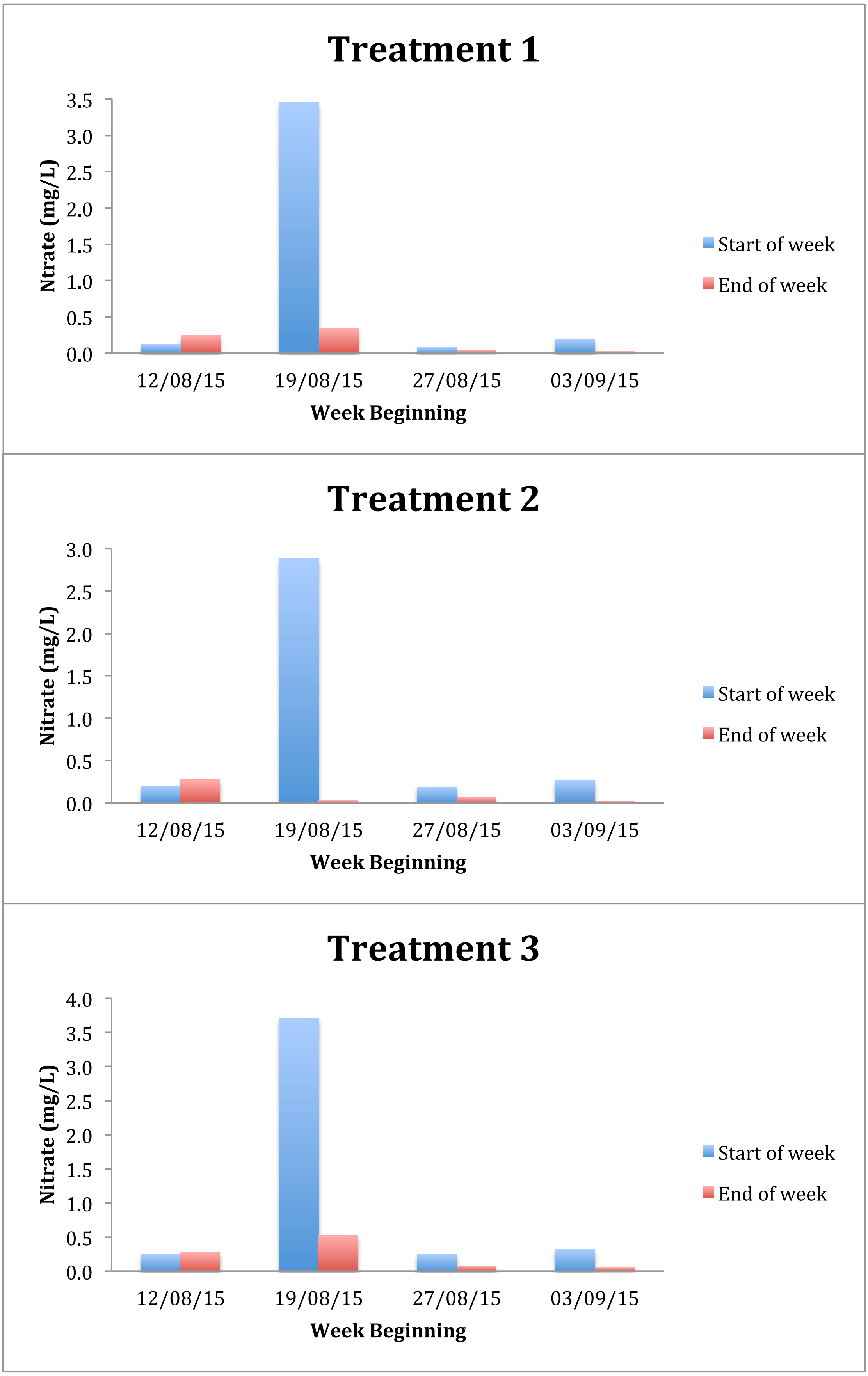

Figure 29: The change in wastewater nitrate levels at the beginning and end of four monitored weeks

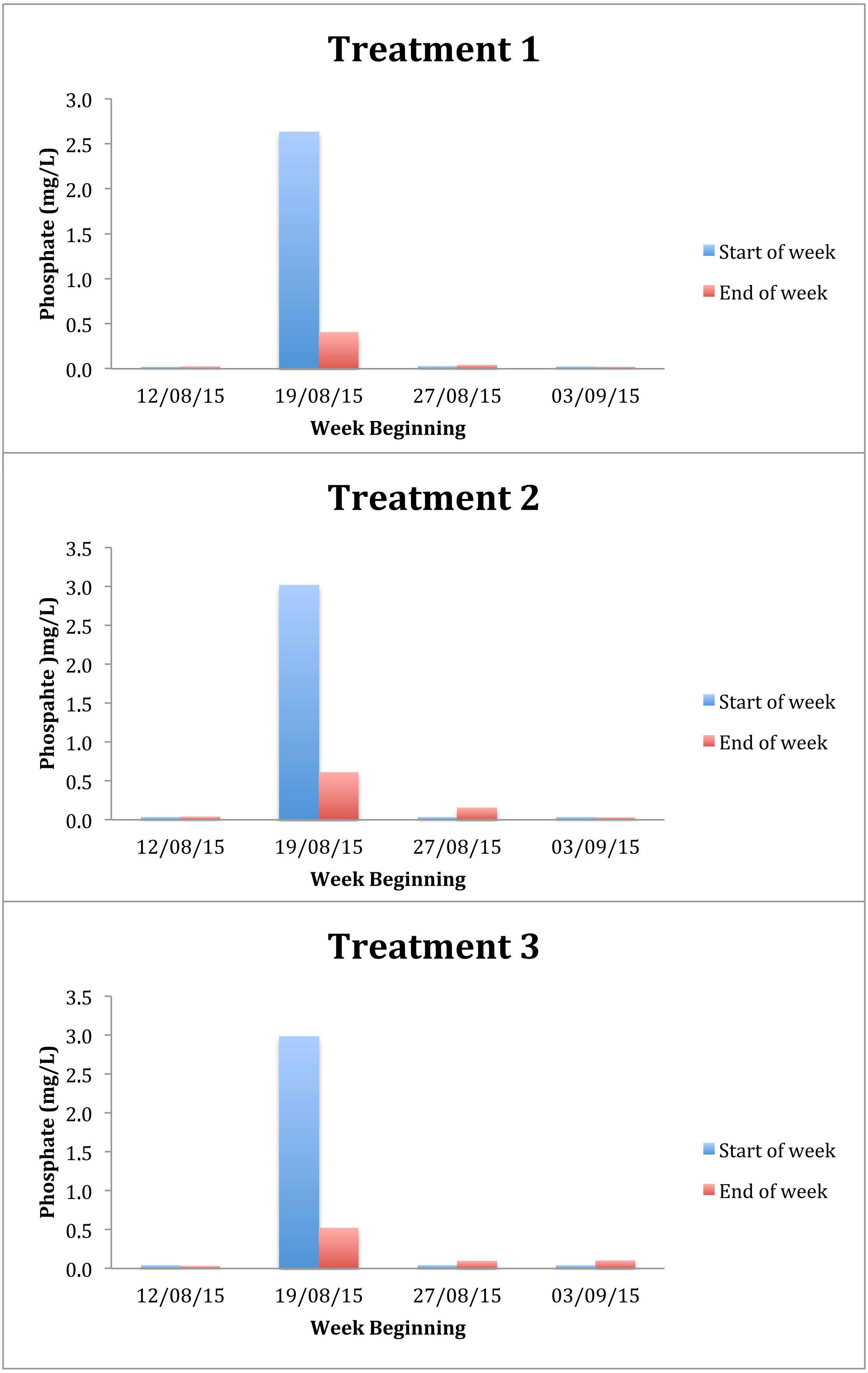

Figure 30: The change in wastewater phosphate levels at the beginning and end of four monitored weeks

5.4 Discussion

In light of these results, one must consider the trade-off between the volume of wastewater that can be treated and the quality and quantity of marsh samphire that is achievable. For instance, the samphire growing in the treatment 3 solution (100% saline wastewater) was effective at removing excess nutrients, but suffered poor growth and high mortalities. Samphire growing in the treatment 2 solution (66.66:33.33% saline wastewater:freshwater) had a lower overall growth than treatment 1 (33.33:66.66% saline wastewater:freshwater), however, the difference was not significant ($p>0.05$). Therefore in treatment 2 a lower percentage of the wastewater needed to be diluted and resulted in only a very small reduction in overall biomass. Treatment 2 also had less mortality than treatment 1, producing a larger number of individual plants. There did not seem to be a correlation with the size at which seedlings were transferred to the aeroponic propagators (small, medium, and large – height) and mortality, with seedlings from each size class for each treatment experiencing mortality. It is likely that some of the mortalities may be due to "dry zones" that were identified within the propagators. These "dry zones" are areas that did not get a sufficient level of spray from the spray bar, and usually occurred at the corner areas of the trays. The potential for "dry-zones" should be considered when purchasing aeroponic propagators or when designing a bespoke propagator. The spray bar should be altered or designed in such a manner that all seedlings will receive a sufficient level of spray. Some of the seedling mortalities may have been due to natural causes.

From the point of view of wastewater treatment and samphire growth, the ratio of saline wastewater:freshwater of treatment 2 would be the best option for this specific hatchery.

It is important to note that the saline wastewater is the main source of nutrients for the samphire, the additional freshwater adding very little. As the level of mortality seen in treatment 1 increased over the duration of the trial, this may be an indication that as the plants grew larger and required more nutrients, the level of nutrients in the treatment 1 solution may not have been sufficient to facilitate the growth of all specimens. The nutrients present in the treatment 2 solution were higher, however, the salinity is also higher, and the growth of samphire is inhibited at salinities approaching full seawater levels. This may explain why the growth was slightly lower in treatment 2 than treatment 1, yet experienced lower mortalities. There were more nutrients present, however, the higher salinity may have restricted growth (Lv *et al*. 2012 and Aghaleh *et al*. 2009). This theory would also explain the growth and mortality levels seen in treatment 3. Treatment 3 had the highest level of nutrients, however, at full salinity, there was a high level of mortalities and poor growth in those that survived.

Each hatchery must be considered on a case-by-case basis. For example, a different hatchery may have a much higher nutrient load in its wastewater, subsequently impacting the levels of growth achieved at varying degrees of dilution. However, we would recommend using hatchery/aquaculture wastewater at a ratio of 30:60-60:30% saline wastewater:freshwater.

Unfortunately due to a delay in getting this trial started, the first step (cold-stratification) took place a month later than suggested in Section 3.1 of the manual. For the aeroponic medium trial (Section 3.4.2) conducted in the summer of 2014 at UCC, the trial started approximately 1 month earlier than the hatchery trial, and the average height, number of branches and nodes (of the samphire grown in coconut coir) at the end of the trial was 26.1cm, 27cm and 11cm respectively (Figure 31) in comparison to the best averages (treatment 1) from the 2015 hatchery trial, at 11.02cm, 12cm, and 7cm respectively. Although the 2014 trial was not conducted with wastewater and took place in a well-sheltered greenhouse, it indicates that a greater biomass may have been achieved in the hatchery trial with an extra month of growth or with artificial light and heat that could extend the growth period.

As this trial was experimental, we did not have a harvesting regime in place. However, for commercial production of samphire, we would recommend a harvesting regime similar to the ones detailed in Section 2.1 and 2.2.

Note: The 2014 trial was preliminary and the seedlings were kept at the seedling development stage for 10 weeks to assess the ideal length of time for this stage. It was found that after 4-6 weeks, growth of seedlings greatly slows down at this stage and should be transferred to the aeroponics stage after this time period. If the seedlings were transferred to the aeroponic stage after 4-6 weeks in the 2014 trial, the final growth level achieved could have been even higher.

Figure 31: Samphire plants at the end of the 2014 aeroponic propagator medium trial

6. Other halophytes with commercial potential

Note: See Section 9.5 for list of halophyte plant and seed suppliers

There are many other species of halophytes that grow in saline waters across Ireland, and these species may have potential for commercial growth and/or the treatment of marine aquaculture wastewaters. The following section details a few of these species.

6.1 Sea kale (Crambe maritima)

Sea kale, of the family Brassicaceae, is a large, fleshy, perennial halophyte that is quite rare in Ireland (Devlin, 2015a and De Vos, *et al*. 2010), mainly being confined to the south coast, with only a few locations on the east and west coast documented (Figure 34) (Devlin 2015a and BSBI 2015). It is mainly found at coastal habitats with well-drained soils in northwest Europe, however, it has been recorded on cliffs and its geographical distribution extends from the North Atlantic to the Black Sea. It mainly grows above the high tide line on shingle and sandy beaches (Devlin, 2015a, De Vos, 2010, and Scott and Randall, 1976). It is a wide plant, only reaching approximately 70cm in height, which resembles a big cabbage, with broad, succulent, waxy, wavy leaves that are green-grey in colour. From May to July, sea kale bears a cluster of while flowers, which produce a fragrant aroma that attract insects for pollination. It is completely hairless and on occasion it contains a tinge of purple (Figure 32) (Devlin, 2015a). Sea kale is very tolerant of sea spray, however, it is sensitive to excessive salinities (above 100mM NaCl) at the roots (De Vos, 2010). At the end of the growing season (October –

Figure 32: Sea Kale (*Crambe maritima*) (Source: Devlin, 2015a)

November) the leaves and inflorescence die off, leaving only the underground root system and tap-root during the winter (De Vos, 2010 and Scott and Randall, 1976).

The sprouts (young shoots) that grow from the tap-root are widely regarded as a tasty vegetable when consumed in an etiolated (whitened or pale through lack of light) form and have been consumed by humans in this fashion for at least 300 years (Figure 33) (De Vos, 2010 and Péron, 1990). These shoots are commonly served in a similar fashion to asparagus, steamed or blanched, with a flavour somewhat like hazelnuts, but with a slight bitterness. Less commonly, the young leaves are eaten raw or cooked (Sanyal *et al*. 2015).

Figure 33: Etiolated sprouts of seakale (source: www.scrops.com)

Sea kale is rich in vitamin C (ascorbic acid), starch, sugars, mineral salts, sulphur and iodine. It also contains sulphur heteroside, which is recognised as having anti-cancer properties. As well as its culinary use, it has been used to prevent viral infection (due to its high vitamin C content), as a purifier, diuretic, antiseptic, and antifungal. The leaves have been used for healing wounds, and the raw juice of the seeds used to fight gastritis and gastric ulcers (Table 5) (Sanyal and Decocq, 2015, Péron *et al*. 1991, and Péron 1990).

Table 5: Nutritional composition of raw etiolated sprouts of seakale (Péron *et al.* 1991 and Péron 1990)

Components	Quantity per 100g of fresh, raw, etiolated sprouts
Protein (g)	2.10
Caloric value (kcal)	16.9
Carbohydrates (g)	1.6
Lipids (g)	0.2
Fibre (g)	3.1
Sucrose (g)	0.1
Reducing sugars (g)	2.4
Sulphur (mg)	28
Potassium (mg)	430
Calcium (mg)	73
Sodium (mg)	3.6
Nitrate (mg)	17
Trace elements (copper, iron, manganese, zinc) (mg)	0.5, 0.6, 0.2, and 0.3
Ascorbic acid (mg)	27
Pyridoxine (mg)	0.21
Riboflavin (mg)	0.05
B-carotene (mg)	0.01
Thiamine (mg)	0.27
Folic Acid (mg)	0.10
Valine (mg)	0.072
Histidine (mg)	0.034
Tryptophane (mg)	0.047
Phosphates (mg)	37
Sulphates (mg)	45

Sea kale is easy to propagate in deep, rich, sandy soils, and can be grown from root cuttings or seed that are available from specialist nurseries in the UK and Europe (Cramb-admin, 2012)

Sea kale has been wild harvested for thousands of years across Atlantic coasts of Europe, before being first cultivated in the 1600s. In the 1800s, it became a popular garden vegetable in Europe and North America. The commercial production of sea kale in Europe has all but died out, coming to an end around the time of World War II. However, Sandy and Heather Pattullo of Eassie Farm in Angus, Scotland, have been producing sea kale on a commercial scale for almost 30 years. Most of their sea kale is harvested from January to March and is sold at London's Covent and Borough markets (The List, 2015, Deane, 2014b, Temperate Climate Permaculture, 2013, and The College for Enlightened Agriculture, 2011).

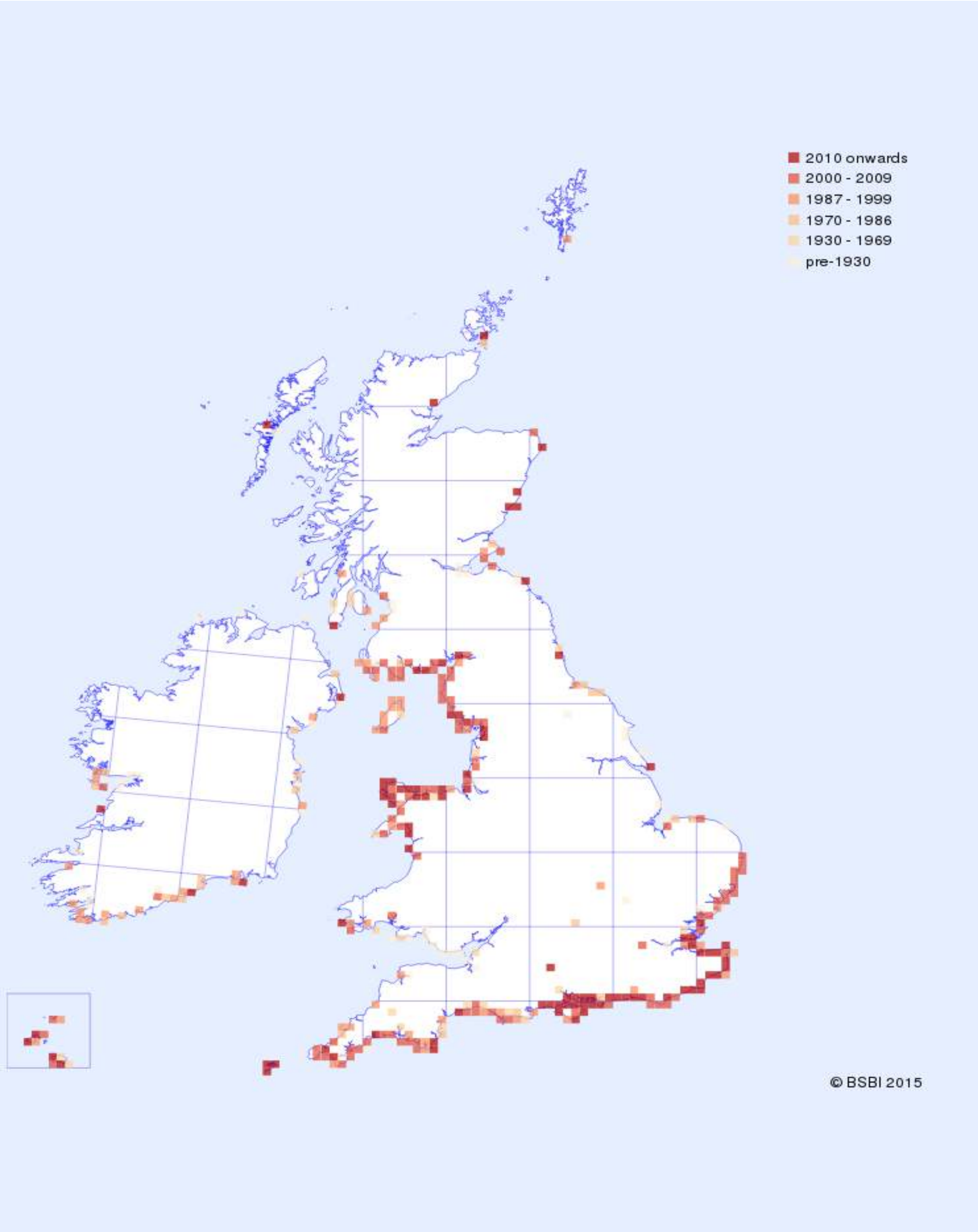

Figure 34: Distribution of *Crambe maritima* across the UK and Ireland; cream squares represent recorded sightings from pre 1930; light orange/orange squares represent recorded sightings from 1930-2009; red squares represent recorded sightings from 2010 onwards (Source: BSBI 2015)

6.2 Sea aster (Tripolium pannonicum)

Sea aster is a biennial to short-lived perennial halophyte of the Asteraceae family that grows in the upper salt marshes and coastal areas of temperate regions, particularly northwestern Europe. In some regions, it can be found on cliff faces in very little soil or on rocks and in inland saline areas (e.g. the Burren, Co. Clare) (Devlin, 2015b, Ramani, 2006, and Clapham *et al.* 1942). It grows in a variety of soil types, ranging from sands to clays and in peaty silts common in the marshes of southwest Ireland (Clapham *et al.* 1942). Sea aster is a native species to Ireland and can be found frequently around our coasts, however, in some areas the distribution is localised (Figure 35 & 36) (BSBI 2015, Devlin 2015b, and The Irish Species Register, 2015).

Sea aster can reach heights of 1m, is fleshy, and has pale, purple-blue insect-pollinated flowers that are similar to daisies in appearance and which bloom from July to October (Figure 35). The leaves are dark green and are linear, with a prominent midrib (Devlin 2015b and Clapham *et al.* 1942). It is very salt-tolerant, being able to grow at levels equivalent to two-thirds the strength of seawater (300mM NaCl) (Ventura *et al.* 2013).

Figure 35: Sea Aster (*Tripolium pannonicum*) leaves and flowers (Source: Devlin 2015b)

The leaves of sea aster are edible, having a salty taste, and due to their high nutritional value (Table 6), they are valuable as a health food (Wagenvoort *et al.* 1989). Interest in producing sea aster commercially has grown in recent years and initial studies are promising (Buhmann *et al.* 2015 and Ventura *et al.* 2013). Ventura *et al.* (2013) conducted growth trials in Israel in a temperature controlled (20°-33°C), plastic covered greenhouse. Four week old sea aster plants were transplanted into sand-dune soil plots (96% sand, 0.8% silt, 3.1% clay, <0.1% organic matter, pH 8, 1 x 2.25m size). There were 12 plots in total, with 4 plots per treatment (control {0mM NaCl}, 50mM NaCl, 100mM NaCl). The salt treatments and irrigation were supplied via a drip irrigation system, three times per day. All treatments were supplemented with a commercial NPK fertiliser (5-3-8 & microelements; Haifa Chemicals Ltd) in the irrigation water.

It was found that the sea aster plants growing at the highest salinity treatment produced the most biomass, however, there was no significant difference between the control, 50mM, and 80mM NaCl treatment. The chemical composition of the leaves exhibited higher levels of electrical conductivity, total soluble solutes, and the antioxidant compounds ascorbic acid and polyphenols in comparison to plants grown without any NaCl supplementation (control). The levels of polyphenols were also significantly greater in the plants grown in 80mM NaCl in comparison to the control (Table 6) (Ventura *et al*. 2013).

Table 6: Yield and leaf constituents of sea aster grown under greenhouse plot conditions (control, 50mM NaCl, 80mM NaCl); values followed by different letters are significantly different (p<0.05); n.d. = not determined (Ventura *et al*. 2013)

	Irrigation water salinity		
	Control	50 mM NaCl	80 mM NaCl
Total yield (kg m^{-2} year^{-1})	16.5±0.7a	18.3±1.2a	18.6±1.2a
Marketable yield (kg m^{-2} year^{-1})	10.0±0.5a	11.5±1.1a	11.7±1.0a
pH	6.11±0.03a	6.10±0.07a	6.22±0.02a
EC (dS m^{-1})	20.3±0.39c	22.5±0.43b	24.2±0.57a
TSS (%)	3.26±0.07a	3.40±0.27a	3.60±0.11a
Ascorbic acid (mg 100 g^{-1} FW)	13.7±1.7a	n.d.	15.1±1.4a
Polyphenols (mg 100 g^{-1} FW)	31.6±2.2b	37.0±2.7b	45.9±2.2a
β-carotene (μg g^{-1} FW)	36.9±5.8a	32.6±2.9a	30.5±3.1a
Chlorophyll (μg g^{-1} FW)	198.6±36.8a	153.6±25.5a	140.0±19.0a

Buhmann *et al*. (2015) conducted hydroponic (method of growing plants using mineral nutrient solutions, in water, without soil) growth trials on 8-week old sea aster plants in a greenhouse with temperatures of c. 20°/15°C day/night and artificial lighting (12h light/dark rhythm) from October to May. A number of 35-day trials were conducted to assess the impacts of nutrient addition and salinity on the biofiltering and growth capacity of sea aster. Before these trials took place, Buhmann *et al* (2015) conducted an experiment to investigate the influence of different culture modes (sand culture, expanded clay {clay pebbles}, and hydroponic) on the growth of sea aster (salinity of solution was 15psu). Some of the main findings of this research were: 1) plants cultured in expanded clay and hydroponic culture showed a higher biomass gain and uptake of nitrogen than those cultured in sand (not significantly different); 2) the hydroponic culture treatment displayed significantly higher uptake of phosphorus than the other two treatments; 3) plants grown in hydroponic and expanded clay had a lower chlorophyll (252 and 525 μg g^{-1}) and carotenoid content (42 and 78 μg g^{-1}) than those grown in sand (812 and 123 μg g^{-1}); 4) biomass gain was significantly higher at the lowest (15psu) salt concentration; and 5) uptake of nitrogen and phosphorus declined with increasing salt concentration (not significant). Varying quantities of nitrate and phosphate additions to the hydroponic solutions were assessed to determine appropriate nitrate-N and phosphate-P concentrations in the solution for effective biofilter performance. It was found that there was little difference seen among treatments with 10-100mg NO_3-N l^{-1}, however, the treatment with 1mg NO_3-N l^{-1} had significantly less biomass gain, plants took up less nitrogen, phosphorus, chlorophyll, and carotenoid. For phosphate, gain of biomass and uptake of nitrogen did not show any difference between treatments. However, uptake of phosphorus and phosphorus content of plants declined significantly with decreasing phosphate-P concentration in the solution. There was also lower chlorophyll and carotenoid content in the treatments with 0.3-3.3 mg l^{-1} phosphate-P when compared to those with 5.0-16.3 mg l^{-1} phosphate-P in the solution (Buhmann *et al*. 2015).

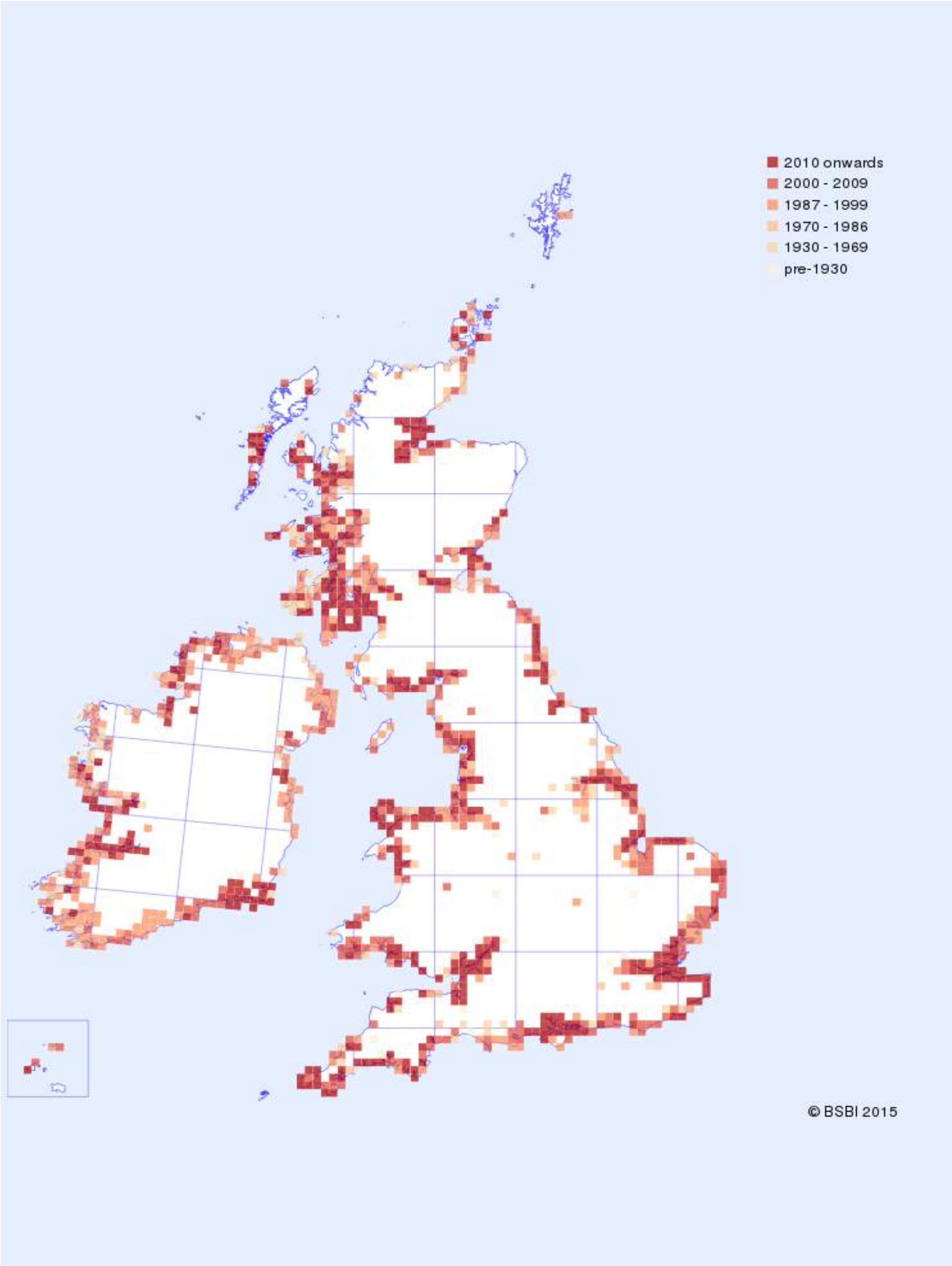

Figure 36: Distribution of *Tripolium pannonicum* across the UK and Ireland; cream squares represent recorded sightings from pre 1930; light orange/orange squares represent recorded sightings from 1930-2009; red squares represent recorded sightings from 2010 onwards (Source: BSBI 2015)

6.3 Sea purslane (Atriplex portulacoides)

Sea purslane is a sprawling, perennial, greyish-green halophytic shrub of the Chenopodiaceae family. It can be found growing in saltmarshes (usually colonising the lower and mid marsh) and estuaries along the coasts of Europe, North Africa, and Southwest Asia in a variety of substratum: shingle, sand, sandy mud, silt, and clay (Devlin, 2015c, Cott *et al.* 2013, Neves *et al*. 2007, and Chapman, 1950). It is native to Ireland and is found mainly on the east and south coast (Devlin, 2015c and BSBI, 2015).

Figure 37: Sea Purslane leaves (*Atriplex portulacoides*) (Source: Devlin 2015c)

This species can be found on the west coast of Ireland, however, it is notably absent from saltmarshes on peat substrates (Figure 38). A study conducted by Cott *et al*. (2013) found that sea purslane is intolerant of high soil moisture, a characteristic of peaty soils. It has a granular appearance and texture with branches that can spread over 100cm and can range in height from 20-50cm (Figure 37). The flowers, which bloom from July to October, are wind-pollinated (Devlin, 2015c, Neves, 2007, and Chapman, 1950). Sea purslane grows optimally in 200mM NaCl water, however, it can survive salinities of 1,000mM, which is twice the salinity of seawater (Benzarti *et al*. 2014). The thick and succulent grey-green ovate young leaves are edible, with a crunchy texture and natural saltiness that can be pickled, eaten raw in salads, steamed, or boiled (PFAF Plant Database, 2012).

Neves *et al*. (2007) conducted field-studies of sea purslane growing in the Castro Marim salt marsh of Southern Portugal from autumn 2001 – autumn 2002 and found that these plants had a very strong level of growth, with a mean above ground biomass of 598g m^{-2} yr^{-1}. The maximum above ground biomass occurred in spring, reaching 1,077g m^{-2} yr^{-1}. The most commonly eaten part of sea purslane, the leaves, provide a healthy level of nitrogen, phosphorus, potassium, calcium, and magnesium. The quantity of these nutrients in the leaves varies with the season and the minimum and maximum levels of the nutrients monitored and the season in which these levels were achieved are listed in Table 7 below (Neves *et al*. 2007).

Table 7: Min/max nutrient content of the edible leaves of sea purslane

Components	**Quantity (mg g^{-1})**
Nitrogen	8.9 (autumn) / 14.6 (summer)
Phosphorus	1.1 (summer) / 1.4 (spring)
Potassium	17 (summer) / 25 (autumn)
Calcium	7 (summer) / 9 (spring)
Magnesium	7 (winter) / 10 (spring)

Other studies on the health benefits of sea purslane are limited, however, a recent study by Ksouri *et al*. (2011) found that a species of the same genus of sea purslane, *Atriplex halimus* (Mediterranean saltbrush), was a source of flavonol aglycones and flavonoid sulphates, both of which have antioxidant activity, helping with glycaemic control in diabetic patients (Ksouri *et al*. 2011). As sea purslane is closely realted to *A. halimus*, it is very likely that it also contains these antioxidant properties.

There are no records available of commercial-scale production of sea purslane, nevertheless, as it is a tasty, salty, and novel source of essential nutrients, there is a potential niche market opportunity for its culture in Ireland for the national and international market (Ksouri *et al*. 2011 and Neves *et al*. 2007).

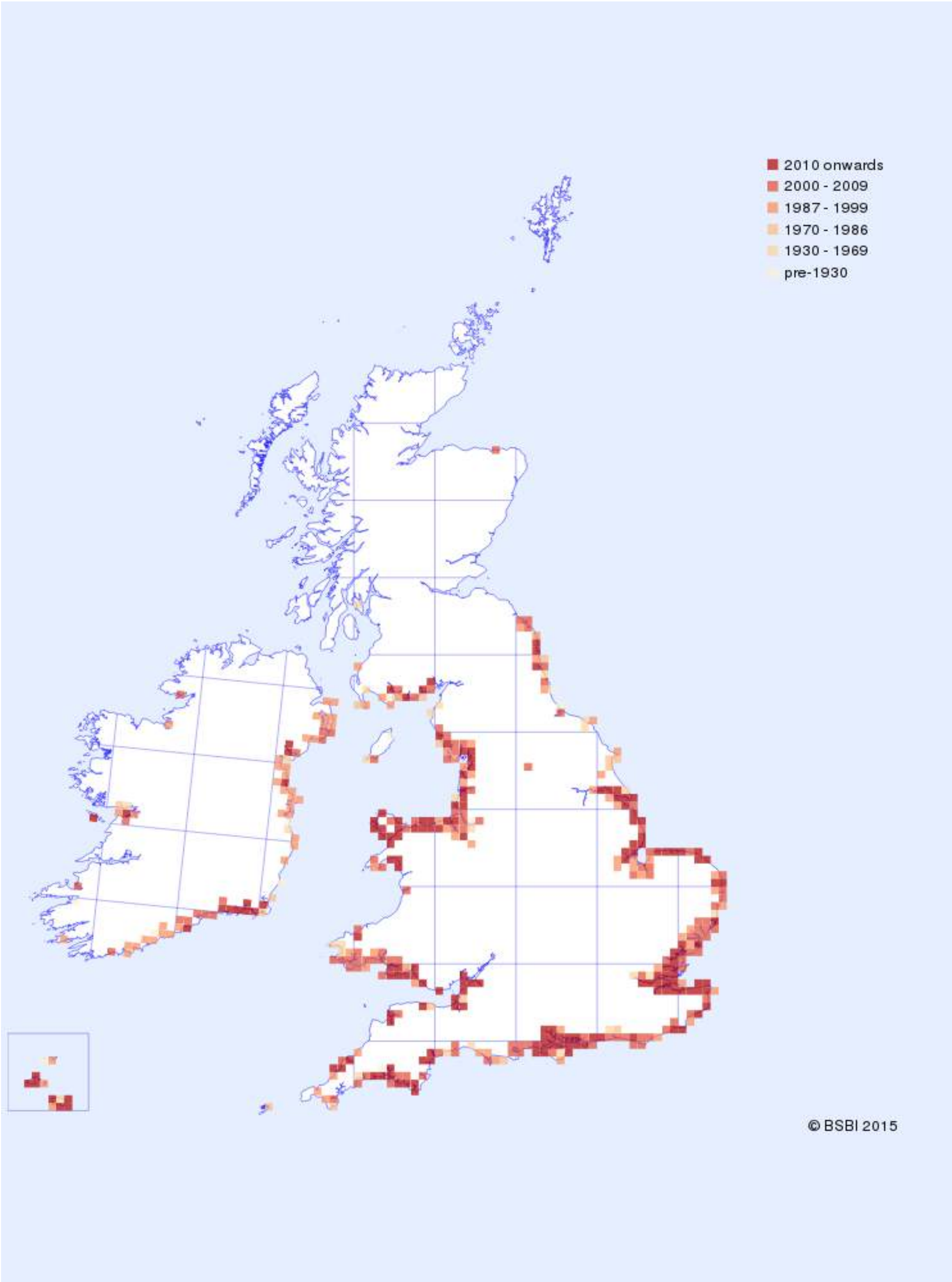

Figure 38: Distribution of *Atriplex portulacoides* across the UK and Ireland; cream squares represent recorded sightings from pre 1930; light orange/orange squares represent recorded sightings from 1930-2009; red squares represent recorded sightings from 2010 onwards (Source: BSBI 2015)

7. Conclusion

In the most arid regions of the word the cultivation of halophytes as an alternative to conventional crops is gaining significant popularity. Globally, halophytes are being noted for their nutritional, culinary, oilseed, forage/ feed crop, medicinal, and wastewater treatment potential. In an Irish context, samphire (*S. europaea*) is becoming increasingly popular on the menus of restaurants and the counters of fishmongers and health-food stores across the country. Also, as the need to reduce the potential environmental impact of industry (e.g. onshore aquaculture) is of the utmost importance for sustainable and environmentally responsible development, the potential for halophytes to treat wastewater is a very promising prospect. The oyster hatchery wastewater trial (Section 5) showed that samphire exhibits successful growth when exposed to varying levels of wastewater and can reduce the ammonia, nitrate, nitrite, and phosphate levels being released to the sea.

The UK and Irish *Salicornia* market is primarily dominated by wild gathering, consequently, the quality and quantity of the product is inconsistent and the supply is limited. Also, the areas in which samphire can be found are often designated SACs, and the picking of any plants in these areas is forbidden. With this in mind, and the fact that samphire is continuing to increase in popularity, there is enormous potential for a commercial samphire industry in Ireland from an economic and environmental remediation perspective. However, to fully realise this potential there are a number of aspects of its cultivation that still need attention.

Firstly, the main impediment to large-scale cultivation of halophytes, including samphire, is the prevalence of undesirable crop characteristics (e.g. non-uniform flowering and ripening) in wild germplasm. Further research into the selective breeding of samphire for traits that are desirable for commercial cultivation e.g. uniform flowering and ripening) would be hugely beneficial (Section 2.6). Secondly, it must be noted that the trials conducted for this manual were relatively small in scale, and further work is required to assess the larger scale aeroponic production of samphire. The propagators used in the Irish trials (Section 3.4.2 & 5) (Figure 15) are ideal for small-scale production (e.g. small shop/hobbyist/road-side sale). For large-scale production the general design principle would be the same (Figure 14; Section 3.4.2), however, modifications required to scale up would need to be made on a site-specific basis. Such modifications would be dependent on a number of factors such as scale of production planned, funding, available space and resources, etc. Other forms of production mentioned in this manual, but which have not yet been trialled in Ireland (e.g. constructed wetlands and subsurface flow-through systems), should also be assessed to find the most suitable system for Ireland. The most suitable system will be dependent on the type of production site being implemented (this can range from a heat and light controlled greenhouse to fully outdoors with no control over environmental conditions).

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9. Appendices

9.1 List of Israeli exporters of Salicornia

1) Flora Export S.E. Israel Ltd (http://www.flora-sg.com/)
2) Farmers Direct Ltd (http://www.farmersdirect.co.il/index.html)
3) Agrexco (http://www.agrexco.com/)
4) Bacto Sil Ltd (http://www.bactosil.com/#In_English_)

9.2 Techniques for measuring growth

Height was measured from the base to the tip of the main stem. The number of nodes refers to the number of nodes on the main stem. The node is the part of the stem from which a leaf, branch, or aerial root grows. The number of branches refers to the number of branches that have grown from the main stem. The branches can be seen growing from the nodes on the main stem (Figure 39).

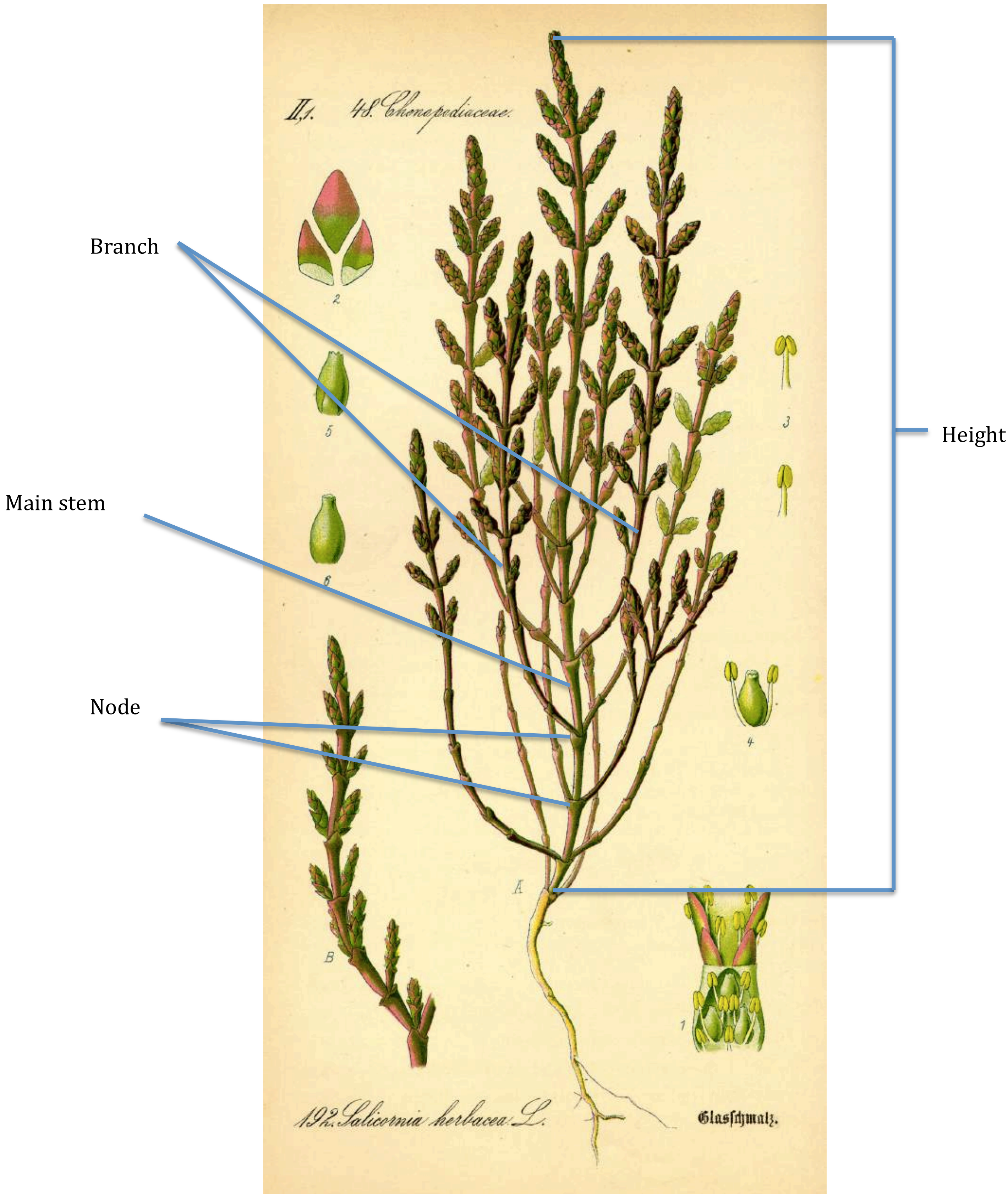

Figure 39: Common features of samphire that are measured to assess growth (source: Biolib.de)

9.3 How to use Excel to randomise a data set

Step 1: Enter *=RAND()* into the first cell of the column adjacent to the data you want to randomise. This will create a random number in this cell.

Step 2: Click the corner of this cell and drag down to the cells below (as far as the end of the adjacent column with the data set that you wish to randomise).

Step 3: Select both columns

Step 4: Select data>sort>custom sort

Step 5: In the "sort by" column, select the column of randomised numbers and click "ok"

Websites for assistance:

http://www.extendoffice.com/documents/excel/644-excel-random-cell.html

http://www.excel-easy.com/examples/randomize-list.html

https://www.youtube.com/watch?v=q8fU001P2lI

9.4 Ammonia, nitrite, nitrate, and phosphate levels in oyster hatchery wastewater before and after treatment with samphire

Note: Numbers highlighted in green represent reduction in level

Table 8: Ammonia (mg/L) levels in each treatment at the beginning and end of a 1 week* treatment period

Start of week				End of week			
Date	**T1**	**T2**	**T3**	**Date**	**T1**	**T2**	**T3**
12/8/15	0.023	0.119	0.165	19/8/15	0.041	0.089	0.134
19/8/15	2.692	2.889	3.254	27/8/15	0.110	0.069	0.556
27/8/15	0.028	0.094	0.203	3/9/15	0.039	0.041	0.077
3/9/15	0.061	0.090	0.131	18/9/15	0.039	0.065	0.192

Table 9: Nitrite (mg/L) levels in each treatment at the beginning and end of a 1 week* treatment period

Start of week				End of week			
Date	**T1**	**T2**	**T3**	**Date**	**T1**	**T2**	**T3**
12/8/15	0.003	0.012	0.028	19/8/15	0.005	0.007	0.014
19/8/15	0.058	0.047	0.123	27/8/15	0.005	<0.001	0.109
27/8/15	0.002	0.008	0.028	3/9/15	0.001	0.020	0.025
3/9/15	0.004	0.011	0.016	18/9/15	0.001	0.001	0.027

Table 10: Nitrate (mg/L) levels in each treatment at the beginning and end of a 1 week* treatment period

Start of week				End of week			
Date	**T1**	**T2**	**T3**	**Date**	**T1**	**T2**	**T3**
12/8/15	0.121	0.204	0.246	19/8/15	0.246	0.275	0.274
19/8/15	3.453	2.882	3.713	27/8/15	0.341	0.028	0.532
27/8/15	0.080	0.188	0.251	3/9/15	0.041	0.065	0.081
3/9/15	0.198	0.271	0.319	18/9/15	0.023	0.024	0.056

Table 11: Phosphate (mg/L) levels in each treatment at the beginning and end of a 1 week* treatment period

Start of week				End of week			
Date	**T1**	**T2**	**T3**	**Date**	**T1**	**T2**	**T3**
12/8/15	0.020	0.035	0.039	19/8/15	0.024	0.040	0.031
19/8/15	2.635	3.016	2.984	27/8/15	0.403	0.610	0.521
27/8/15	0.028	0.032	0.041	3/9/15	0.042	0.152	0.095
3/9/15	0.024	0.032	0.038	18/9/15	0.021	0.029	0.102

*Note: Wastewater added to the propagators on the 3/9/15 remained in the propagators for 2 weeks, therefore the "end of week" sample was taken 2 weeks later

9.5 List of halophyte plant and seed suppliers

Crambe maritima (Sea Kale):

1) Nursery: Victoriana Nursery, Kent, UK

Cost: £1.30 per plant/£3.25 for 20 seeds

Link: https://www.victoriananursery.co.uk/Seakale_Plant_Lillywhite/ (plant)

https://www.victoriananursery.co.uk/Sea_Kale_Seed_Lillywhite/ (seed)

2) Nursery: Special Plants Nursery, Wilts, UK

Cost: £2 for 10 seeds

Link: http://www.specialplants.net/shop/search/crambe_maritima/

Other nursery sources for sea kale in the UK can be found at:

https://www.rhs.org.uk/Plants/Nurseries-Search-Result?query=4710&name=%3Ci%3ECrambe%20maritima%3C/i%3E

Tripolium pannonicum (Sea Aster):

Nursery: Scrops – Serra Maris bvba, Ninove, Belgium

Cost: Seeds - contact nursery for quote (email: info@scrops.com phone: +32 54 329093)

Link: http://www.scrops.com/Seeds.htm

Atriplex portulacoides (Sea Purslane):

Nursery: Pennard Plants, East Pennard, Somerset, UK

Cost: £1.75 for approximately 600 seeds

Link: https://www.pennardplants.com/products.php?cat=234