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Feasibility study for integrated multitrophic aquaculture in southern Australia



**K. H. Wiltshire, J. E. Tanner, C.F.D. Gurgel
and M. R Deveney**

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**SARDI Aquatics Sciences
PO Box 120 Henley Beach SA 5022**

December 2015

Report to the Fisheries Research & Development Corporation



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**PREMIUM
FOOD AND WINE FROM OUR
CLEAN
ENVIRONMENT**



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EXECUTIVE SUMMARY

Integrated multitrophic aquaculture (IMTA) involves strategic co-culture of organisms so that wastes from one species are used to grow another, providing environmental and economic benefits. Seaweeds can be used in IMTA systems to remove and utilise dissolved inorganic nutrients from fish aquaculture, allowing environmentally sustainable expansion of this industry. Farming seaweeds is also of interest due to increasing demand for seaweed products, of which Australia is a net importer. Seaweed farming is, however, not an established industry in Australia, and to date no offshore seaweed aquaculture has been performed. Here we report a study performed by the South Australian Research and Development Institute (SARDI) to investigate the aquaculture potential of several native seaweeds with little to no previous aquaculture history, and to implement the first offshore seaweed aquaculture trials in South Australia (SA).

Background

Several finfish species are farmed in Australia, and production is increasing to meet growing demand for seafood both nationally and internationally. Finfish aquaculture in SA involves off-shore farming of two main species in Spencer Gulf: Southern Bluefin Tuna, *Thunnus maccoyii*, and Yellowtail Kingfish, *Seriola lalandi*. These predatory fish release around 10 times more dissolved nitrogen per tonne of production than farmed salmonids. The biogeochemical models used to set limits on stock levels to maintain water quality show that dissolved nitrogen wastes are the limiting nutrient for environmentally sustainable production of these fish species in SA. The industry is keen to increase stocking densities and to reduce costs by employing automated feeding, but both of these would likely result in greater localised nutrient inputs to the environment. To avoid increased nutrient loading, either feed conversion ratios would need to be improved, or nutrients removed, e.g. by growing seaweed in an IMTA system.

Australia is a net importer of seaweed products and few Australian macroalgae species have been commercially cultivated. Off-shore cultivation is yet to be developed. Seaweed aquaculture should use local species to ensure they are appropriate for the habitat and to avoid the risks involved with introduced species. Adoption of IMTA in Australia is therefore likely to require development of novel species for aquaculture. This project investigated several species of seaweed native to SA's fish farming region to determine potentially suitable species and farming systems for the development of IMTA in Australia.

Objectives

1. Review available published and unpublished literature and databases, and liaise with international research teams to assess potentially suitable species and farming techniques for use in IMTA;
2. Trial selected macroalgae species in tanks to improve understanding of their biology and develop appropriate propagation techniques for later open-water grow-out, based on knowledge gained from Objective 1;
3. Undertake a field trial of IMTA, to assess macroalgal growth rates, determine optimal spatial configuration to maximise growth, and commercial potential;
4. Assess the potential for macroalgal species trialled to act as reservoirs for parasites/pathogens of other species used in the system;
5. Provide improved parameter estimates for biogeochemical modelling of IMTA, enabling its consequences for regional nutrient enrichment to be determined;
6. Provide recommendations to industry on what species to farm, with what culture systems, and in what densities, to optimise both nutrient extraction and economic returns.

Methodology

A literature review identified several native seaweed species potentially suitable for aquaculture. We investigated growth rates, nutrient removal capability, culture techniques, and feasibility of propagation for eight of these in initial laboratory trials at SARDI and in a pilot field trial near metropolitan Adelaide in 2012-13. The best performing species from initial trials were then used in on-farm trials conducted on Yellowtail Kingfish lease sites near Port Lincoln, SA, in 2014-15. The on-farm trials also investigated whether seaweed farming infrastructure could harbour eggs of skin and gill flukes. Propagation techniques, and light, temperature, and nutrient responses of selected species were also studied in the laboratory to obtain further information on optimum culture conditions. Data from these experiments will also enable nutrient extraction by seaweeds to be incorporated into biogeochemical models used to predict environmental carrying capacity of fish farming.

Results

The carrageenan-producing red seaweed *Solieria robusta*, and the common kelp, *Ecklonia radiata*, showed the greatest aquaculture potential of the tested species. *Solieria robusta* performed well in laboratory experiments and showed promising, although highly variable, growth in field trials. *Ecklonia radiata* was the best performing brown seaweed in field trials and was successfully reproduced and seeded onto string. The agar-producing red seaweed, *Gelidium australe*, is another potential candidate. It had lower maximum potential growth than *S. robusta*, but performed best in the pilot field trial and may be better suited to culture in certain

conditions. We were able to reproduce this species using tissue culture techniques, which have not yet been attempted for *S. robusta*. Overgrowth by fouling organisms negatively impacted seaweed growth at field sites, but given their small scale, the trials may not be representative of larger scale seaweed aquaculture at these sites. Trials conducted with greater initial biomass may enable seaweed to out-compete fouling growth, and more regular monitoring and maintenance of trials would assist in averting problems. Seaweed culture infrastructure entangled few fluke eggs and is unlikely to impact on fluke management. We obtained information on optimal conditions (temperature, light and nutrient) for *S. robusta* growth in the laboratory, which will help to determine the best depths and seasons for culture, and provide information to refine biogeochemical models to include nutrient removal by seaweeds.

Implications

IMTA can decrease net nutrient release from fish farms, or facilitate higher stocking densities with the same environmental footprint for finfish aquaculture. Industry and communities can benefit from IMTA through reduced economic risks from product diversification, improved productivity, and amelioration of environmental impacts. This project contributes the first practical information on offshore seaweed culture and implementation of IMTA in Australia, but additional research and development are required before IMTA using seaweeds can be applied on a large scale or commercially.

Recommendations

Development of IMTA at a large scale requires further investigation of seaweed biology, culture systems and locations. Propagation and nursery techniques need refining and up-scaling to produce adequate biomass for seed stock for large scale seaweed culture. Suitable sites for seaweed culture need to be identified and culture systems refined. Potential markets for products from farmed seaweeds need to be developed, and future culture trials should investigate product quantity and quality as well as seaweed growth and nutrient removal.

Keywords

Aquaculture, seaweed, IMTA, nutrients, *Ecklonia radiata*, *Cystophora subfarcinata*, *Sargassum linearifolium*, *Scytothalia dorycarpa*, *Gelidium australe*, *Pterocladia capillacea*, *Solieria robusta*, *Plocamium angustum*, South Australia, Southern Bluefin Tuna, *Thunnus maccoyii*, Yellowtail Kingfish, *Seriola lalandi*.

INTRODUCTION

Integrated multi-trophic aquaculture (IMTA) is a system involving co-culture of organisms at complimentary trophic levels, such that wastes from one (e.g. finfish) are recycled and utilized by others, such as filter-feeders (e.g. bivalves), which remove particulate wastes, and autotrophs (e.g. seaweeds), which remove dissolved inorganic nutrients (Soto 2009). The seaweeds, bivalves or other extractive species used in IMTA are also crops of commercial value, leading to reduced economic risk through diversification of product farmed (Ridler *et al.* 2007; Barrington *et al.* 2009; Soto 2009). Extractive species in IMTA systems grow faster than in monoculture, leading to greater overall profitability (Petrell and Alie 1996; Troell *et al.* 2003; Whitmarsh *et al.* 2006). IMTA can also lead to greater social acceptance of aquaculture activity (Ridler *et al.* 2007), and IMTA seafood can be marketed at a premium price (Whitmarsh and Wattage 2006). With growing concerns about environmental impacts of aquaculture, such as eutrophication and benthic enrichment (Silvert 1992), governments worldwide are increasingly regulating aquaculture activities, and there is growing interest in IMTA (Neori *et al.* 2004; Barrington *et al.* 2009).

Several finfish species are farmed in Australia, and there is community concern about impacts of finfish aquaculture, and a strong emphasis on management of the industry to ensure environmental sustainability (Rimmer and Ponia 2007). Finfish aquaculture in South Australia (SA) involves off-shore farming of two main species in Spencer Gulf: Southern Bluefin Tuna (tuna), *Thunnus maccoyii*, and Yellowtail Kingfish (kingfish), *Seriola lalandi*, with annual production of tuna ranging between 5 800 and 9 757 tonnes from 2005/06 to 2013/14, and production of kingfish being between 579 and 3 757 tonnes from 2008/09 to 2013/14 since beginning in 2007 (Econsearch 2015). Tuna production is projected to increase by ~7% per annum from 2015-2017, while kingfish production is projected to increase by 26% annually (Econsearch 2015). For every tonne of production, tuna release as much as 500 kg of nitrogen (N), with ~90% in dissolved form (Fernandes *et al.* 2007), and kingfish release up to 200 kg, with ~70% dissolved (Fernandes and Tanner 2008). Tuna are fed baitfish and have a higher food conversion ratio (FCR) than kingfish, which are fed a pellet diet, and both have greater FCRs than other farmed fish, e.g. salmonids, which release 42-57 kg N per tonne of production (Fernandes *et al.* 2007; Fernandes and Tanner 2008). Primary Industries and Regions SA (PIRSA) Fisheries and Aquaculture use biogeochemical models (e.g. Collings *et al.* 2007; Tanner *et al.* 2007; Middleton *et al.* 2013) to set limits on stock levels to maintain water quality, with dissolved nitrogen wastes being the nutrient determining maximum stock levels for both tuna and kingfish. Industry is keen to increase stocking densities and to reduce costs by employing automated feeding (Cleanseas pers comm.), but both of these would likely result in

greater localised nutrient inputs to the environment. There is also interest from both industry and government in expanding production, and opening up new areas to aquaculture (Econsearch 2015; PIRSA pers. comm). To avoid increased nutrient loading, either FCRs would need to be improved, or nutrients removed, e.g. by growing macroalgae in an IMTA system (Neori 2008).

In addition to the biomitigation potential of seaweeds used in IMTA (Chung *et al.* 2002; Neori 2008; Troell *et al.* 2009), interest in growing seaweeds is also being driven by the increasing demand for seaweed products globally. Seaweed aquaculture comprises almost half of global aquaculture production in terms of biomass, and although seaweed farming occurs predominantly in Asia, this growing demand for seaweed products, combined with diminishing wild harvests, has led to the expansion of seaweed culture in many countries (FAO 2010). Major uses of seaweeds are for human consumption (especially the kelp species *Saccharina japonica* and *Undaria pinnatifida*, and red algae of the genera *Pyropia* and *Porphyra*), and for their hydrocolloids: alginate, carrageenan and agar (McHugh 2003; Bixler and Porse 2011; White and Wilson 2015). Seaweed hydrocolloids are used as gelling and emulsifying agents in a wide range of food products, including confectionary, dairy and processed meat, have numerous industrial applications including in textile printing and paper manufacture, have several pharmaceutical applications, and form gels that are used as culture media and for electrophoresis (McHugh 2003; Bixler and Porse 2011). Many seaweeds produce compounds with bioactive properties, including anti-tumor, anti-viral, anti-bacterial, and anti-fungal activities, that may be of benefit in functional foods, medicines, and pesticides (Smit 2004; Holdt and Kraan 2011; Lorbeer *et al.* 2013). The use of seaweed biomass for production of biofuels (Buchholz *et al.* 2012; Wei *et al.* 2013) is also being developed. Australia has few established algal industries, and nearly all seaweed products are imported, with the remainder coming from harvests of natural populations or beach cast material. Total imports of seaweed products into Australia were ~5 000 tonnes, with a value over AUD\$17 million, in 2008-9, and are increasing by almost 30% per annum (Lee 2010).

Few Australian macroalgae species have been commercially cultivated, and off-shore cultivation is yet to be developed (Lee 2010). Farmed seaweeds should, however, be local to ensure they are appropriate for the habitat and to avoid the risks involved with introduced species (Soto 2009). Application of seaweeds to IMTA in Australia is therefore likely to require development of novel species for aquaculture. Australia, and in particular, southern Australia, has high macroalgal diversity, with a large proportion of endemic species (Phillips 2001). The potential value of this unique macroalgal diversity has been recognised, further supporting the development of a local seaweed industry using novel species (Lee 2010; Lorbeer *et al.* 2013). Other drivers for the establishment of a local seaweed aquaculture industry include increasing

demand for fresh local product from the restaurant industry, and an increasing demand in major markets such as China and Japan for fresh product that does not have contamination concerns associated with it. Currently, these markets are largely supplied from locally grown seaweed, often grown in polluted waters in China, and since the Fukushima nuclear disaster, in potentially radioactively polluted waters in Japan. This project investigated several species of seaweed native to South Australia's fish farming region to determine potentially suitable species and farming systems for the development of IMTA in Australia.

Objectives

1. Review available published and unpublished literature and databases, and liaise with international research teams, to assess potentially suitable species and farming techniques for use in IMTA;
2. Trial selected macroalgae species in tanks to improve understanding of their biology and develop appropriate propagation techniques for later open-water grow-out, based on knowledge gained from Objective 1;
3. Undertake a field trial of IMTA, to assess macroalgal growth rates, determine optimal spatial configuration to maximise growth, and commercial potential;
4. Assess the potential for macroalgal species trialled to act as reservoirs for parasites/pathogens of other species used in the system;
5. Provide improved parameter estimates for biogeochemical modelling of IMTA, enabling its consequences for regional nutrient enrichment to be determined;
6. Provide recommendations to industry on what species to farm, with what culture systems, and in what densities, to optimise both nutrient extraction and economic returns.

1. SELECTING POTENTIALLY SUITABLE SPECIES

Species used in IMTA systems need to fulfil several criteria: they should be local to ensure they are appropriate for the habitat and to avoid the risks involved with introduced species; cultivation technology needs to be available; and they should have an established or potential market value. Suitable species should also be able to achieve high biomass in order to provide adequate nutrient removal, although a high value species that is slower growing could also be suitable, with the trade-off of reduced biomitigation (Soto 2009).

To determine the potential suitability of southern Australian seaweeds for aquaculture, the 1168 species described in the “Marine Benthic Flora of Southern Australia” series (Womersley 1984, 1987, 1994, 1996, 1998, 2003) were systematically reviewed (Appendix IV). Fish farming in SA occurs in moderately to relatively exposed regions of Spencer Gulf, therefore, only species with native ranges spanning this region, that were not listed as rare or uncommon, and that were not restricted to calm conditions, were considered. Each species distribution within SA was determined from the State Herbarium of South Australia’s online plant distribution mapper¹. Since there is no established market for the majority of southern Australian seaweeds, a review of commercial uses of seaweeds globally was undertaken in order to determine which species may have market value. Further to having a potential market value, species were only retained for further consideration if they routinely grow to >20 cm (suggesting that they might be capable of forming at least a moderate biomass in open sea cultivation), and were likely to be able to be cultured using existing technologies. The resulting list of 89 species was further reduced based on expert knowledge of the species characteristics. When multiple species from a single genus were still retained, an attempt was then made to choose the two that were considered the most likely candidates for further examination.

A total of seven species of brown seaweeds (Phaeophyceae) and nine species of red seaweeds (Rhodophyta) were regarded as being worthy of further investigation (Table 1), although it was noted that, especially for the genera *Cystophora*, *Sargassum* and *Plocamium*, related species were also likely to be suitable. This list was further refined based on the accessibility of species for collection, availability of sufficient biomass for initial experiments, and amenability to transport and handling.

Culture methods, reproduction, and growth patterns differ between red and brown seaweeds. Red seaweeds such as the commonly cultivated Gigartinales and Gracilariales are generally able to be grown vegetatively from fragments, while most brown seaweeds of the main farmed

¹ <http://www.flora.sa.gov.au/mapper2.shtml>

orders Laminariales and Fucales do not successfully regenerate or reattach from cuttings, and need to be reproduced from spores or gametes respectively (Sahoo and Yarish 2005; Titlyanov and Titlyanova 2010). Initial trials therefore did not seek to compare all species, but rather to compare species within these two groups, to determine the best candidate species of each. A pilot field study was carried out at Grange on the Adelaide coast to look at adaptability to field culture and seasonal patterns in growth and nitrogen content of both red and brown seaweeds, and to compare culture methods for the red seaweeds. We further investigated growth and nitrogen content of red seaweeds in the laboratory, and attempted reproduction of brown seaweeds by methods used for related farmed species.

1.1. Determining species availability

1.1.1. Methods

A review of published literature, SARDI databases, and herbarium records was conducted to determine potential collecting sites for the short-listed species. The SARDI data sets used included those compiled from Reef Health (Turner *et al.* 2007; Collings *et al.* 2008) and biodiversity surveys (Rowling *et al.* 2009). Herbarium records were accessed from Australia's Virtual Herbarium². Literature searches were performed in Scopus and Google Scholar. Searches were performed using each species name and known synonyms plus appropriate geographical terms.

Between April and September 2012, 23 locations around South Australia were visited and, where suspected shortlist species were found, algal material was collected and brought back to the South Australian Aquatic Sciences Centre (SAASC) at West Beach, where it was housed in 2 000L outdoor tanks. Tanks were supplied with aeration and continuous flow-through filtered natural seawater sourced from the adjacent Gulf St Vincent at ambient temperature and salinity. Illumination was by natural sunlight, filtered through medium green shade cloth.

Where multiple representatives of a genus of interest were found, their relative abundance and ease of collection were noted. Specimens that could not be readily identified were morphologically examined at the State Herbarium of SA by relevant experts, and compared to herbarium specimens to confirm their identity.

² <http://avh.ala.org.au>

Table 1. Shortlist of South Australian seaweed species with potential for IMTA developed from the literature review (Appendix IV)

Order	Family	Species	Species or relative farmed	Possible products
Ochrophyta: Phaeophyceae				
Fucales	Sargassaceae	<i>Cystophora platylobium</i>	Sargassaceae	Terpenoids, polyphenols
		<i>Cystophora subfarcinata</i>	Sargassaceae	Terpenoids, polyphenols
		<i>Sargassum fallax</i>	<i>Sargassum</i> spp.	Terpenoids, polyphenols
		<i>Sargassum linearifolium</i>	<i>Sargassum</i> spp.	Terpenoids, polyphenols
	Seirococcaceae	<i>Scytothalia dorycarpa</i>	Fucales	Terpenoids, polyphenols
		<i>Seirococcus axillaris</i>	Fucales	Terpenoids, polyphenols
Laminariales	Lessoniaceae	<i>Ecklonia radiata</i>	<i>Ecklonia</i> spp.	Terpenoids, polyphenols
Rhodophyta: Florideophyceae				
Bonnemaisoniales	Bonnemaisoniaceae	<i>Asparagopsis taxiformis</i>	<i>Asparagopsis</i> spp.	Abalone feed, bioactives
Gelidiales	Gelidiaceae	<i>Gelidium australe</i>	<i>Gelidium</i> spp.	Agar, abalone feed
	Pterocladaceae	<i>Pterocladia lucida</i>	Gelidiales	Agar, abalone feed
Gigartinales	Solieriaceae	<i>Solieria robusta</i>	Solieriaceae	Carrageenan, abalone feed
	Cystocloniaceae	<i>Hypnea ramentacea</i>	<i>Hypnea</i> spp.	Carrageenan, abalone feed
Gracilariales	Gracilariaceae	<i>Gracilaria chilensis</i>	Farmed species	Agar, abalone feed
		<i>Gracilaria cliftonii</i>	<i>Gracilaria</i> spp.	Agar, abalone feed
Plocamiales	Plocamiaceae	<i>Plocamium mertensii</i>	None known	Abalone feed, bioactives
		<i>Plocamium preissianum</i>	None known	Abalone feed, bioactives

Note: Taxonomic classifications and scientific names in this table have been updated from those shown in Appendix IV to those currently accepted according to AlgaeBase (Guiry and Guiry 2012).

1.1.2. Results

Not all short-listed species were found in sufficient quantity to undertake experimental work, with no *Gracilaria cliftonii*, few isolated specimens of *Hypnea ramentacea*, and only one drift specimen of *Cystophora platylobium* collected. *Asparagopsis taxiformis* was also ruled out of further consideration: although found abundantly this species rapidly decomposed when transferred to tanks. *Gracilaria chilensis* was found only in late spring to summer and also did not survive well in holding tanks. Some specimens of this species were out-planted at the site later used for the pilot field trial (see section 1.2); specimens were tied onto rope following the culture method used elsewhere for this species (Sahoo and Yarish 2005; Abreu *et al.* 2009), but none remained after two months. When grown at sea, *Gracilaria* spp. are generally cultured in low-energy environments such as sheltered bays (Sahoo and Yarish 2005; Titlyanov and Titlyanova 2010), so it is likely that this species is not well suited to cultivation in exposed offshore conditions.

Several *Plocamium* species were collected, including the shortlisted *P. mertensii* and *P. preissianum*, but *P. angustum* showed best survival when held in outdoor tanks, so was selected as the best candidate of this genus. Of the Fucales, three shortlisted species were commonly located in the field, these being: *Cystophora subfarcinata*, *Sargassum linearifolium* (Sargassaceae), and *Scytothalia dorycarpa* (Seirococcaceae). The short-listed species *Sargassum fallax* (Sargassaceae) and *Seirococcus axillaris* (Seirococcaceae) were also located, but were less abundant at collecting locations than their relatives. *Sargassum linearifolium* could be identified year-round by the distinctive shape of its basal leaves, while most *Sargassum* spp. cannot be distinguished from close relatives when not fertile (Womersley 1987). Other species of *Cystophora*: *C. moniliformis*, *C. monilifera*, *C. expansa* and *C. siliquosa* were commonly found, although none were as abundant as *C. subfarcinata*. Most of these species also did not appear as amenable to transport and handling as *C. subfarcinata*, with *C. moniliformis* and *C. expansa* in particular rapidly decaying after collection. No attempt to collect and maintain *C. siliquosa* was made, aside from a few specimens for identification. This species is very similar in appearance to *C. retorta*, making reliable field collection difficult, and it is also dioecious, which is likely to make reproducing this species more complicated than other *Cystophora* spp., which are monoecious (Womersley 1987). The fertile season of *C. subfarcinata* is longer than that of many of its congeners (Klemm 1988; Hotchkiss 1999), providing further evidence that this species is the best candidate from this genus. *Ecklonia radiata* is a common and abundant species, found at nearly all potential collecting sites investigated.

From the short-listed species, the most readily available and amenable to handling were therefore the brown seaweeds: *E. radiata* (Laminariales), *C. subfarcinata*, *Sargassum*

linearifolium, and *Scytothalia dorycarpa* (Fucales); and the red seaweeds: *Gelidium australe*, *Pterocladia lucida* (Gelidiales), *Solieria robusta* (Gigartinales), and *P. angustum* (Plocamiales). *Ecklonia radiata* belongs to the same brown algal order (Laminariales, commonly known as kelps) as common cultivated species such as *Saccharina japonica* and *Undaria pinnatifida*, which are farmed primarily for human consumption but also used as a source of alginates (McHugh 2003; FAO 2010; White and Wilson 2015). Several species of *Ecklonia* are also utilised as food globally (White and Wilson 2015). *Scytothalia dorycarpa* (family Seirococcaceae), *C. subfarcinata*, and *Sargassum linearifolium* (both Sargassaceae) belong to the Fucales, another order of large brown seaweeds commonly used for food and alginates (e.g. *Ascophyllum*, *Sargassum*, *Durvillea* and *Fucus* spp.) (White and Wilson 2015). These two orders of brown seaweed are also known to produce polysaccharides (e.g. Fucoidan) and secondary metabolites, including several polyphenols, that exhibit a range of biological activities (e.g. anti-oxidative, anti-viral, anti-cancer and anti-inflammatory), and have potential application in medicines, functional foods and cosmetics (Smit 2004; Holdt and Kraan 2011; Thomas and Kim 2011; Lorbeer *et al.* 2013). Laminariales and Fucales are also used to produce plant growth stimulators (Craigie 2011; Briceño-Domínguez *et al.* 2014), animal and aquaculture feeds (Dworjanyn *et al.* 2007; Hwang *et al.* 2009; Evans and Critchley 2014), and are a potential source of biomass for biofuel production (Buchholz *et al.* 2012; Wei *et al.* 2013).

Pterocladia lucida is an agarophyte commercially wild-harvested in New Zealand (Brasch *et al.* 1984), while the southern Australian endemic *Gelidium australe* is also a known agar producer (Gordon-Mills *et al.* 1990). Gracilariales are the main red seaweeds farmed for food-grade agar, due to their ease of cultivation and rapid growth, but agar from Gelidiales has stronger gelling properties and is preferred for bacteriological and pharmaceutical applications (Bixler and Porse 2011). *Solieria robusta* belongs to the same family (Solieriaceae) as the predominant farmed carrageenophytes, and produces ι-carrageenan with a high pyruvate and sulphate content (Chiovitti *et al.* 1999). Extracts from *Solieria robusta* show anti-cancer (Yen *et al.* 2014), hypolipidaemic (Ara *et al.* 2002) and anti-fungal (Khazada *et al.* 2007) activity. This species also has a history of human consumption in the Philippines (Tito and Liao 2000) and Pacific islands (Novaczek 2001). *Plocamium angustum* is of potential commercial interest as a feed for farmed abalone (Kirkendale *et al.* 2010), and as a source of bioactives, including anti-bacterial and anti-fungal agents (Timmers *et al.* 2012).

1.2. Pilot field trial

The seaweed species identified as potentially suitable in the literature review (Appendix IV), and found to be available and amenable to handling, have virtually no previous history of aquaculture, although related species are farmed. It is therefore unknown if these species are

suitable for culture in the sea and so a pilot field trial was conducted in Gulf St Vincent, at Grange on the Adelaide metropolitan coast, to obtain a preliminary determination of adaptability to culture and compare growth rates and nitrogen content between species and seasons. Culture methods, reproduction, and growth patterns differ between red and brown seaweeds (Sahoo and Yarish 2005; Titlyanov and Titlyanova 2010); therefore, the two types were considered separately, with comparisons made within each group rather than between types.

Ecklonia species are not commercially farmed, but experimental culture of some species has been carried out (Hwang *et al.* 2009; Neill *et al.* 2009), including of *Ecklonia radiata* in New Zealand (Neill *et al.* 2009). These studies applied established culture techniques used for related farmed Laminariales. Several *Sargassum* species are farmed, including *S. fusiforme*, *S. horneri*, *S. thunbergii* and *S. fulvellum* (Hwang *et al.* 2007; Pang *et al.* 2007; Pang *et al.* 2009; Li *et al.* 2010; Zou *et al.* 2012), but there is no history of culture of *Cystophora* or *Scytothalia*, which are found only in Australia and New Zealand (Womersley 1987). Farmed Laminariales and Fucales are reproduced sexually, with spores or gametes respectively settled directly onto rope substrates, or seedlings threaded onto rope for out-planting following a period of nursery culture (Sahoo and Yarish 2005; Titlyanov and Titlyanova 2010). In contrast to brown seaweeds, many farmed red seaweeds can be vegetatively propagated. Cultivated Solieriaceae are grown predominantly from fragments tied to ropes, although some culture using mesh bags or tubes also occurs (Ask and Azanza 2002; Góes and Reis 2011). Gelidiales are not commercially farmed (Bixler and Porse 2011), but several cultivation techniques have been trialled, including using fragments attached to ropes, shells, stones or concrete cylinders, or in mesh bags (Friedlander 2008; Ganesan *et al.* 2011). Plocamiales are not cultivated, but cultivation methods used for other Rhodophyta may be applicable (Kirkendale *et al.* 2010).

1.2.1. Methods

The brown seaweeds used were *Ecklonia radiata*, hereafter *Ecklonia*, *Scytothalia dorycarpa*, (*Scytothalia*), *Cystophora subfarcinata* (*Cystophora*), and *Sargassum linearifolium* (*Sargassum*). The red seaweeds used were *Gelidium australe*, hereafter *Gelidium*, *Pterocladia lucida* (*Pterocladia*), *Plocamium angustum* (*Plocamium*) and *Solieria robusta* (*Solieria*). Specimens were collected from the sites shown in Table 2 and housed in 2 000L outdoor stock tanks at SAASC for 1-4 weeks before use. Stock tanks were supplied with aeration and continuous flow-through filtered natural seawater sourced from the adjacent Gulf St Vincent at ambient temperature and salinity. Illumination was by natural sunlight, filtered through medium green shade cloth.

The field experiment was carried out in Gulf St Vincent, at Grange on the Adelaide metropolitan coast (34° 54' 14" S, 138° 28' 16" E), from October 3rd 2012 to October 4th 2013, and consisted

of six deployments of approximately 2 months each (Table 4). Each deployment will be referred to hereafter by an abbreviation of its starting month. On the same day that each new set of specimens was deployed, all specimens from the prior deployment were collected.

Table 2. Collecting localities

Site	GPS coordinates	Depth (m)	Species
Chinaman's Hat	35° 17' 19" S, 136° 55' 5" E	2-5	<i>Pterocladia lucida</i> , <i>Gelidium australe</i> , <i>Scytothalia dorycarpa</i>
Hallett Cove	35° 04' 25" S, 138° 29' 40" E	4-6	<i>Cystophora subfarcinata</i> , <i>Sargassum linearifolium</i>
Outer Harbor	34° 48' 14" S, 138° 28' 24" E	2-5	<i>Solieria robusta</i> , <i>Ecklonia radiata</i>
Rapid Bay	35° 31' 18" S, 138° 11' 09" E	2-5	<i>Ecklonia radiata</i> , <i>Cystophora subfarcinata</i>
Granite Island	35° 33' 59" S, 138° 37' 41" E	3-8	<i>Gelidium australe</i> , <i>Plocamium angustum</i> , <i>Pterocladia lucida</i> , <i>Scytothalia dorycarpa</i>

For the red seaweeds, two culture methods were trialled: ties and bags, based on the 'tie-tie' and 'bag net' methods used for farmed Solieriaceae (Ask and Azanza 2002). Tied specimens were attached to polyethylene rope using loops of bricklayers' line, while bagged specimens were placed in drawstring mesh bags (Land and Sea sports Australia) that had small styrofoam floats attached. The holdfast of brown seaweeds was threaded twice through the lay of weighted ropes. Ropes with bags, tied specimens or brown seaweeds attached were suspended at approximately 5 m low tide water depth on anchored PVC frames. The number of replicate specimens used for each treatment ranged from four to six depending on availability of material, with specimens randomly assigned to treatments and positions, and new specimens used for each deployment. Algal fresh weights were obtained 24 hours prior to each deployment and within 24 hours after retrieval for each specimen. Fresh weights were measured after gently patting dry the specimens on paper towel to remove excess water, and used to calculate specific growth rate (SGR, as % d⁻¹) assuming exponential growth, i.e. $SGR = 100 * \ln(FW_t/FW_0)/t$, where FW_t = final fresh weight, FW_0 = initial fresh weight, and t is time in days. Some specimens were lost and for these, SGR is undefined. Additionally, red specimens that had SGR less than -3 and brown specimens that had SGR less than -1 were regarded as functionally lost and excluded from analysis of SGR. These levels were chosen based on observations of specimens and on data visualisation. The cut-off varied between the reds and browns due to the dependence of SGR on initial weight, and the much larger initial weight of brown compared to red specimens (average 52.2 g for browns and 13.5 g for reds). Samples for nitrogen analysis were taken from each specimen after weighing at the end of each deployment.

To compare environmental conditions between deployments, water temperature data were obtained from control site monitoring for the Adelaide desalination plant (SA Water unpublished data), and daily climate data (insolation, wind speed and direction) from the Australian Bureau of Meteorology (BoM) (www.bom.gov.au/climate/data) for the weather station nearest to the field trial location (Adelaide Airport, station number 023034). Insolation recorded at Adelaide airport is well correlated with subsurface photosynthetically active radiation (PAR) in adjacent Gulf St Vincent (Collings *et al.* 2006) and was used to compare relative light availability between deployment periods. Gulf St Vincent is protected from ocean swell, so waves on the Adelaide coast are largely generated by local winds, with westerly winds having the greatest fetch and being directly incident onto the coast, causing the largest waves (Pattiaratchi *et al.* 2007). Frequency of strong ($>13 \text{ ms}^{-1}$) westerly winds was therefore used as a proxy for the relative likelihood of rough sea conditions in each deployment.

Samples for algal tissue nitrogen content were frozen at -20°C , freeze-dried overnight, and then ground to a fine powder using a Fritsch stainless steel ball mill grinder. A 100 mg aliquot was analysed on a LECO Truspec CNS Elemental Analyser (LECO, St Joseph, MI, USA).

Data from the reds and browns were analysed separately. Statistical analyses were performed in R (R Core Team, 2013). SGR and nitrogen content were analysed using the “lm” routine and the “car” package (Fox and Weisberg, 2011), with Type III sums of squares. Normality was confirmed by QQplot and homoscedasticity by Levene’s test. Nitrogen content data for the brown seaweeds were log transformed to achieve normality and homoscedasticity. A three-way ANOVA was used for the reds to test effects of species, deployment and attachment, with two-way ANOVA used to test the effect of species and deployment for the browns. Pairwise t-tests were used to compare factor levels where main effects were significant, with control of false discovery rate (Benjamini *et al.*, 2006; Verhoeven *et al.*, 2005) implemented using the Excel program of Pike (2011). Where significant interaction terms were found, pair-wise tests were performed between factors within levels of the interacting factor. The frequency of specimen loss was analysed by logistic regression using the “glm” routine and binomial (logit) link function, with model selection using the Akaike Information Criterion. An α of 0.05 was used in all cases.

1.2.2. Results

Of the red seaweeds, a total of 27 out of 176 specimens were lost completely across the deployments, and a further seven were regarded as functionally lost, while for the browns, five out of 95 specimens were completely lost and a further 10 functionally lost (Table 3).

For the retrieved red seaweeds, there were significant differences in SGR with both species and attachment method, contingent upon the deployment period, as shown by significant deployment x species and deployment x attachment method terms in the ANOVA (Table 5).

Greatest growth was achieved in the Oct deployment for all four red species, while many specimens lost biomass in Nov, Jan and Mar (Figure 1). *Plocamium* was only used in the first three deployments due to difficulty in obtaining enough biomass. *Gelidium* was the fastest growing species in Oct, May and Jul.

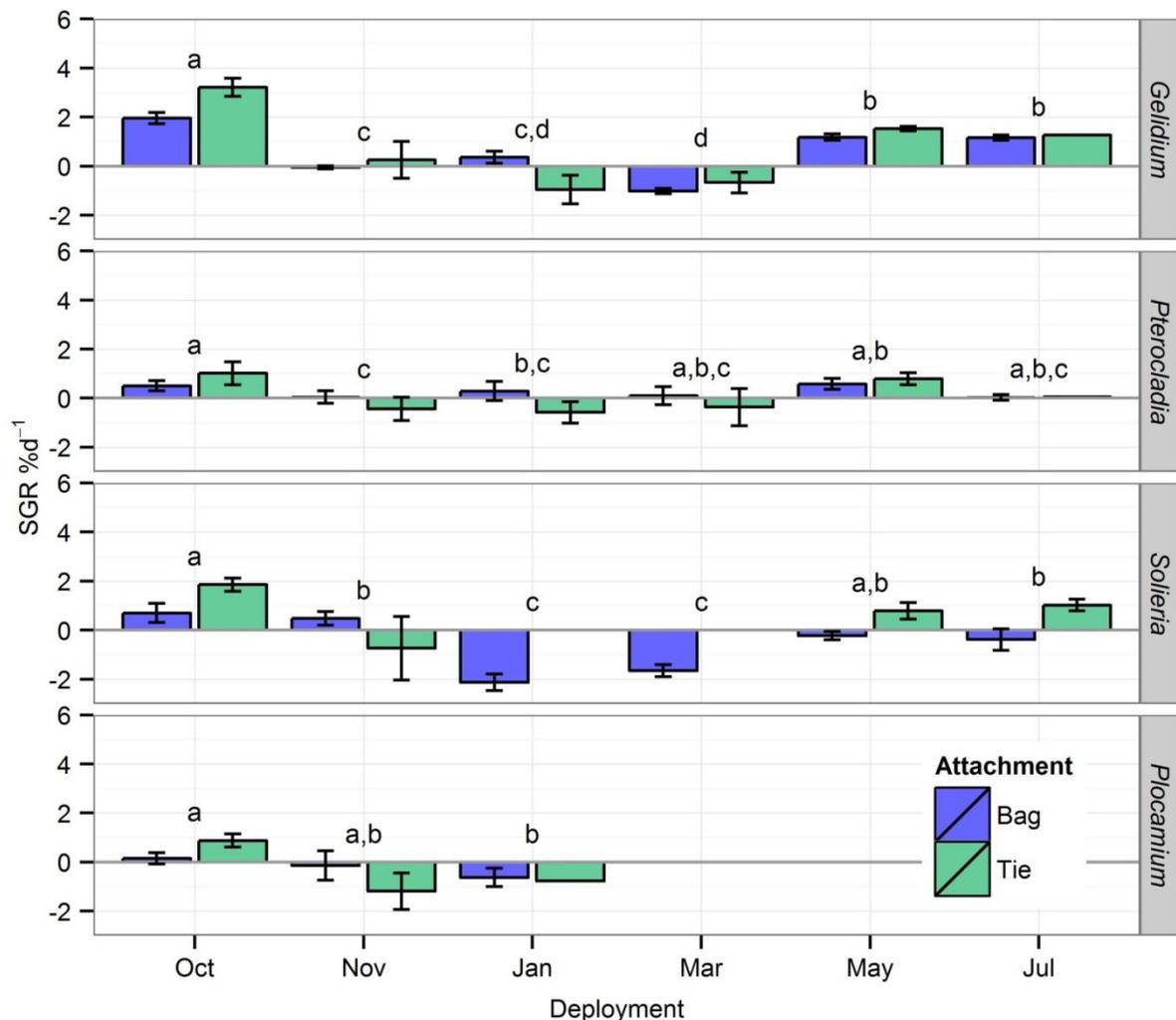


Figure 1. Mean SGR over the six field deployments for the red seaweeds. Error bars show standard error (n = number of retrieved specimens - see Table 3). Shared letters indicate no significant differences between deployments (across attachment method) within each species. Note, no tied specimens were retrieved for *Solieria* in Jan or Mar; *Plocamium* was used only in Oct, Nov, Jan.

For the browns, ANOVA showed a significant interaction of species and deployment on SGR (Table 5). *Ecklonia* showed lowest growth in Jan and Mar, while *Sargassum* showed lowest growth in Nov and Mar (Figure 2). SGR of *Cystophora* and *Scytothalia* did not vary with deployment. Note that *Scytothalia* was used only in Oct and Nov, and *Sargassum* was not used in May due to insufficient biomass of these species being available. *Scytothalia* had also demonstrated poor survival in holding tanks when collected for the first two deployments and

so its use was discontinued. SGR was not significantly different between species except in the Jul deployment, where SGR of *Ecklonia* > *Sargassum* > *Cystophora*.

Table 3. Number of specimens retrieved for each deployment (total deployed shown in brackets). Specimens were regarded as functionally lost if SGR < -3 for reds or < -1 for browns and excluded from counts of retrieved specimens and SGR analysis. Shaded cells indicate that species was not used in the deployment.

Species	Attachment	Deployment					
		Oct	Nov	Jan	Mar	May	Jul
<i>Gelidium</i>	Bag	4 (4)	4 (4)	4 (4)	6 (6)	3 (4)	4 (4)
	Tie	4 (4)	4 (4)	3 (4)	3 (6)	3 (4)	1 (4)
<i>Pterocladia</i>	Bag	4 (4)	4 (4)	4 (4)	4 (4)	3 (4)	4 (4)
	Tie	4 (4)	3 (4)	4 (4)	4 (4)	3 (4)	1 (4)
<i>Solieria</i>	Bag	4 (4)	4 (4)	3 (4)	6 (6)	3 (4)	4 (4)
	Tie	4 (4)	2 (4)	0 (4)	0 (6)	3 (4)	3 (4)
<i>Plocamium</i>	Bag	4 (4)	4 (4)	4 (4)			
	Tie	4 (4)	4 (4)	1 (4)			
<i>Cystophora</i>		4 (4)	3 (4)	5 (5)	4 (5)	5 (5)	6 (6)
<i>Ecklonia</i>		4 (4)	4 (4)	5 (5)	3 (5)	6 (10)	6 (6)
<i>Sargassum</i>		4 (4)	2 (4)	1 (5)	5 (5)		6 (6)
<i>Scytothalia</i>		3 (4)	1 (4)				

Similar to the patterns in SGR for the reds, the pattern of losses (actual + functional) varied with both species and attachment method contingent upon the deployment period (logistic regression: deployment x attachment method $\chi^2_{10} = 26.48$, $p=0.003$; deployment x species $\chi^2_5 = 17.47$, $p=0.004$). *Plocamium* was not included in this analysis due to it being used in only 3 deployments. Given the small sample sizes, this analysis should be interpreted with caution, but it was clear that tied specimens were lost more often than bagged: a total of 30 tied specimens (plus 3 tied *Plocamium*) were lost compared to 4 bagged specimens, with losses of tied specimens occurring mainly in Jan (8, + 3 *Plocamium*), Mar (9) and Jul (7), while most losses of bagged specimens (3) occurred in May. A total of 17 of 55 specimens of *Solieria* were lost, mainly in Jan (5) and Mar (6), compared to 10 of 55 specimens of *Gelidium*, mainly in Mar (3) and Jul (4), and 6 of 48 *Pterocladia* (3 in Jan).

Table 4. Deployment dates and summary of environmental conditions (mean \pm standard error, n= number of days) for the field trial.

Deployment	Start date	Days	Water Temp (°C)	Insolation (MJ)	Freq strong W wind
Oct	3 Oct 2012	47	16.7 (\pm 0.2)	25.0 (\pm 0.8)	31%
Nov	19 Nov 2012	65	20.5 (\pm 0.1)	29.4 (\pm 0.6)	37%
Jan	23 Jan 2013	55	22.2 (\pm 0.1)	23.3 (\pm 0.7)	24%
Mar	19 Mar 2013	71	19.4 (\pm 0.2)	13.9 (\pm 0.6)	13%
May	29 May 2013	48	15.3 (\pm 0.2)	8.9 (\pm 0.4)	4%
Jul	16 Jul 2013	80	14.2 (\pm 0.1)	15.5 (\pm 0.7)	20%

Table 5. ANOVA results for SGR and nitrogen content (N%) data from the field trial. Significant results are highlighted in bold.

Factor	SS		df		F		p	
	SGR	logN%	SGR	N%	SGR	logN%	SGR	logN%
Browns								
Deployment	5.27	0.889	5	5	8.30	5.94	<0.001	<0.001
Species	1.95	1.75	3	1	5.13	58.39	0.003	<0.001
Deployment x Species	4.74	0.845	10	5	3.74	5.65	<0.001	<0.001
Reds								
Deployment	1.19	10.073	5	5	16.31	35.76	<0.001	<0.001
Species	41.38	0.521	3	1	4.91	9.31	0.002	0.003
Attachment	7.47	0.232	1	1	0.03	4.15	0.520	0.046
Deployment x Species	0.01	0.695	12	5	3.63	2.48	0.003	0.042
Deployment x Attachment	22.12	0.460	5	5	3.47	1.64	0.005	0.164
Species x Attachment	8.81	0.003	3	1	0.25	0.061	0.714	0.807
Deploy x Species x Attach	0.38	0.660	10	4	0.93	2.95	0.521	0.028

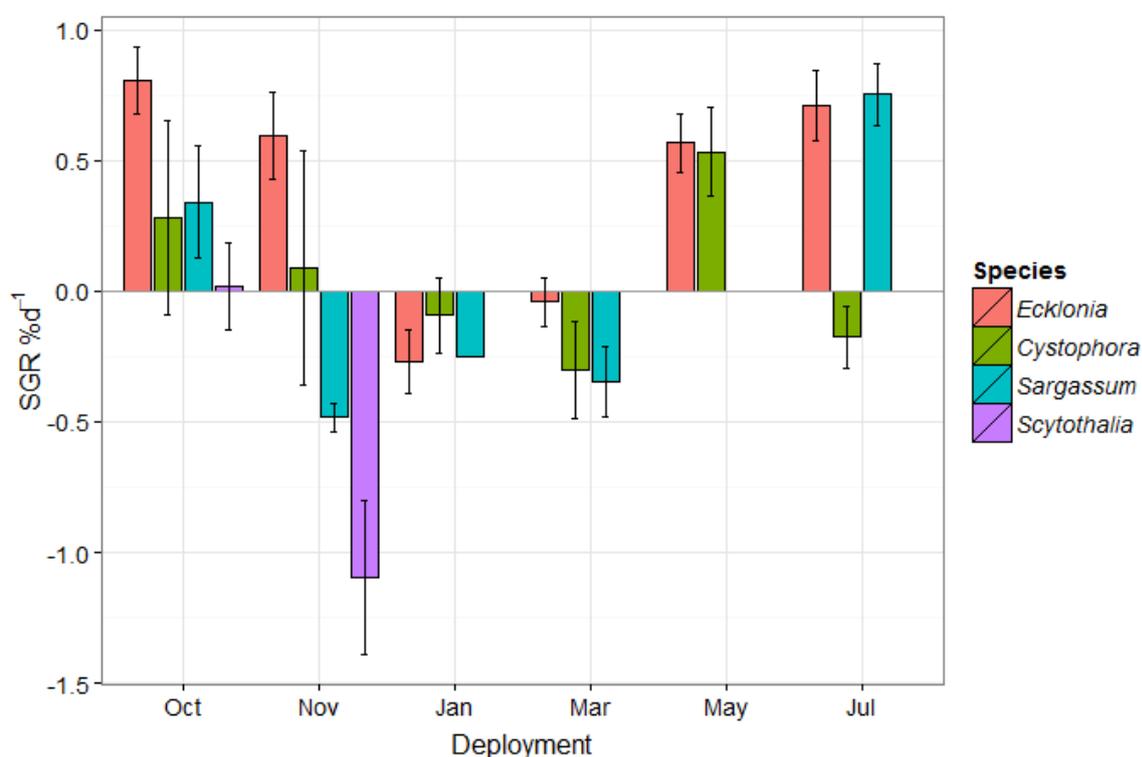


Figure 2. Mean SGR over the six field deployments for the brown seaweeds. Error bars show standard error (n = number of retrieved specimens - see Table 3). Note, *Scytothalia* was used only in Oct, Nov, *Sargassum* was not used in May.

For the browns, the likelihood of loss varied with deployment ($\chi^2_5 = 14.58$, $p=0.012$), but was not significantly different between species ($\chi^2_2 = 2.18$, $p=0.336$). *Scytothalia* was not included in this analysis due to it being used in only two deployments, but 4 of the 8 specimens of this species were lost (1 in Oct, 3 in Nov). For the other species, most losses occurred in May (5), followed by Jan (4), with 3 specimens lost in each of the Mar and Oct deployments. A total of 6 of 24 specimens of *Sargassum* were lost, 3 of 30 *Cystophora* and 6 of 30 *Ecklonia*. Most losses of *Sargassum* occurred in January, and involved the shedding of spent reproductive branches, leaving only the small vegetative base. The seasonal development and loss of fertile branches is typical for southern Australian members of this genus (Womersley 1987).

Nitrogen content was analysed for the reds *Gelidium* and *Solieria*, and the browns *Ecklonia* and *Cystophora*, as these species showed the best potential for IMTA across both field and laboratory trials (see sections 1.3 and 1.4). Nitrogen in *Gelidium* and *Solieria* was highly variable, with a significant deployment x species x attachment method interaction (Table 4). Nitrogen content of *Gelidium* was generally greater than that of *Solieria*, while bagged specimens had higher nitrogen content than tied in several deployments (Tables 4, 6).

Table 6. Mean nitrogen content \pm standard error for *Gelidium* and *Solieria* (reds) and *Cystophora* and *Ecklonia* (browns) from the Grange field trial.

Deployment	Species	n		N (% d.w.)	
		Bag	Tie	Bag	Tie
Oct	<i>Gelidium</i>	4	4	1.42 (\pm 0.06)	1.05 (\pm 0.1)
	<i>Solieria</i>	4	4	0.91 (\pm 0.14)	0.96 (\pm 0.18)
Nov	<i>Gelidium</i>	4	4	1.98 (\pm 0.03)	1.57 (\pm 0.07)
	<i>Solieria</i>	4	1	1.1 (\pm 0.04)	1.09
Jan	<i>Gelidium</i>	4	3	2.78 (\pm 0.04)	2.51 (\pm 0.08)
	<i>Solieria</i>	4	1	2.44 (\pm 0.1)	1.31
Mar	<i>Gelidium</i>	5	5	1.96 (\pm 0.07)	1.68 (\pm 0.22)
	<i>Solieria</i>	6	2	1.42 (\pm 0.09)	1.95 (\pm 0.02)
May	<i>Gelidium</i>	3	3	1.56 (\pm 0.21)	1.76 (\pm 0.1)
	<i>Solieria</i>	2	4	1.45 (\pm 0.09)	1.34 (\pm 0.16)
Jul	<i>Gelidium</i>	1	4	1.01	0.94 (\pm 0.08)
	<i>Solieria</i>	3	4	0.81 (\pm 0.03)	1.42 (\pm 0.06)
Oct	<i>Cystophora</i>		4		0.82 (\pm 0.31)
	<i>Ecklonia</i>		4		0.54 (\pm 0.09)
Nov	<i>Cystophora</i>		4		0.7 (\pm 0.04)
	<i>Ecklonia</i>		4		0.5 (\pm 0.15)
Jan	<i>Cystophora</i>		5		0.94 (\pm 0.16)
	<i>Ecklonia</i>		5		0.5 (\pm 0.07)
Mar	<i>Cystophora</i>		5		1.14 (\pm 0.24)
	<i>Ecklonia</i>		3		0.6 (\pm 0.09)
May	<i>Cystophora</i>		5		0.87 (\pm 0.14)
	<i>Ecklonia</i>		6		0.83 (\pm 0.07)
Jul	<i>Cystophora</i>		6		0.72 (\pm 0.13)
	<i>Ecklonia</i>		6		0.67 (\pm 0.05)

There was a significant deployment x species interaction for nitrogen content of *Ecklonia* and *Cystophora* (Table 4). *Cystophora* had highest nitrogen in Mar, while *Ecklonia* had highest nitrogen in May (Tables 4, 6). Nitrogen of *Cystophora* was greater than *Ecklonia* in Jan and Mar.

Although seasonal patterns varied between species, nitrogen status did not correspond to growth patterns in either red or brown seaweeds, with nitrogen content being generally lower over the deployments where better growth was observed. A nitrogen content of <2% is regarded as indicative of N limitation in macroalgae (Hanisak 1990), with values we found being often ~1% or less. This indicates that specimens were likely nitrogen limited throughout the field trial, but factors other than nutrient limitation appear to have negatively impacted growth in the summer period.

Environmental parameters (Table 4) also did not appear to explain the observed differences in growth and specimen loss between deployments. Seaweed growth can improve with increased water movement, but rough conditions may cause loss by breakage (Ask and Azanza, 2002; Friedlander, 2008). Strong westerly winds, which cause rough conditions on the Adelaide coast, were most common in Nov, but only slightly less frequent in Oct, when the best growth was observed for all species and no specimens were lost. Lower light availability and temperature may have contributed to slower growth in May-Jul compared with Oct, but light availability does not appear to explain poor growth over summer, when insolation was similar to Oct. Insolation data, while correlated with PAR, does not, however, account for water turbidity, which can vary seasonally due to riverine inputs, which decrease light availability after rain, or to phytoplankton blooms, which are common in spring-summer (Collings *et al.* 2006). Increasing water temperature increases seaweed growth up to a physiological optimum for each species (Eggert, 2012), but temperature responses of these species have not been studied, so their optima are unknown. Seasonal growth patterns in *Ecklonia* and *Cystophora* have been previously reported. In natural populations, *Ecklonia* typically shows fastest rates of elongation and biomass increase in spring to early summer, with erosion and biomass loss occurring in late summer-autumn (Novaczek 1984a; Miller *et al.* 2000; Wernberg and Goldberg 2008). *Cystophora* in southern Australia shows maximum growth in spring or autumn, with erosion in summer (Klemm 1988; Hotchkiss 1999). Increased light availability is suggested to lead to increased growth in spring, with adverse effects of high temperature and hydrodynamics leading to slowed growth and biomass losses in summer (Novaczek 1984a; Hotchkiss 1999; Wernberg and Goldberg 2008); development of reproductive biomass in autumn also contributes to seasonal growth patterns in *Cystophora* species (Klemm 1988). While seasonal growth in *Sargassum linearifolium* has not been reported, related *Sargassum* spp. show greatest growth in winter-autumn, corresponding to development of reproductive biomass; adult plants shed branches after reproducing, with only vegetative bases remaining in January (Kendrick and Walker 1994). We did not find any previous studies of growth patterns in *Scytothalia* or other Seirococcaceae. *Scytothalia* does not naturally occur in the Adelaide region or in the mid to upper reaches of Spencer Gulf, although it extends further north (to ~30°S) in Western Australia. It is, however, less common at lower latitudes (Wernberg *et al.* 2011) and in warmer areas (Smale *et al.* 2010). It is therefore possible that this species was adversely affected by temperature over summer. We also found no reports of seasonal growth patterns for any of the red seaweeds used. It is possible that conditions during the summer period of the field trial exceeded the physiological tolerances of these species, especially since sea surface temperatures in southern Australia were the highest on record in January-February 2013, and temperatures more than 0.5°C above average persisted from November 2012 until May 2013 (Bureau of Meteorology, 2014).

1.3. Laboratory comparison of red seaweeds

The red seaweeds were used in a laboratory trial to compare their growth rates and nitrogen storage potential under light, temperature and nutrient conditions expected in the tuna farming zone near Port Lincoln, SA, an area in which IMTA may be applied. Tuna are stocked seasonally in this area and fed over autumn and winter (Fernandes *et al.* 2007), with biogeochemical modelling predicting elevated dissolved nutrient levels around cages from April to July (Tanner and Volkman 2009). Water temperature in the zone is typically around 17-18°C in April, dropping to 14°C in July (Tanner and Volkman 2009). Initial laboratory trials to compare red seaweeds were conducted at the nutrient and temperature conditions expected in April since the performance of the algae at elevated nutrient levels is of interest, and the slightly warmer temperatures may promote growth, as well as being more feasible to achieve in the laboratory than July temperatures.

1.3.1. Methods

The experiment was conducted in November 2012 using 18 L conical-bottomed aquaria with filtered (10 µm) natural seawater supplied at 8 L h⁻¹ at 17.5°C and with aeration. Lighting of 160 µmol photons m⁻² s⁻¹ at the water surface with 10:14 h light:dark regime was supplied by cool-white LED lamps filtered through medium density green shade cloth. Nutrients were added from a stock solution of (NH₄)₂SO₄, KNO₃ and KH₂PO₄ via IV microburettes (B Braun Exadrop), with drip rates adjusted to provide aquaria with a continuous concentration (mean ± s.e., n= 20) of 46 ± 4 µg N L⁻¹ as ammonia, 79 ± 2 µg N L⁻¹ as oxidized nitrogen and 14 ± 1 µg P L⁻¹ as phosphate, when mixed with incoming water. Nutrient concentrations were based on those predicted by the biogeochemical modelling of the tuna farming zone near Port Lincoln (Tanner and Volkman 2009) and were verified using water nutrient samples collected three times weekly from the experimental system. Water nutrient samples were kept frozen at -20°C until analysis on a Lachat QuickChem 8000 Automated Ion analyser (Lachat, Loveland, CO, USA). Ammonia (NH₃ + NH₄⁺) was determined using the indophenol blue method (Lachat 2003b), oxidized nitrogen (NO₂⁻ + NO₃⁻) by the sulfanilamide method after reduction of nitrates using a cadmium column (Lachat 2003a), and phosphate (PO₄³⁻) by the ascorbic acid method (Lachat 2003c).

The seaweeds used were the same four red species as used in the field trial: *Gelidium australe*, hereafter *Gelidium*, *Pterocladia lucida* (*Pterocladia*), *Plocamium angustum* (*Plocamium*) and *Solieria robusta* (*Solieria*). Clean specimens of each species were selected from the stock collections. Algal fragments of 5-8 cm were excised with a sterile scalpel blade, and physically cleaned with a soft toothbrush in filtered seawater to remove micro-epiphytes. Approximately 7 g of plant fresh weight was placed into each aquarium, suspended at 10 cm

water depth on 5 mm mesh knotless nylon netting, and allowed to acclimate under experimental light and temperature conditions for 16 days with no nutrient addition. After the acclimation period, samples were taken for initial nitrogen content and between 4.5 g and 5 g fresh weight returned to each aquarium for the start of the experiment.

Algal fresh weights in each aquarium were measured at the start of the experiment and weekly thereafter for four weeks after gently patting dry the specimens on paper towel. Specific growth rates (SGR, as % d⁻¹) were calculated as per the field trial (see section 1.2.1).

Samples for final nitrogen content were collected after recording fresh weights at the end of the experiment (week four). Nitrogen analyses were performed as described for the field trial.

Statistical analyses were performed in R (R Core Team 2015). SGR was analysed with a linear mixed model to test the effect of species, including tank as a random effect with autoregressive correlation structure. A mixed model was also used to assess differences in nitrogen between species and times (i.e. initial and final samples) with tank as a random effect. These analyses were performed using “lme” in the “nlme” package (Pinheiro *et al.* 2014). Where main effects were found to be significant, post-hoc tests were performed using “glht” in the “multcomp” package (Hothorn *et al.* 2008). In all cases, normality was confirmed by QQplot and homoscedasticity by Levene’s test, and an α of 0.05 was used. Control of false discovery rate (Benjamini *et al.*, 2006; Verhoeven *et al.*, 2005) was applied to pairwise comparisons using the Excel program of Pike (2011).

1.3.2. Results

Growth rates (SGR) of the four species were significantly different over the four week experiment ($F_{3,16} = 28.29$, $p < 0.001$). Post-hoc tests indicated that SGR of *Solieria* (5.3%) > *Gelidium* (2.5%) > *Plocamium* (1.4%), with *Pterocladia* (1.8%) intermediate between and not significantly different to the latter two species (Figure 3).

All species increased their nitrogen content over 4 weeks ($F_{3,16} = 155.9$, $p < 0.001$, Table 7). There were also differences between species ($F_{3,16} = 92.91$, $p < 0.001$), with nitrogen content of *Plocamium* = *Pterocladia* > *Gelidium* > *Solieria*.

The total N assimilated by each species was calculated using initial and final fresh weights and nitrogen content data. *Gelidium* removed the most nitrogen over the 28 day experiment (12.2 mg N g⁻¹ initial fresh weight, FW), followed by *Pterocladia* (7.9 mg N g⁻¹ FW), *Solieria* (7.3 mg N g⁻¹ FW), and *Plocamium* (5.3 mg N g⁻¹ FW). Potential nitrogen assimilation was calculated using the average SGR and final nitrogen content for each species. This showed that, for an equivalent 1 kg starting biomass, *Solieria* would assimilate the most nitrogen in a 60-day culture period, overtaking the nitrogen removal of *Gelidium* by day 42 (Figure 4).

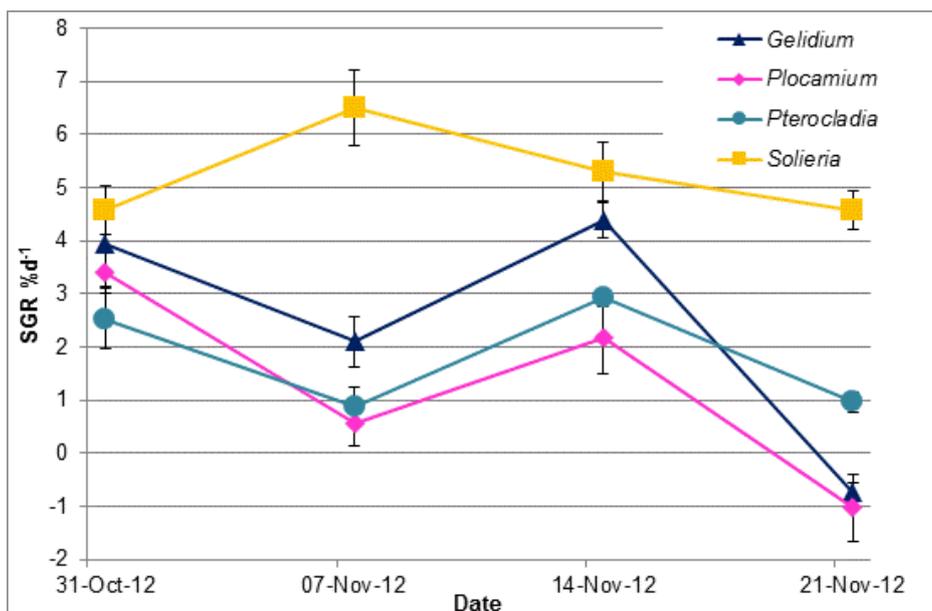


Figure 3. SGR for the four weeks of the laboratory experiment. Error bars show standard error (n=5)

Table 7. Mean tissue nitrogen (% d.w.) content ± standard error (n=5) from the laboratory experiment.

Species	Start	End
<i>Gelidium</i>	2.34 (±0.10)	3.22 (±0.10)
<i>Pterocladia</i>	2.69 (±0.03)	3.41 (±0.05)
<i>Solieria</i>	2.69 (±0.09)	3.18 (±0.08)
<i>Plocamium</i>	1.52 (±0.05)	2.12 (±0.10)

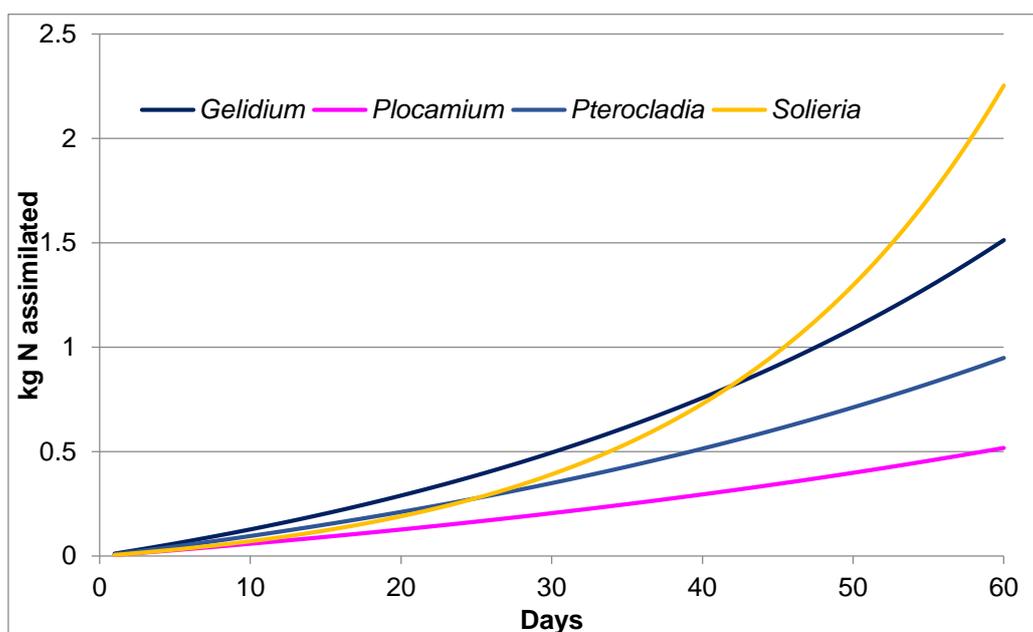


Figure 4. Potential total nitrogen assimilation (kg N) by seaweed over 60 days for each species, assuming 1 kg initial biomass, average SGR and water content, and final nitrogen content from the laboratory experiment.

1.4. Propagation of brown seaweeds

The predominant farmed species of brown seaweeds, including *Saccharina*, *Laminaria* and *Undaria* species (White and Wilson 2015), belong to the order Laminariales. Laminariales show distinct differences between alternate generations, with a large conspicuous sporophyte and microscopic filamentous gametophyte. Motile spores are produced in sori located on the central blade and/or laterals (Womersley 1987) or, in the case of *Undaria* species, in fertile structures located near the holdfast (Sahoo and Yarish 2005). Spores develop into gametophytes, with male gametophytes releasing motile male gametes that fertilise the sessile female gametes, which remain attached on the female gametophytes. The zygotes then develop into the next generation of sporophytes. Spores are obtained by allowing sporophytes' fertile tissue to partially dry and then re-immersing in seawater to stimulate spore release (Sahoo and Yarish 2005). Farming of Laminariales typically involves seeding spores onto string or rope that is maintained in nursery conditions until young sporophytes develop, or otherwise nursery-grown sporophytes are manually inserted onto culture ropes for out-planting in the sea (Sahoo and Yarish 2005; Titlyanov and Titlyanova 2010). Gametophytes may also be vegetatively cultured in flasks prior to being seeded onto rope (Sahoo and Yarish 2005; Forbord *et al.* 2012).

Fucales do not show alternating generations and have no gametophyte stage, with adult plants producing male and female gametes directly (Womersley 1987). *Sargassum* is globally the largest genus in the Fucales (Guiry and Guiry 2012) and several *Sargassum* species are farmed, including *S. fusiforme* (known as Hijiki), *S. horneri*, *S. thunbergii* and *S. fulvellum* (Hwang *et al.* 2007; Pang *et al.* 2007; Pang *et al.* 2009; Li *et al.* 2010; Zou *et al.* 2012). Most of these species are dioecious (i.e. have separate male and female plants), and synchronization of reproduction in male and female plants is an important consideration in their culture (Pang *et al.* 2005; Pang *et al.* 2006). Eggs are fertilised on the surface of the female reproductive structures, where, in nature, they remain attached for one to a few days before being released and settling (De Wreede 1978; Deysher and Norton 1981; Monteiro *et al.* 2009). In culture, zygotes are collected by rubbing or washing them from the parent and seeding them onto string (Pang *et al.* 2005; Hwang *et al.* 2007; Zhao *et al.* 2008). Seedlings are allowed to develop over a period of nursery culture prior to planting in the sea (Hwang *et al.* 2007; Pang *et al.* 2008).

The genus *Ecklonia* is not commercially farmed, but trials of production have been carried out for *E. stolonifera* in Korea (Hwang *et al.* 2009), and *E. radiata* in New Zealand (Neill *et al.* 2009), and laboratory reproduction of *Ecklonia* spp. has been performed for various experimental purposes (Papenfuss 1942; Jennings 1967; Novaczek 1984b; Bolton and Levitt

1985). Both Neill *et al.* (2009) and Hwang *et al.* (2009) used very similar methods, typical of those used for farmed Laminariales, to obtain *Ecklonia* spores and seed them onto string. Sori of *E. radiata* are located mainly on the central blade but extend onto laterals, often being extensive but relatively inconspicuous (Womersley 1987). The fertile season of this species is unclear; with peak fertility reportedly occurring in winter-spring in New Zealand (Novaczek 1984a) but in summer-autumn in southern Australia (Mohring *et al.* 2013).

Although related fuclean species are farmed, there is no history of culture for *Sargassum linearifolium*, *Cystophora subfarcinata* or *Scytothalia dorycarpa*. In contrast to many farmed Fucales, the three species considered in the current study are all monoecious, with male and female gametes both produced by each plant (Womersley 1987). No published information on reproduction in *Sargassum linearifolium* is available, but several accounts for closely related species from southern Australia exist, including *S. spinuligerum*, *S. podacanthum* and *S. distichum* (Kendrick and Walker 1991; Kendrick and Walker 1994). These species were found to be fertile from September to January, with peak reproductive biomass present in November; mature plants were observed to have visible zygotes attached to the reproductive structures, which are developed in fertile branches (Kendrick and Walker 1991; Kendrick and Walker 1994). No species of *Cystophora* are farmed, but reproduction in the laboratory has been carried out in a small number of studies, all of which used manipulation of light and temperature to stimulate gamete release in fertile plants (Klemm and Hallam 1987; Klemm 1988; Taylor and Schiel 2003). *Cystophora subfarcinata* is known to be fertile from July to December in southern Australia, with peak fertility in October-November (Klemm 1988). Fertile structures develop on upper branches in this species (Womersley 1987). There are few published studies on reproduction in Seirococcaceae, with most on *Phyllospora comosa*, but fertile structures are known to occur in branch axes of *Scytothalia* and to be present year-round (Womersley 1987). Gametes have been obtained from *Phyllospora comosa* using light and temperature manipulation as per *Cystophora* spp. (Burridge *et al.* 1993; Burridge and Hallam 1993; Schoenwaelder and Clayton 2000).

Feasibility of propagation is an important consideration in determining the suitability of brown seaweeds for aquaculture. Therefore, reproduction of the short-listed species was attempted, using protocols from related species, where fertile material was obtained.

1.4.1. Methods

Specimens of *Ecklonia radiata* (hereafter *Ecklonia*), *Scytothalia dorycarpa*, (*Scytothalia*), *Cystophora subfarcinata* (*Cystophora*), and *Sargassum linearifolium* (*Sargassum*) were collected and examined for the presence of fertile tissue during field work associated with the

pilot field trials between September 2012 and June 2013. Descriptions from Womersley (1987) were used to identify fertile structures.

Where fertile material was found, reproduction was attempted using the following techniques:

Ecklonia: Clean sections of the central blade with fertile tissue were selected and rinsed in filtered seawater before being allowed to desiccate in dark humid conditions for one hour, and then placed in filtered seawater in a shallow tray. Gentle agitation was applied periodically over a period of four hours. Water samples were examined under a compound microscope for the presence of spores.

Sargassum: Fertile branches were excised and placed in glass aquaria with filtered seawater and aeration provided to keep branches in constant motion. Fertile structures were examined daily under a dissecting microscope for the presence of zygotes.

Cystophora and *Scytothalia*: Clean fronds with mature fertile structures were excised and rinsed in filtered sea water, refrigerated at 4°C in the dark for 16 hours, then placed in petri dishes of filtered seawater and exposed to ambient light in the laboratory and allowed to warm slightly to stimulate gamete release. Water samples were examined under a dissecting microscope for the presence of zygotes.

1.4.2. Results

Freshly collected *Ecklonia* with sori were observed in March 2013. Sori were also observed on collected plants that were held at SAASC from April to May 2013. Reproduction was attempted using samples from both sources. In both cases, spores were obtained.

Fertile plants of *Sargassum* were found between October 2012 and January 2013 and again in June 2013. Reproduction was attempted three times: November 2012, December 2012, June 2013, but no zygotes were obtained.

Fertile *Cystophora* was found in September 2012 and zygotes were successfully obtained.

Scytothalia with apparently mature fertile structures present was collected in September and October 2012. This species, however, showed poor survival in holding tanks, in addition to poor growth performance in the field (see section 1.3.2). It was therefore not considered further and reproduction was not attempted.

1.5. Discussion

Of the red seaweeds, *Solieria* was the fastest growing species in the laboratory, with an SGR of 5.3 % d⁻¹, while *Gelidium* was the best performing in the field, exhibiting an SGR of up to 3.2 % d⁻¹. Growth rates compare favourably to published values for related species, given that culture techniques have not been optimised for the species we used. Farmed *Kappaphycus*

species show SGRs of 2-6 % d⁻¹, although up to 10 % d⁻¹ has been achieved in the field (de Paula *et al.* 2002) and 14 % d⁻¹ in the laboratory (Ask and Azanza 2002). Experimental cultivation of other Gelidiales has achieved SGRs of 3-7 % d⁻¹ in the field and >20 % d⁻¹ in the laboratory (Friedlander 2008; Ganesan *et al.* 2011). *Gelidium* assimilated the most nitrogen in the 4-week laboratory experiment, however, given a similar starting biomass and assuming the SGR and final nitrogen content for each species from the laboratory trial, the nitrogen removal of *Solieria* would exceed that of *Gelidium* after 42 days due to its faster growth rate. Conditions at our pilot field site were probably sub-optimal for growth of *Solieria*, resulting in SGR <2% d⁻¹ during the field trial. Solieriaceae are typically cultured at depths <1 m (McHugh 2003) and growth of this species may improve with shallower culture than we used, and optimization of other parameters important for growth, including nutrients (Ask and Azanza 2002).

The better performance of *Gelidium* compared to *Solieria* in the field may indicate that *Gelidium*, which was primarily collected at >5 m depth, is physiologically better suited to lower light conditions than *Solieria*, which was primarily collected at 1-2 m depth. The compact and cartilaginous texture typical of Gelidiales may also be more resistant to herbivory and breakage than *Solieria*, which has relatively soft branches filled with filaments and mucilage (Womersley 1994). The field site was located adjacent to seagrass beds, and bridled leatherjackets *Acanthaluteres spilomelanurus*, a herbivorous species common in seagrass (Hutchins 1999), were observed around specimens in the field and found inside some bags from the Nov and Jan deployments. Herbivory is therefore a likely cause for biomass losses in the Nov-Mar deployments, although other causes cannot be ruled out, particularly supra-optimal summer temperatures. Culture in mesh bags in the current study may have afforded some protection against losses of red seaweeds, but during the deployment when all species grew best (Oct), tied specimens grew significantly better than bagged. This difference may be due to the light attenuation that occurs within bags. Reduced water exchange in bags may also have contributed by limiting gas exchange due to reduced water movement. Use of a larger size mesh may offer the advantages of both methods by allowing extra light penetration and gas exchange while providing protection.

The SGR of *Pterocladia* and *Plocamium* in laboratory and field experiments was consistently <2% d⁻¹, so they are unlikely to be commercially viable, even if the value of their nitrogen removal for IMTA application is considered. Although these species had greater initial nitrogen content than that of *Solieria* and *Gelidium*, this difference reduced with nutrient addition, and their slow growth means that more biomass per tonne of fish production would be required to remove equivalent nitrogen.

There was little difference in the performance of the brown seaweeds *Ecklonia*, *Cystophora*, and *Sargassum* over the 12 months of the pilot field trial, although *Ecklonia* showed marginally better growth in some deployments. Results for seasonal growth of *Ecklonia*, *Cystophora* and *Sargassum* in the current experiment are consistent with previous observations of poor growth in summer due to erosion or shedding of spent reproductive branches (Novaczek 1984a; Klemm 1988; Kendrick and Walker 1994; Miller *et al.* 2000; Wernberg and Goldberg 2008). The seasonal development and shedding of reproductive branches in *Sargassum* also means that this species would only be suitable for culture for part of the year, and failure to harvest at an appropriate time could lead to large losses of material. In reproduction trials, *Ecklonia* and *Cystophora* were able to be reproduced in the laboratory, but no gametes were obtained from *Sargassum*, so this species will not be considered further. Herbivory cannot be completely ruled out as a contributing factor to biomass losses, but many southern Australian brown seaweeds, including *Ecklonia* and several *Cystophora* and *Sargassum* species, contain terpenoid compounds that make them unpalatable to many herbivores (Steinberg and Altena 1992). Overall growth rates for the brown seaweeds were lower than those recorded for commercially cultivated kelps, which show SGR of 6-9 %d⁻¹ under optimal conditions (Tabrizi 1992). The culture depth used for kelps is typically <2 m (Scoggan *et al.* 1989; Hwang *et al.* 2009; Neill *et al.* 2009), and *Ecklonia stolonifera* was found to grow around three times faster at 1.5 m compared with 4 m depth (Hwang *et al.* 2009). A depth of between 1-3 m is optimal for the cultured *Sargassum fulvellum* (Hwang *et al.* 2007). The growth rate of kelps and Fucales also increases with nutrient addition (Tabrizi 1992; Petrell and Alie 1996; Schaffelke and Klumpp 1998). It is therefore likely that better growth rates can be achieved by optimising culture depth and nutrient conditions.

2. LABORATORY TRIALS OF SELECTED SPECIES

2.1. Temperature responses of *Solieria robusta* and *Gelidium australe*

Initial trials (see Chapter 1) identified *Solieria robusta*, hereafter *Solieria*, and *Gelidium australe* (*Gelidium*) as the red seaweed species with greatest potential for aquaculture and application to IMTA. Little, however, is known about the biology of these species or the optimum conditions for their growth. Temperature is an important factor determining seaweed growth, and while temperature response often correlates to local temperature regime for a species due to local adaptation, the breadth of temperature tolerance and optima may vary between and within species (Eggert 2012). Understanding temperature responses will help to determine suitable seasons for culture of *Solieria* and *Gelidium*. Data on seasonal growth is also needed to incorporate nutrient removal by seaweeds into the biogeochemical models that are used to determine aquaculture carrying capacity if IMTA is applied.

2.1.1. Methods

The experiment was conducted in September 2013 using 3 L plastic tubs filled with artificial seawater (Dupla Marin) at a salinity of 36, with nutrient added from a stock solution of $(\text{NH}_4)_2\text{SO}_4$, KNO_3 and KH_2PO_4 to provide 0.08 mg N L^{-1} as ammonia, 0.02 mg N L^{-1} as oxidized nitrogen and $0.008 \text{ mg P L}^{-1}$ as phosphate, based on expected nutrient concentration near farms in the tuna farming zone of Port Lincoln, SA (Tanner and Volkman 2009). Clean specimens of each species were selected from the stock collections, fragments of 5-8 cm were excised with a sterile scalpel blade, and physically cleaned with a soft toothbrush in filtered seawater to remove micro-epiphytes. Approximately 3.5 g of plant fresh weight was used in each of ten tubs per species. Algal fresh weights in each tub were measured at the start of the experiment and weekly thereafter for four weeks after gently patting dry the specimens on paper towel. Specific growth rates (SGR, as $\% \text{ d}^{-1}$) were calculated as per initial trials (see section 1.2.1). Salinity in the tubs was checked daily and distilled water added as necessary to compensate for evaporation. Water and nutrients in the tubs were replaced twice weekly, and aeration was supplied continuously. Lighting of $160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at the water surface with 10:14 h light:dark regime was supplied by cool-white LED lamps. The tubs were placed in aquaria that acted as water baths to maintain temperature. Each species was randomly assigned to 10 aquaria, which were maintained at temperatures of 12, 13, 14, 16, 18, 20, 22, 23, 24 and 25°C , corresponding to the typical annual range in South Australian gulf areas (Petruševics 1993). The annual range of water temperatures in the tuna farming zone is 14 to 20°C (Tanner and Volkman 2009), but other potential aquaculture areas may

experience the wider range used here. Temperature was recorded in each tub five times per week throughout the four-week long experiment using a calibrated digital thermometer. Temperature was also logged for a period of between 72 and 96 hours in each tub during the experiment using TPS 90-C temperature and conductivity loggers.

The response of both species to temperature was found to be non-linear, but resolution of the data was not great enough to permit reliable fitting of a temperature response curve (e.g. Norberg 2004). Generalised additive modelling (GAM) was therefore used for statistical analysis with temperature fitted as a smooth effect. The effect of species was tested by comparing a model of the overall temperature response for both species with one that also included a term for the species difference (Wood 2006). To avoid overfitting, the number of knots used was chosen to be less than half the number of data points. Analysis was performed using the *mgcv* package (Wood 2006) in R (R Core Team, 2013) and an α of 0.05 was used.

2.1.2. Results

Growth rates (SGR) of both species were significantly affected by temperature ($p < 0.001$), and the temperature response was significantly different between the two species ($p < 0.001$) (Figure 5). *Gelidium* grew faster than *Solieria* at temperatures below 14°C; its growth increased slightly with temperature, but at greater than 21°C, specimens showed very poor to no growth and became bleached and brittle. By the fourth week of the experiment, these specimens appeared to have died and were starting to disintegrate. The growth of *Solieria* increased rapidly from 12 to 16°C, and was similar between 16 and 22°C, declining slightly at higher temperatures, although plants still appeared healthy. GAM predicted a maximum SGR of 4.1% d⁻¹ at 20°C for *Solieria*, and of 2.8% d⁻¹ at 18°C for *Gelidium*.

2.2. Tissue culture of *Gelidium*

Authors: Dandan Wang, Kathryn Wiltshire, Jason Tanner, Xiaoxu Li

The red algal species in the genus *Gelidium* (Rhodophyta, Gelidiales) are widespread, economically important seaweeds, and are the preferred sources of bacteriological-grade agar and agarose, which have wide-ranging industrial, technological and research applications (Friedlander 2008; Bixler and Porse 2011). *Gelidium australe* was identified as a potentially suitable candidate species for IMTA during the initial field and laboratory trials (see sections 1.2 and 1.3).

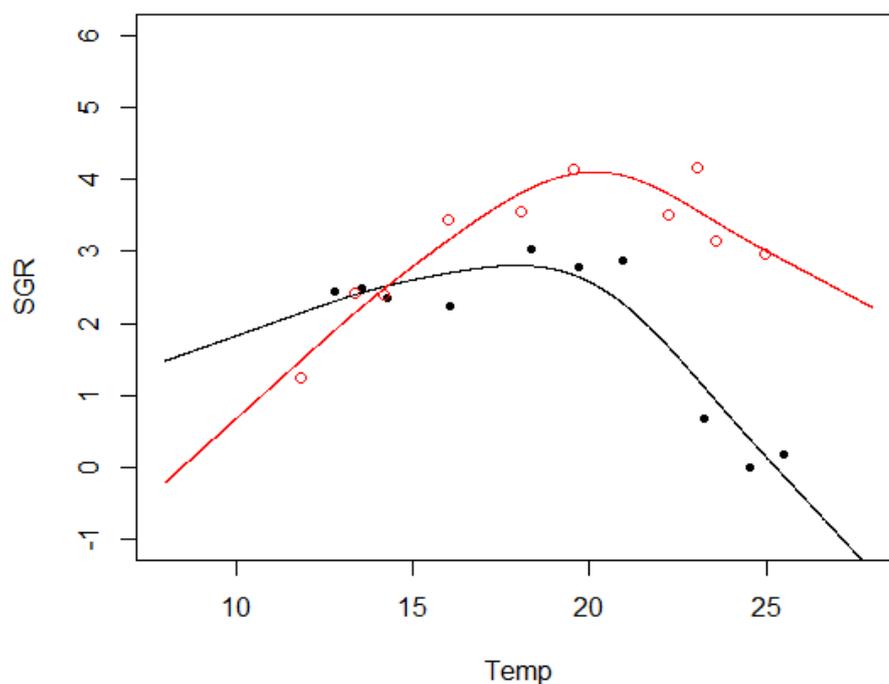


Figure 5. Specific growth rate (SGR, %d⁻¹) with temperature for *Solieria* (red, open circles) and *Gelidium* (black, solid circles) and response curves fitted by GAM.

Aquaculture of many red seaweeds, including those farmed for phycocolloids, utilises vegetative reproduction to produce seedlings (Sahoo and Yarish 2005; Titlyanov and Titlyanova 2010) but micropropagation techniques, such as tissue culture, are being investigated for strain selection and production of seedling biomass, including for *Gelidium* species (Pei *et al.* 1996; Titlyanov and Titlyanova 2006; Titlyanov *et al.* 2006; Reddy *et al.* 2008). The use of tissue culture for production of seedlings reduces the need for collection of material from natural beds (Yokoya and Yoneshigue-Valentin 2011), an important consideration for the establishment of seaweed aquaculture in Australia. Tissue culture of *Gelidium* species may also facilitate production of seedlings with rhizoids that could be attached to substrates for planting in the sea (Titlyanov and Titlyanova 2006; Titlyanov *et al.* 2006).

Tissue culture for production of seedlings was trialled for *G. australe*, with explants grown under a range of temperatures, light levels and salinities to determine optimum conditions for seedling production.

2.2.1. Methods

Specimens of *G. australe* were collected on 1st November 2013 from between 3–8 m deep at Granite Island, Victor Harbor, SA (35° 33' 59"S, 138° 37' 41"E), and maintained in an outdoor flow-through aquarium facility under ambient light, temperature and salinity, until required for

use. Undamaged plants were selected, and cleaned of any sediment and fouling organisms with brushes in sterilised and filtered seawater (SFSW). The SFSW was filtered to 5 μm and autoclaved at 120°C for 30 minutes. To prevent contamination, individual explants were prepared for culture on a bench sterilized by wiping with 75% alcohol and exposure to UV light for 20 min. The external surface of each *G. australe* frond was sterilized by placing it in 100% alcohol for 10 s, then transferring into 1% sodium hypochlorite (NaClO) for 10 mins. Fronds were then washed in SFSW 10 times to remove alcohol and NaClO. Individual apical and stem explants of $\sim 2 \times 2 \times 2$ mm were excised from each frond and placed in SFSW prior to being moved to 250 mL flasks with 125 mL SFSW for culture. Stem specimens were cut 3 cm from the base of the fronds, while apical specimens were cut 3 cm from frond tips.

To examine the influence of temperature on growth and survival, explants were cultured at five different temperatures (12, 14, 16, 18, 20°C) for 60 days, with temperature maintained by water baths. The temperature range was based on the previous experiment (section 2.1), which showed that this was the range suitable for growth of this species. Three independent replicate flasks were cultured at each temperature, with 10 explants in each. Cultures were maintained with a light intensity of 20-30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ provided by cool white LED lamps, a photoperiod of 12L:12D, and a salinity of 36. The culture medium was renewed every 5 days. The proportion of explants with adventitious buds, the number of adventitious buds, and mean length of the adventitious buds on each explant were recorded every 20 days.

To examine the effects of light intensity and salinity on development and growth, a second set of stem explants were cultured for 60 days at six different light intensities (0, 10, 20, 30, 40, 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and three different salinities (33, 36, 39) of artificial seawater (Dupla Marin sea salt). Illumination was provided by cool white LED lamps with shading by layers of medium shade cloth. Three independent flasks were maintained under each combination of light and salinity. The experimental protocol otherwise followed that described above, except that all cultures were maintained at 20°C.

Mixed models were used to assess the effects of temperature, explant type and time for the first experiment, and of light, salinity and time in the second experiment, with flask as a random effect in each case. These analyses were performed in R (R Core Team, 2013) using “lme” in the “nlme” package (Pinheiro *et al.* 2014). Where main effects were found to be significant, post-hoc tests were performed using “glht” in the “multcomp” package (Hothorn *et al.* 2008), with control of false discovery rate (Benjamini *et al.*, 2006; Verhoeven *et al.*, 2005) implemented using the Excel program of Pike (2011). Where significant interaction terms were found, pair-wise tests were performed between factors within levels of the interacting factor.

2.2.2. Results

There was a clear increase in the proportion of both stem and apical explants with adventitious buds with increasing temperature and over time (Figure 6). The effect of time was contingent on temperature ($p < 0.001$) and explant type ($p = 0.019$). At day 20 there was no difference in the proportion of explants with buds between temperatures ($p = 0.322$) or explant types ($p = 0.347$), but by day 40 the effect of temperature was significant ($p = 0.004$), with more explants developing buds at 18 and 20°C compared to 12°C. At day 60, there were significant differences with both temperature ($p < 0.001$) and explant type ($p = 0.023$), with a higher proportion of stem than apical explants developing buds.

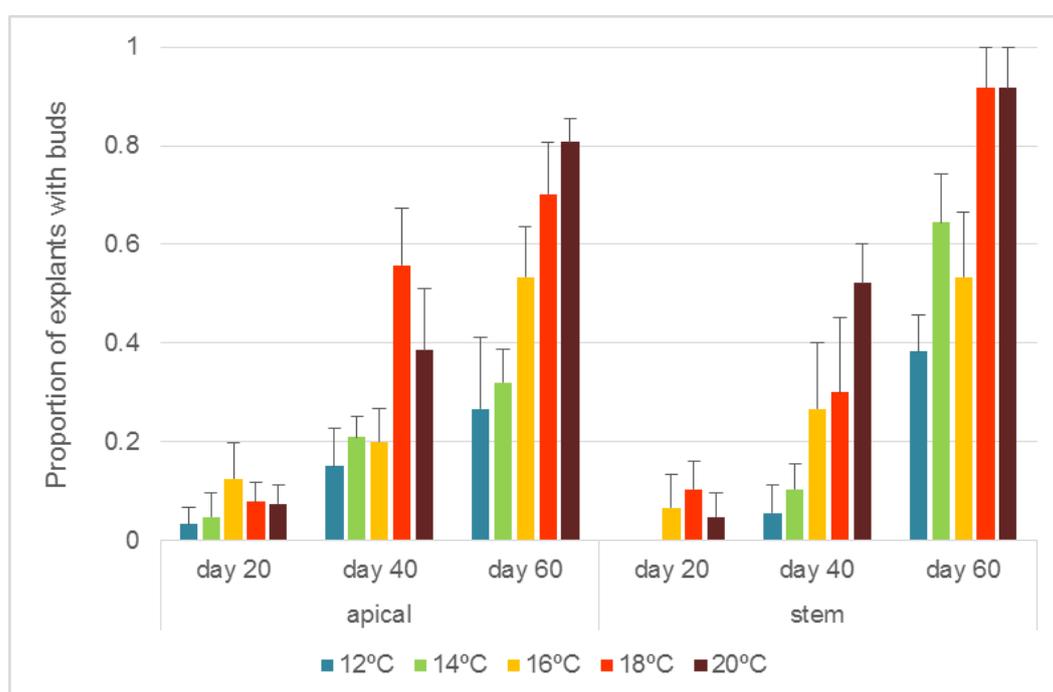


Figure 6. Effect of temperature and time on the mean proportion of explants of *Gelidium* with adventitious buds showing mean \pm s.e. ($n=3$) for apical and stem explants.

The number of buds also increased with time and temperature, contingent on explant type (Figure 7), with the three-way interaction temperature \times type \times time being significant ($p = 0.039$). There was no difference in the number of buds between treatments at day 20 or 40, but at day 60 the interaction of temperature \times explant type was significant ($p = 0.039$). There was no effect of temperature on number of buds for apical explants ($p = 0.431$), but there was for stem explants ($p = 0.036$), with fewer buds at 12 and 14°C compared with higher temperatures. There were more buds in stem than apical explants at 14, 18 and 20°C.

The length of buds also increased with time contingent on temperature (temperature \times time $p < 0.001$), but with no difference between explant types ($p = 0.271$). There were significant

differences between temperature treatments by day 20 ($p=0.002$), with buds being smaller at 12 and 14°C (1.5- 2.5 mm) compared with higher temperatures ($>3.5\text{mm}$). The difference between temperature treatments became more pronounced over time (Figure 8).

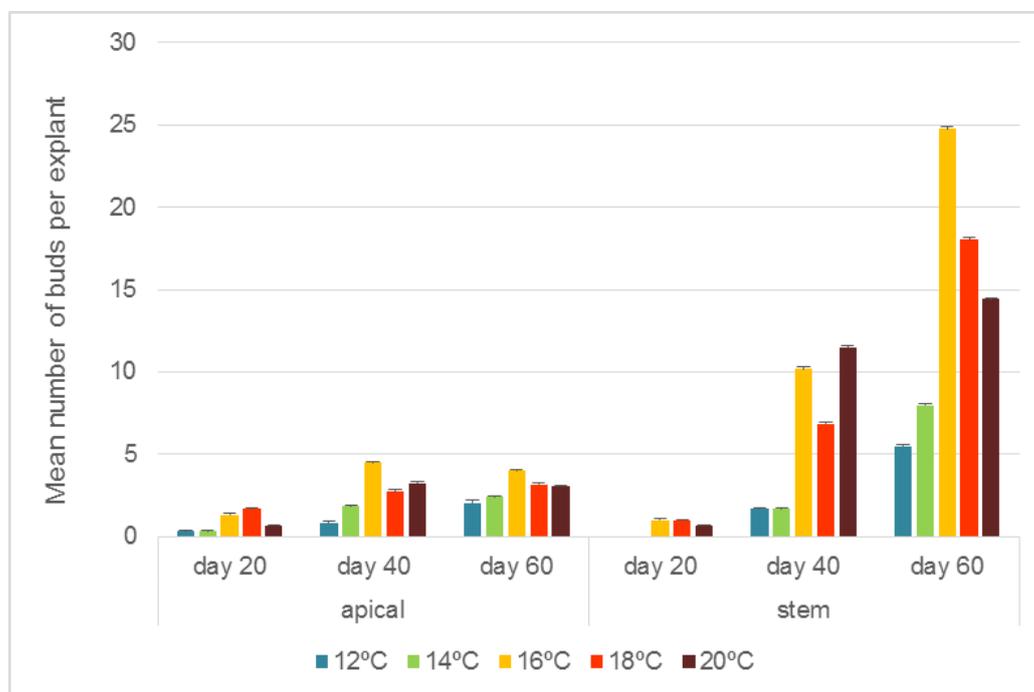


Figure 7. Effect of temperature and time on the mean number of adventitious buds per *Gelidium* explant showing mean \pm s.e. ($n=3$) for apical and stem explants.

In the second experiment, the proportion of explants with adventitious buds increased with light intensity and over time (Figure 9), with significant interactions of light and time ($p<0.001$), salinity and time ($p=0.002$) and salinity and light ($p=0.002$). Effects of light were significant by day 20 ($p<0.001$), with more explants having buds at 30 and 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ compared to other light treatments. By day 60, the ranking of proportion of explants with buds was 20=30=40>50>10>0 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Salinity treatments showed different rates of bud development and minor differences in the effects of light, but by day 60 nearly all explants had developed buds at light levels between 20 and 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$.

The number of buds also varied with time and light (Figure 10), with significant interactions of light and time ($p<0.001$), salinity and time ($p=0.002$) and salinity and light ($p=0.003$). Effects of light were significant by day 20 ($p<0.001$), with more buds at 30 and 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ compared to other light treatments. By day 60, ranking of the proportion of explants with buds was 30=40>50>20>10>0 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Explants at a salinity of 39 developed most buds, with the difference to other salinity treatments becoming more pronounced over time, however, differences between salinity treatments were only significant at suboptimal light levels (0, 10 and 20 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$).

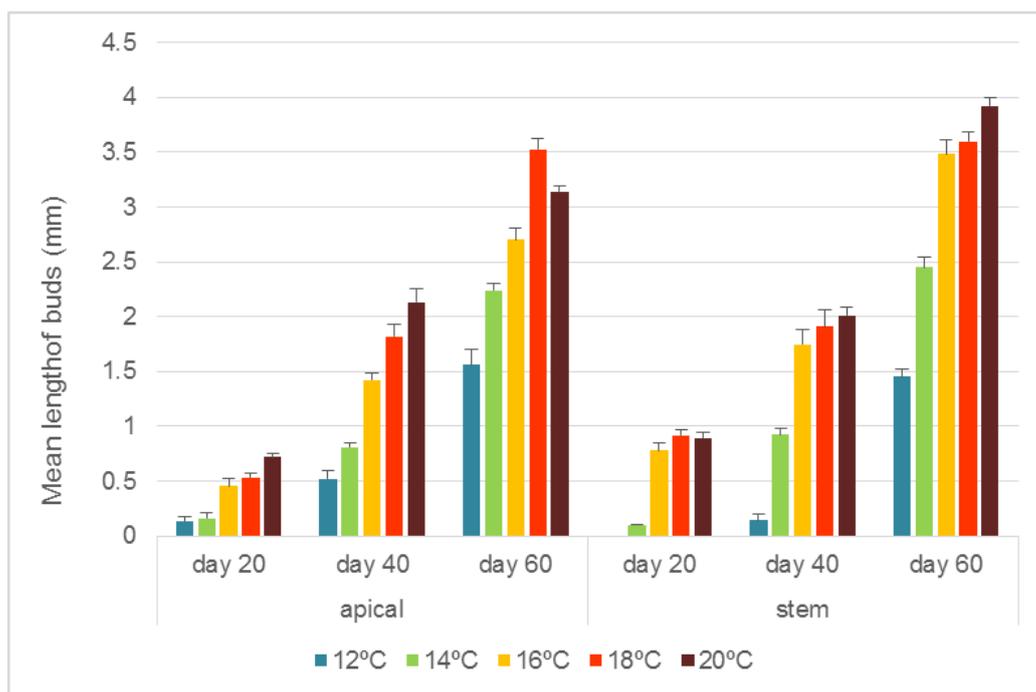


Figure 8. Effect of temperature and time on the mean length of adventitious buds on *Gelidium* explants showing mean \pm s.e. (n=3) for apical and stem explants.

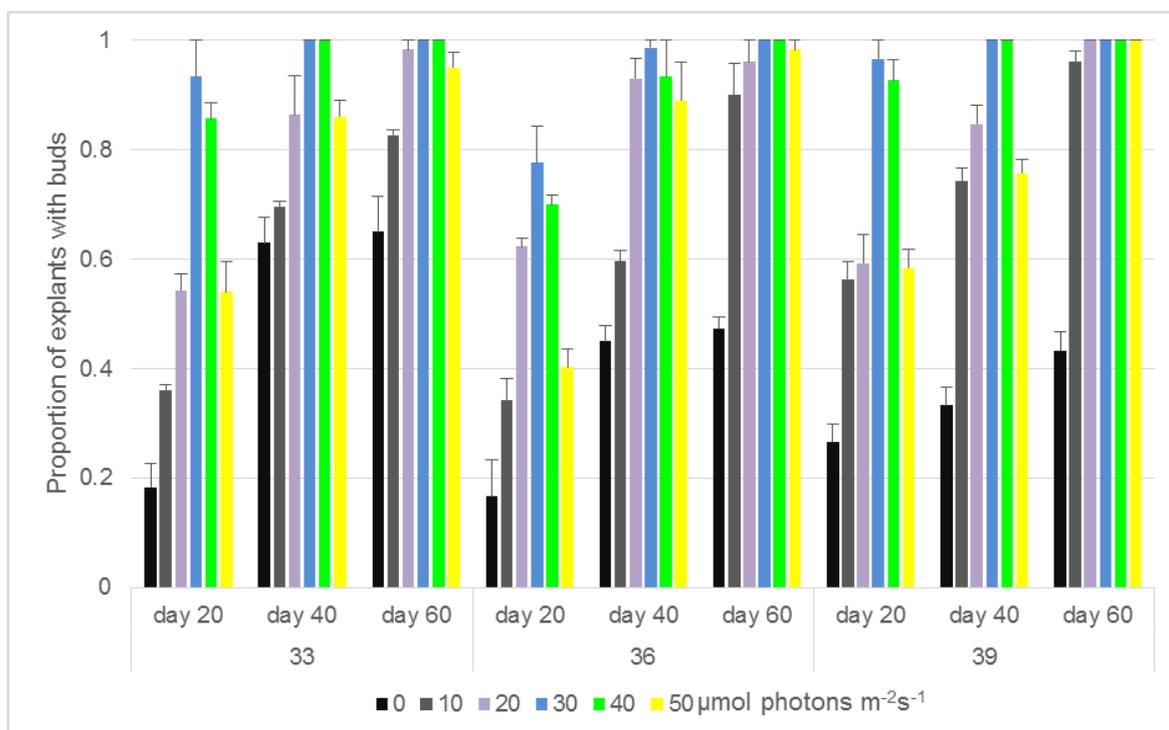


Figure 9. Effect of light intensity, salinity and time on the mean proportion of explants of *Gelidium* with adventitious buds showing mean \pm s.e. (n=3) for stem explants at three salinities (33, 36, 39).

The length of buds varied with time and light (Figure 11), with a significant three-way interaction of light, salinity, and time ($p=0.006$). At day 20, there was no difference between salinity treatments, except at a light level of $0 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, while by day 40 there were differences at 0 and $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and at day 60 the length of buds was different between salinities at all light levels except 20 and $30 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. Initially, buds were longer at a salinity of 33, but by day 60, they were generally longest at a salinity of 36. Effects of light were significant within all combinations of time and salinity, with the longest buds at $30\text{-}40 \mu\text{mol photons m}^{-2}\text{s}^{-1}$.

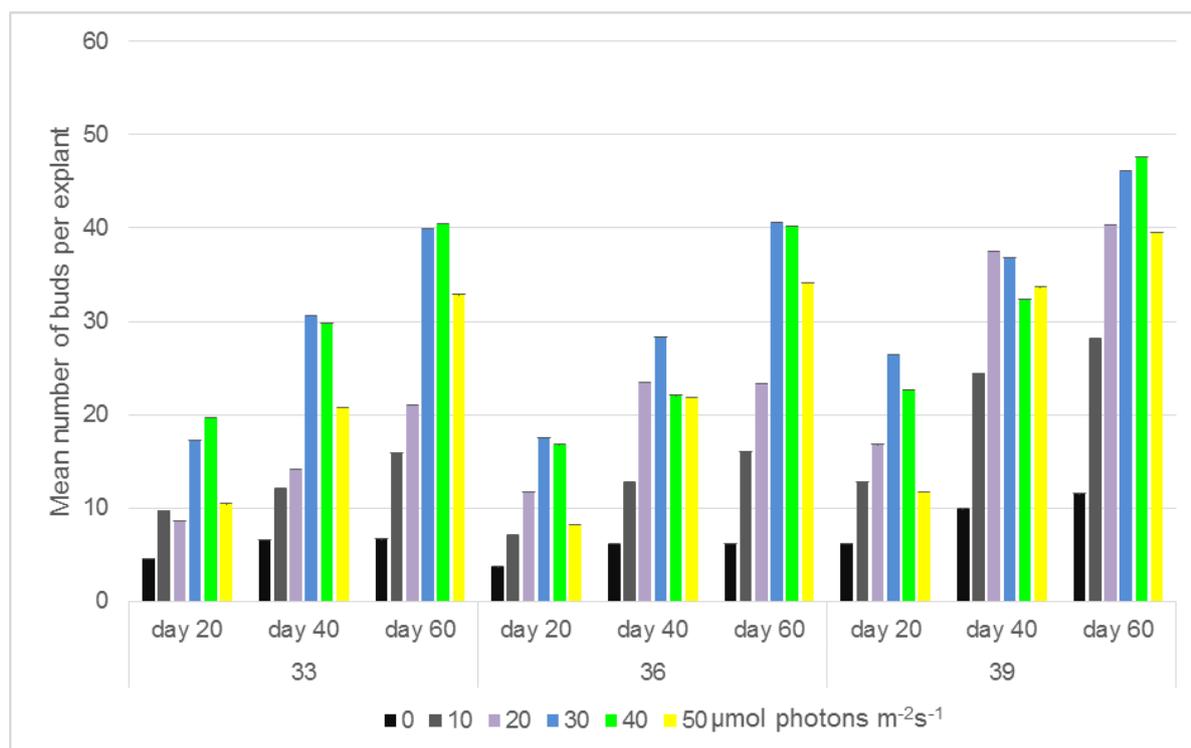


Figure 10. Effect of light intensity, salinity and time on the mean number of adventitious buds per *Gelidium* explant showing mean \pm s.e. ($n=3$) for stem explants at three salinities (33, 36, 39).

We have shown for the first time the possibility of obtaining seeding material of *Gelidium australe* through tissue culture. From the temperature experiment, both stem and apical explants produced adventitious buds across all temperatures, but the best results were obtained at $18\text{-}20^\circ\text{C}$. Stem explants performed better than apical explants, particularly in regards to the number of buds produced. This is in contrast to Titlyanov and Titlyanova (2006), who found apical explants had more regeneration capacity than the explants from stems. There are several possible explanations for this difference. One is that we took apical explants from 3 cm below branch tips, but the exact source of the equivalent fragments in their experiment is unclear. We additionally found that the apical explants were thinner than stem explants in *Gelidium australe*, which means stem explants have more resources for

adventitious bud growth than apical explants; this may have differed from the unspecified *Gelidium* species used by Titlyanov and Titlyanova (2006). The thinner apical explants may also have been more affected by our use of NaClO for disinfection than stem explants.

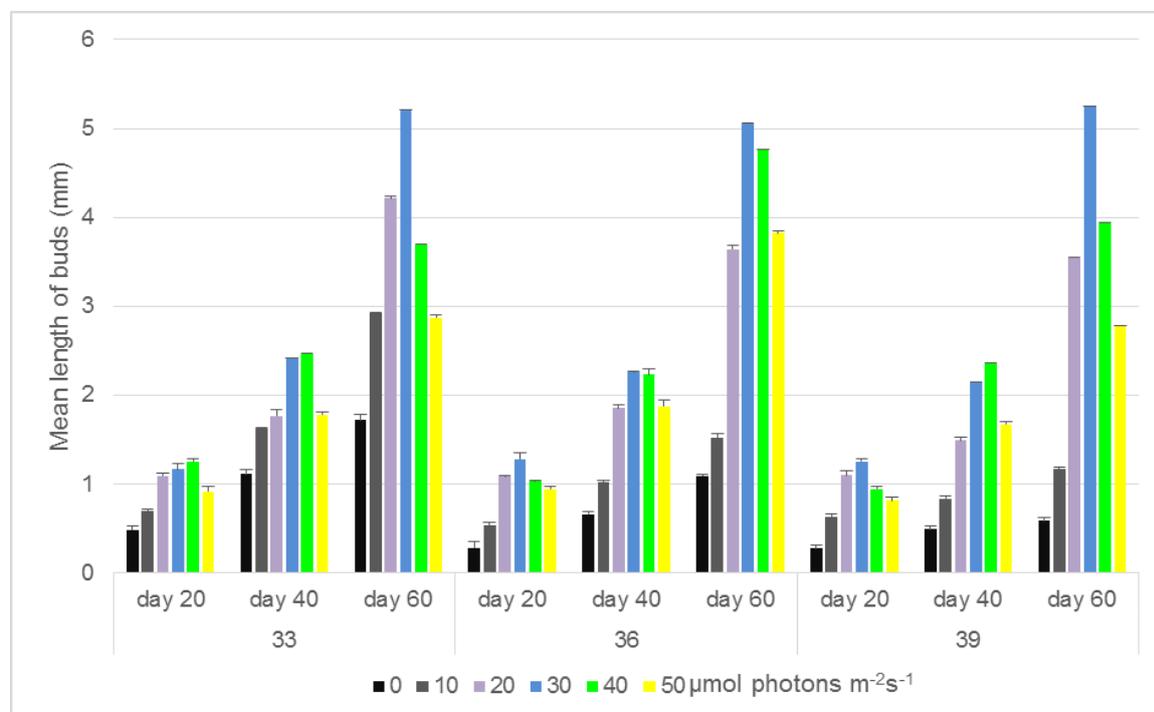


Figure 11. Effect of light intensity, salinity and time on mean length of adventitious buds showing mean \pm s.e. ($n=3$) for stem explants at three salinities (33, 36, 39).

Rhizoid development was not observed in our experiments. This may be due to different experimental conditions and explant preparation. Titlyanov and Titlyanova (2006) were successful in the stimulation of rhizoid formation in excised portions of *Gelidium* sp. having the basal parts partly shaded by plastic nets in bubbling culture; while Titlyanov *et al.* (2006) only obtained plantlets with rhizoids after applying a freeze-thaw method to meristem tissue. In other studies, rhizoids of *Gelidium* spp. were developed where explants were taken from branch tips and grown in contact with a carbonate-based substrate such as shells or limestone pieces (Salinas 1991; Salinas and Valdés 1993; Pei *et al.* 1996). We did not use the very tip of branches due to the fine morphology of *G. australe*, which made obtaining explants from branch tips impractical.

In the light and salinity experiment, we found the optimal light intensity for tissue culture to be 30-40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Although salinity moderated the effects of light, differences between salinity treatments were minor and primarily evident at suboptimal light levels.

2.3. Light and nutrient responses of *Solieria robusta*

Light and nutrient are important in determining the growth of seaweeds (Harrison and Hurd 2001; Buschmann *et al.* 2008; Hurd *et al.* 2014). Seaweed photosynthesis increases with increasing photosynthetically active radiation (PAR), to the point where photosynthesis is saturated, but PAR levels above this may cause a decrease in photosynthesis due to photoinhibition. Protective regulatory processes reduce photosynthetic rates, but are reversible, however, prolonged high irradiance (PAR and/or UV) may cause irreversible damage including bleaching of pigments (Hanelt and Figueroa 2012; Hurd *et al.* 2014). In the ocean, PAR availability is affected by incident radiation, which varies seasonally, and water depth, with water clarity impacting the transmission of light to depth (Hurd *et al.* 2014). A knowledge of the optimal PAR range of a seaweed species can help to inform suitable depths for culture (Hwang *et al.* 2007; Buschmann *et al.* 2008), although knowledge of environmental conditions at the culture site is also important.

Carbon, nitrogen and phosphorus are the major nutrients required by seaweeds (Harrison and Hurd 2001). Seaweed growth in nature is often nutrient-limited, with nitrogen limitation being most common, especially in temperate regions (Lignell and Pedersén 1987; Duarte 1992; Lapointe *et al.* 1992). The growth rate of several seaweeds has been shown to improve when they are cultured in IMTA systems due to additional nutrient availability (Petrell and Alie 1996; Troell *et al.* 2003; Abreu *et al.* 2009). Seaweeds generally increase their nitrogen content when grown under higher nitrogen conditions; with nitrogen stored in photosynthetic pigments as well as in internal nitrogen pools and in proteins (Harrison and Hurd 2001; Abreu *et al.* 2009). Light and nutrients often have interactive effects on seaweed growth (Lapointe and Tenore 1981; Lapointe and Duke 1984; Buschmann *et al.* 2008). Increased growth with nutrient addition may only occur where sufficient light is available, while the additional photosynthetic pigments produced under high nitrogen conditions can lead to improved growth under a range of light levels (Lapointe and Tenore 1981; Lapointe and Duke 1984).

Solieria robusta was the fastest growing species in our initial laboratory trial of red seaweeds (section 1.3), and was found to show greater high temperature tolerance and faster growth at most temperatures tested than *Gelidium australe*, the other red seaweed considered as a potential candidate for IMTA from initial trials (section 2.1). We therefore examined light and nutrient responses in *Solieria robusta* (hereafter *Solieria*), using ammonia, since this is the primary nutrient waste from SA fish farms (Fernandes *et al.* 2007; Fernandes and Tanner 2008; Tanner and Volkman 2009).

2.3.1. Methods

The experiment was conducted in September 2015 using 250 mL conical flasks filled with 150 mL low-nutrient artificial seawater (Sigma) at a salinity of 36. Clean specimens of *Solieria* were selected from the stock collections, fragments of 5-8 cm excised with a sterile scalpel blade, and physically cleaned with a soft toothbrush in filtered seawater to remove micro-epiphytes. These fragments were grown in low nutrient artificial seawater for two weeks prior to the start of the experiment. From this material, approximately 0.5 g of algal material was added to each flask. Fresh weights were measured at the start of the experiment and weekly thereafter for three weeks after gently patting dry the specimens on paper towel. Specific growth rates (SGR, as % d⁻¹) were calculated as per initial trials (see section 1.2.1). Nutrient treatments were nil, low and high, with the nil treatment receiving no added nutrient, low receiving a weekly flux of 11.2 µg ammonia-N L⁻¹, and high receiving 168 µg ammonia-N L⁻¹. These fluxes are equivalent to those expected around SA fish farms under 2010-11 stocking levels, and under the maximum carrying capacity, respectively (Middleton *et al.* 2013). Phosphate was added in a 10:1 molar ratio to nitrogen to avoid phosphate limitation, and modified Provasoli Enrichment solution (Harrison and Berges 2005), made without nitrogen or phosphorus, was added to all treatments to supply micronutrients and vitamins. Ammonia doses were added to flasks from a stock solution of (NH₄)₂SO₄ and KH₂PO₄ at the same time as water was replaced 2-3 times weekly, and aeration was supplied continuously. The flasks were placed in aquaria that acted as water baths to maintain temperature at 18°C. Lighting was supplied by cool-white LED lamps with shade cloth used to achieve four PAR levels (mean ± SE for n = 5 tanks each): 52 ± 3, 136 ± 5, 261 ± 7 and 365 ± 13 µmol photons m⁻² s⁻¹. PAR treatments were randomly assigned to aquaria, and each aquarium housed three flasks, being one of each nutrient treatment. Ammonia removal was calculated from samples taken from each flask two days after nutrient addition in the first week of the experiment and four days after nutrient addition in the third week. Water nutrient samples were kept frozen at -20°C until analysis on a Lachat QuickChem 8000 Automated Ion analyser (Lachat, Loveland, CO, USA). Ammonia (NH₃ + NH₄⁺) was determined using the indophenol blue method (Lachat 2003b).

After three weeks culture, effective quantum yield of PSII photochemistry (Genty *et al.* 1989) was calculated for each specimen based on fluorescence values taken three hours after the light regime started using a wireless waterproof Pulse Amplitude Modulated (PAM) fluorometer (Classic Fluorometer, Aquation Pty Ltd, Australia), following Maxwell and Johnson (2000). At the completion of the experiment, specimens were photographed for colour analysis, and nitrogen content was analysed as per the initial laboratory trial of red seaweeds (see section 1.3.1). Colour analysis was performed in FIJI/ImageJ (Schindelin *et al.* 2012) after correcting white balance with the chart white balance plug-in. Red, green and blue values

were extracted and converted to CIE Lab values using the colorspace package (Ihaka *et al.* 2015) for R (R Core Team, 2015). The effect of light and nutrient on CIE Lab values was analysed using permutational multivariate ANOVA (with the PERMANOVA routine) in PRIMER v 6.1.15 (Plymouth Routines in Multivariate Ecological Research) with the PERMANOVA+ add-on v1.0.5 (Anderson *et al.* 2008). Principal components analysis was used to visualise colour data. SGR was analysed with a linear mixed model, including tank as a random effect, using “lme” in the “nlme” package in R (Pinheiro *et al.* 2014). Due to the small size and low nitrogen content of many algal specimens, nitrogen content of some was below the detection limit for analysis. Nitrogen content data were therefore analysed by tobit regression using the AER and Survival packages in R, with tank as a grouping factor (Kleiber and Zeileis 2008; Therneau 2014). Where main effects were found to be significant, post-hoc tests were performed using “glht” in the “multcomp” package (Hothorn *et al.* 2008), with control of false discovery rate (Benjamini *et al.*, 2006; Verhoeven *et al.*, 2005) applied to pairwise comparisons using the Excel program of Pike (2011). In all cases, an α of 0.05 was used.

2.3.2. Results

SGR of *Solieria* was significantly different between ammonia treatments ($p < 0.001$) but was not affected by PAR level ($p = 0.255$) or the interaction ($p = 0.354$). Post hoc tests showed that growth was greater in the high ammonia treatment than the nil or low treatments, which did not differ from each other (Figure 12). Effective quantum yield of PSII (Φ_{PSII}) varied with both ammonia treatment ($p < 0.001$) and light ($p = 0.007$), with no interaction of these effects ($p = 0.282$). As for SGR, Φ_{PSII} was significantly higher in the high ammonia treatment than the nil or low treatments, while specimens under $136 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ had higher Φ_{PSII} than those grown at $261 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 13). Although the difference in SGR with PAR was not significant, specimens in the $136 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR treatment grown with high ammonia appeared to perform best, and it is likely that their better photosynthetic performance would result in higher growth over longer culture periods. Specimens kept under the two highest irradiances and with high nutrient also had noticeable growth of epiphytic algae by the end of the three weeks, while those kept at lower PAR or nutrient levels had no visible epiphytes.

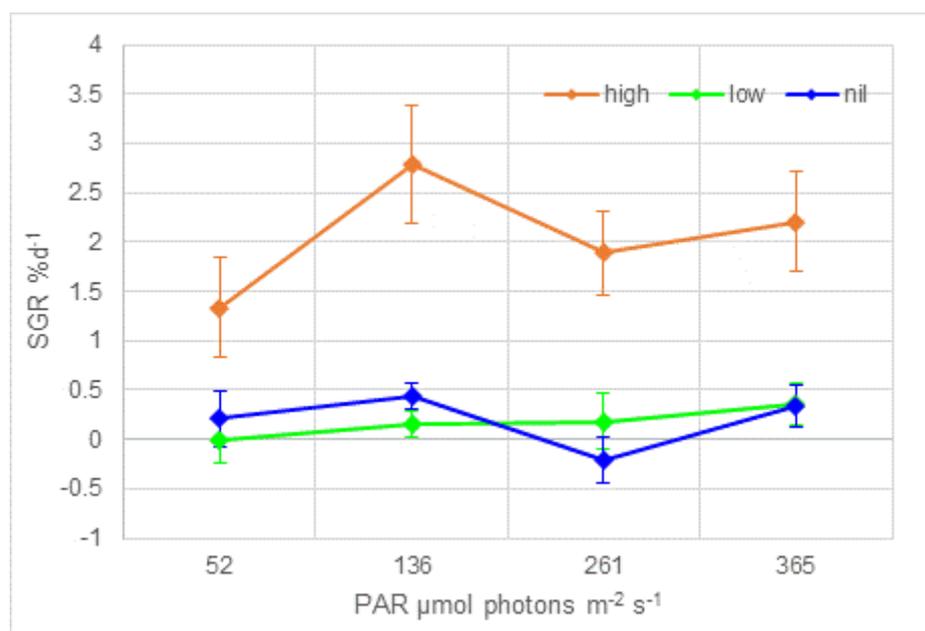


Figure 12. Specific growth rate of *Solieria* under four PAR levels with high, low or no ammonia addition. Error bars indicate standard errors (n=5).

Nitrogen content also varied with ammonia ($p < 0.001$) and PAR level ($p = 0.036$), with no interaction ($p = 0.08$). Specimens in the high ammonia treatment and kept under the lowest light ($52 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) had the highest nitrogen content. Colour analysis was used as there was insufficient material to analyse pigment content. PERMANOVA showed significant effects of ammonia ($p < 0.001$) and PAR ($p < 0.001$) on CIE Lab colour, with no interaction ($p = 0.537$). Post hoc tests showed that the high ammonia treatment was different to the low and nil nutrient treatments, while all PAR treatments were different from one another except the two highest. PERMDISP showed that there was no difference in multivariate dispersion between treatments ($p = 0.992$ for nutrient, $p = 0.956$ for PAR). Specimens grown under high ammonia or low PAR had greater 'a' values, indicating more red/less green, lower 'b' values, indicating more blue/less yellow, and lower 'L' values, indicating less luminance, i.e. darker colour (Figures 14, 15). Red seaweeds may increase their phycobiliprotein content with increased nitrogen availability (e.g. Lignell and Pedersén 1987; Gal-Or and Israel 2004; Carmona *et al.* 2006); these pigments, phycoerythrin and phycocyanin, absorb in the red and blue spectra respectively, and are likely to be responsible for the colour changes observed. Seaweeds also generally increase in photosynthetic and accessory pigment content with increasing depth/decreasing light availability, and excess light may also damage or destroy pigments leading to bleaching at high irradiance (Hanelt and Figueroa 2012; Hurd *et al.* 2014).

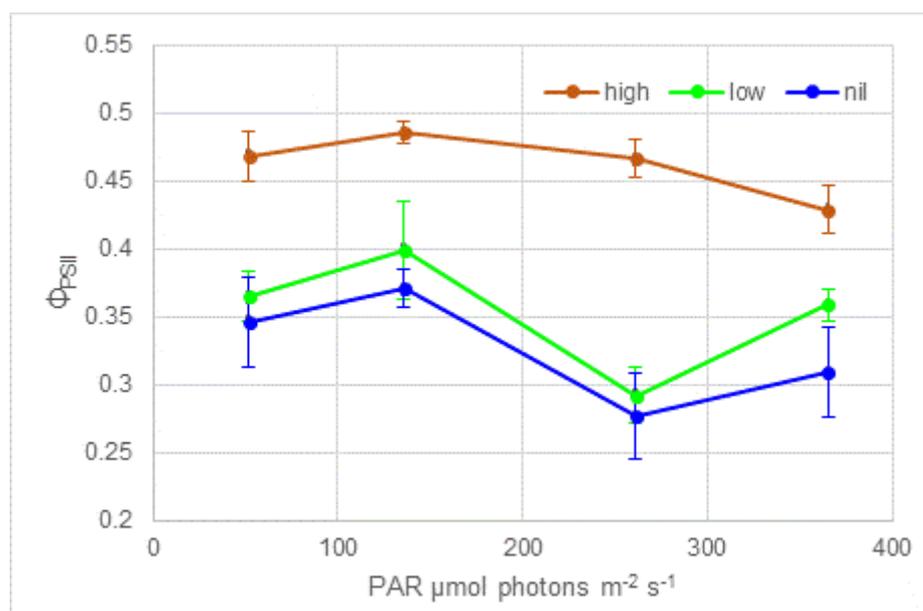


Figure 13. Effective quantum yield of *Solieria* under four PAR levels with high, low or no ammonia addition. Error bars indicate standard errors (n=5).

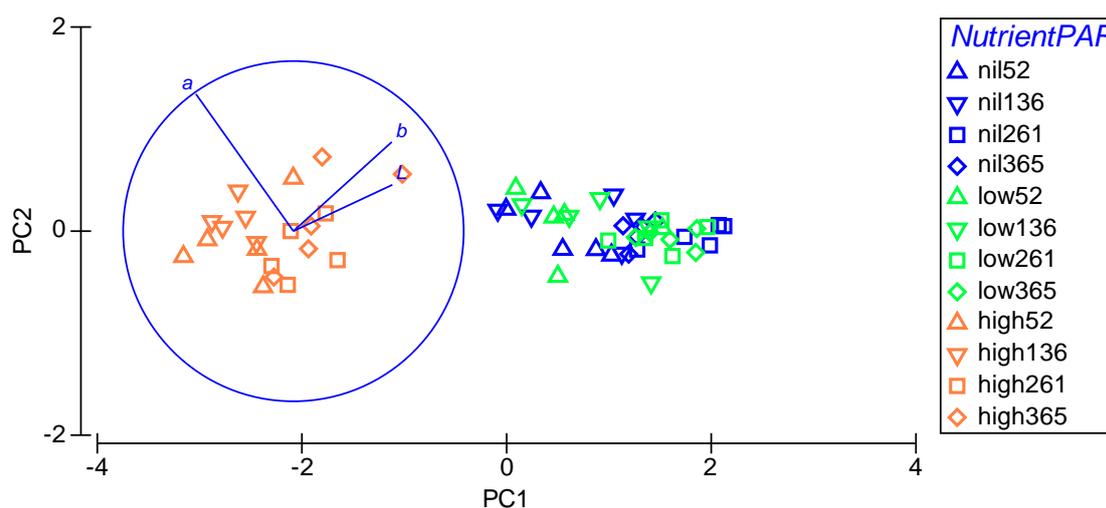


Figure 14. Principal components analysis of *Solieria* CIE Lab colour values. Different nutrient treatments are indicated by marker colour and PAR treatments by marker shape.

Dissolved inorganic nutrient samples taken in week 1 and week 3 showed that, after 2 and 4 days respectively, the residual ammonia concentration in the treatments ranged between 3 and 7 μg ammonia-N L⁻¹. For week 1, this represented removal of 98.9% of the initial dose in the high ammonia treatment, 71.0% in the low and 46.0% in the nil treatment, which contained trace ammonia (<10 μg ammonia-N L⁻¹) prior to use, possibly from contaminants in the salt or mixing vessel. In week 3, when final samples were taken 4 days after ammonia addition, the high, low and nil treatments removed 99.8%, 98.4% and 96.8% of the ammonia added. For the high nutrient treatment, the uptake rate was calculated to be 96 μg ammonia-N gFW⁻¹ d⁻¹ in week 1, and 67 μg ammonia-N gFW⁻¹ d⁻¹ in week 3. The potential uptake rate is likely higher

than this, as we do not know exactly how quickly nutrients were depleted. The lower initial concentrations in the low and nil treatments meant ammonia was even more likely to be depleted and uptake rates therefore not representative, so we did not calculate these. There was no difference in remaining ammonia or uptake rate observed between PAR levels. Although we could not generate a dose-response curve for ammonia uptake, it was clear that *Solieria* was able to effectively remove ammonia, driving concentrations to very low levels within 2-4 days.



Figure 15. Specimens of *Solieria* demonstrating colour differences. From left: examples grown with no ammonia and low PAR, low ammonia and low PAR, high ammonia and high PAR, high ammonia and low PAR.

2.4. Reproduction and string seeding of *Cystophora subfarcinata* and *Ecklonia radiata*

Brown seaweeds of the major farmed order Laminariales do not regrow from cuttings, and while excised portions of Fucales may grow, they generally do not reattach. Seed stock of these seaweeds for aquaculture is therefore obtained through sexual reproduction (Titlyanov and Titlyanova 2010). Spores or zygotes are typically settled onto string or rope and seedlings grown to a suitable size for out-planting (Sahoo and Yarish 2005; Titlyanov and Titlyanova 2010). We were previously successful in obtaining spores of *Ecklonia radiata* (Laminariales) and gametes of *Cystophora subfarcinata* (Fucales) but made no attempt to seed these onto string or grow them into seedlings. Growing seedlings on string will be an important step in production of biomass for aquaculture if IMTA is to be established in Australia.

2.4.1. Methods

Our earlier research (section 1.4) had shown that fertile *Ecklonia radiata* (hereafter *Ecklonia*) was found in the Adelaide area in late summer, and fertile *Cystophora subfarcinata* (*Cystophora*) in early spring. These results correspond to the patterns observed around southern Australia for these species (Hotchkiss 1999; Mohring *et al.* 2013). As the

reproductive periods for these species do not overlap, it was not possible to perform a single experiment to compare seeding success between species, rather, seeding and seedling cultivation was attempted separately for each species.

We collected *Ecklonia* monthly between December 2013 and May 2014. Collected plants were housed in outdoor tanks at SAASC that were supplied with flow-through natural seawater sourced from the adjacent Gulf St Vincent at ambient temperature and salinity. Plants were examined for sori weekly. Over January and February, high air temperature led to the outdoor tanks having elevated temperature, despite the continual water flow, and many of the plants died while others were overgrown with nuisance algae. In March 2013, an indoor tank was set up in a glasshouse receiving filtered sunlight, supplemented by metal-halide lamps. This tank was filled with filtered seawater, which was exchanged weekly, and fitted with a chiller to maintain temperature at 20°C. *Ecklonia* collected in April-May 2014 were housed in the indoor tank.

Zoospore release and string seeding was attempted in May 2014 using specimens collected in April 2014 that had been housed at SAASC. Clean sections of the central blade with sori were selected and rinsed in filtered seawater before being allowed to desiccate in dark humid conditions for one hour and then placed in filtered seawater in an 80 L plastic tub. Gentle agitation was applied periodically over a period of four hours. A water sample was taken to confirm the presence of zoospores. Seedling collectors, made from 8 m of polyethylene rope wound onto 30 cm lengths of PVC drainpipe, were submerged into the zoospore slurry for 30 minutes, then gently transferred to 100 L conical-bottom tanks filled with filtered seawater and provided with illumination of 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ on a 12L:12D cycle by cool white LED lamps. The tanks were maintained in a constant environment room at 18°C. Water exchanges of approximately half the tank volume were performed twice weekly, with modified Provasoli Enrichment solution (Harrison and Berges 2005) added; gentle aeration was applied after the first 10 days. The seeded collectors were maintained for 3 months.

In January 2015 zoospore release and seeding was attempted using freshly collected *Ecklonia* that did not have visible sori. The method followed the one described above, except that the excised blade sections included all clean sections of central blade, and these were wiped with 90% ethanol prior to desiccation. Seedling collectors used three string types: polyethylene, polypropylene, and nylon. Three collectors of each type were seeded and maintained as above except that seawater was passed through an additional stage of filtration to remove particles to 10 μm .

Fertile *Cystophora* was collected in October 2014. Reproductive branches were excised, rinsed in filtered seawater, refrigerated at 4°C in the dark for 16 hours, then placed in an 80 L

plastic tub in a constant environment room at 18°C and exposed to light. A water sample was taken to confirm the presence of zygotes. Seedling collectors, using the three string types as per *Ecklonia*, were submerged into the zygote slurry for 30 minutes, then gently transferred to 100 L conical-bottom tanks. Three collectors of each type were seeded and maintained as per the second set of *Ecklonia*.

After 3 months, the length of all seedlings and weights of 10 seedlings per collector were recorded. The effect of string type on seedling length and weight was analysed in R using linear mixed models, with collector as a random effect.

2.4.1. Results

Ecklonia seedling collectors from the first (May 2014) seeding trial became overgrown with nuisance algae by 3 months, and no *Ecklonia* sporophytes were observed to develop. From the second seeding trial, sporophytes developed on eight of the nine collectors; none were observed on one of the polyethylene collectors. Sporophytes were not evenly distributed on the collectors but occurred in clumps containing up to 14 seedlings each. Collectors with sporophytes had between 11 and 28 clumps per 8 m of string. There was no significant difference in seedling length or weight found between string types, with seedlings reaching an average length (\pm S.E., $n=555$) of 48.7 ± 1.0 mm and weight of 0.16 ± 0.12 g. Mohring *et al.* (2013) found that *Ecklonia* plants could produce spores from early summer, prior to the appearance of visible sori, although spore releases were greatest in late summer and early autumn. These results confirm that *Ecklonia* may be fertile prior to the appearance of sori, but it is likely that spore release was low, leading to the patchy occurrence and overall low numbers of seedlings.

Cystophora zygotes were observed on string collectors but failed to develop. After three months, nuisance algae was beginning to overgrow the collectors and the trial was discontinued.

2.5. Discussion

We investigated temperature responses of *Solieria robusta* and *Gelidium australe* and found that *Solieria* grew faster at temperatures greater than 14°C, and had a greater maximum growth rate and higher temperature tolerance than *Gelidium*. The upper limit for *Gelidium* growth was ~21°C, with specimens kept at higher temperatures becoming bleached and starting to disintegrate after 4 weeks. It therefore appears likely that summer temperatures during our pilot field trial (section 1.2) exceeded the tolerance of this species, contributing to the poor growth observed in that trial over the warmer months. Summer temperatures would have been within the range tolerated by *Solieria*, but higher than its optimal temperature for

growth. Light and nutrient responses of *Solieria* showed that low-level ammonia enrichment did not increase growth or nitrogen storage in this species, but growth, photosynthetic performance, and nitrogen content were increased at higher levels of ammonia addition. *Solieria* was able to remove almost all added ammonia, with residual concentrations after 2-4 days being $<7 \mu\text{g ammonia-N L}^{-1}$. Interactive effects of light and nutrient were not observed, and *Solieria* was able to grow over a range of PAR levels, indicating that this species is able to photoacclimate. Better photosynthetic performance at intermediate light levels, however, suggests that best longer-term growth is likely to be achieved at $<250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. This is likely to correspond to a depth $>2\text{-}3$ m in late spring to summer, based on average insolation from Port Lincoln (Bureau of Meteorology climate data) and the recorded light attenuation in Boston Bay (Middleton *et al.* 2013). Further investigation of the light climate at potential culture sites would be needed to determine suitable depths, however. Specimens grown under lower irradiance also stored more nitrogen, likely incorporating this into photosynthetic pigments as indicated by analysis of specimen colour.

Propagation techniques were investigated for *Gelidium australe*, *Cystophora subfarcinata* and *Ecklonia radiata*. We showed that tissue culture could be used to produce *Gelidium* seeding material, and determined suitable temperature, salinity and PAR for explant culture. While zygotes of *Cystophora* were obtained, these failed to develop when seeded on string. Attempts to culture *Ecklonia* were hindered by limited availability of fertile material and overgrowth of fouling organisms on seedling collectors, but in later trials we were able to obtain viable seedlings from plants early in the reproductive period that did not have visible sori. These were successfully seeded and grown on three string types, all of which appear suitable for use.

3. PORT LINCOLN FIELD TRIALS

The pilot field trial (section 1.2) was conducted on the Adelaide coast in Gulf St Vincent, which is not an area used for aquaculture. That trial tested adaptability to culture of eight seaweed species, including four red and four brown seaweeds, but results may not be transferrable to culture in an IMTA system, where additional nutrient is likely to be available. Environmental conditions are also likely to vary between the Adelaide coast and aquaculture areas, which are located in southern Spencer Gulf, SA.

Based on initial field and laboratory trials, four species were considered as potentially suitable for IMTA, and these were used in a field trial conducted at a finfish aquaculture site near Port Lincoln, SA. The best performing species were then used in a second on-farm trial.

One concern in establishing IMTA in SA is the risk of negative effects of seaweed on farmed fish. Bioactive compounds from seaweed, however may be protective against fish pathogenic bacteria (Bansemir *et al.* 2006). In other types of IMTA, farming mussels adjacent to salmon was shown to reduce disease incidence in the fish (Skar and Mortensen 2007), but disease implications are an important aspect of IMTA that have otherwise been rarely studied (Soto 2009).

External parasitic flatworms (flukes) are an ongoing health issue for culture of yellowtail kingfish (Ernst *et al.* 2002). Skin (*Benedenia seriolae*) and gill (*Zeuxapta seriolae*) flukes occur naturally in wild populations and can proliferate on farmed fish due to the parasites' direct lifecycles and high host fish density (Whittington 2012). Flukes are controlled by bathing fish with hydrogen peroxide, but reinfection occurs from wild fish and eggs (which are resistant to treatment) that attach to fish cage infrastructure (Ernst *et al.* 2002). Although seaweed is not a host for flukes, placing additional culture infrastructure in the vicinity of fish cages, such as in an IMTA system, could result in more fluke eggs being retained, acting as an additional reservoir for infection. The occurrence of fluke eggs on seaweed farming infrastructure was therefore also investigated.

3.1. Boston Island trial

3.1.1. Methods

An on-farm field culture trial was performed on a yellowtail kingfish lease site located near Boston Island, Boston Bay in southern Spencer Gulf (34° 43' 41" S, 135° 55' 44" E), 5 km offshore (east) from Port Lincoln, between March and November 2014.

Two long-lines were set up in the south-eastern corner of the lease site, each approximately 150 m from a stocked cage, with one being located south of the cage, in line with the prevailing

tidal movement (inline treatment), and the other east (offset). Six PVC culture frames were attached to each long-line, with three at each of two depths: approximately 2 and 5 m below the water surface. Three specimens of each species were attached to each frame. The species used were two reds: *Gelidium australe*, hereafter *Gelidium*, and *Solieria robusta* (*Solieria*), and two browns: *Ecklonia radiata* (*Ecklonia*) and *Cystophora subfarcinata* (*Cystophora*). The red seaweeds were held in mesh bags made from nylon mussel netting (Venus products), based on the method of Góes and Reis (2011). The holdfast of brown seaweeds was threaded twice through the lay of weighted ropes.

Specimens were tested over 3 culture periods: deployed on the 25th March and collected on either the 25th August (Mar-Aug) or 28th November 2014 (Mar-Nov), or deployed on the 25th August and collected on 28th November 2014 (Aug-Nov). It was initially planned to have three seasonal deployments ~3 months each, and to have specimens deployed in March collected after 3, 6 and 9 months. Weather and logistical issues, however, meant sampling could not occur before late August and the design was changed accordingly.

Due to breakage of some of the frames, 64 specimens (of a total of 429) were not recovered, and their fate is unknown. These specimens were not included in any analyses. For the remaining samples, specimens were regarded as lost if the associated bag or rope was retrieved but the specimen was missing. Fresh weights were obtained 24 hours prior to each deployment, and after retrieval for each specimen, with SGR calculated as in initial trials. Samples for nitrogen content were taken after weighing from each collected specimen and analysed as described in section 1.2.1.

Two specimens for fluke egg analysis were randomly selected from the three replicate samples of each treatment from two culture periods: Mar-Aug and Aug-Nov. The bags used to house red seaweed specimens and the ropes used for browns were examined under a dissecting microscope for the presence of fluke eggs.

Water temperature data were obtained from a logger (Hobo water temp pro) located on the intake for the Lincoln Marine Science Centre in Boston Bay, approximately 2.5 km southwest of the field trial site. Daily solar exposure (insolation) data was obtained from the Australian Bureau of Meteorology (www.bom.gov.au/climate/data) for the weather station nearest to the field trial location (Port Lincoln South, station number 018205).

SGR data from the reds and browns were analysed separately. Data were highly heteroscedastic even after attempted transformation, therefore, univariate permutational ANOVA (with the PERMANOVA routine) was utilized in PRIMER v 6.1.15 (Plymouth Routines in Multivariate Ecological Research) with the PERMANOVA+ add-on v1.0.5 (Anderson *et al.* 2008). Frame, nested within site and depth, was treated as a random effect, with deployment

and species as fixed effects. Where significant interaction terms were found, pair-wise tests were performed between factors within levels of the interacting factor, and PERMDISP was used to assess if significant differences in multivariate dispersion were present between groups. Euclidean distances were used in all analyses, with 9999 permutations; Monte-Carlo p-values were used if less than 1000 unique permutations occurred. The frequency of specimen loss was analysed by logistic regression in R (R Core Team, 2013), using the “glm” routine and binomial (logit) link function, with model selection using the Akaike Information Criterion. Nitrogen content data was logit transformed to achieve normality and homoscedasticity; effects of depth and site were then analysed by a linear mixed model with frame as a random effect, using “lme” in the “nlme” package (Pinheiro *et al.* 2014). An α of 0.05 was used in all cases.

3.1.2. Results

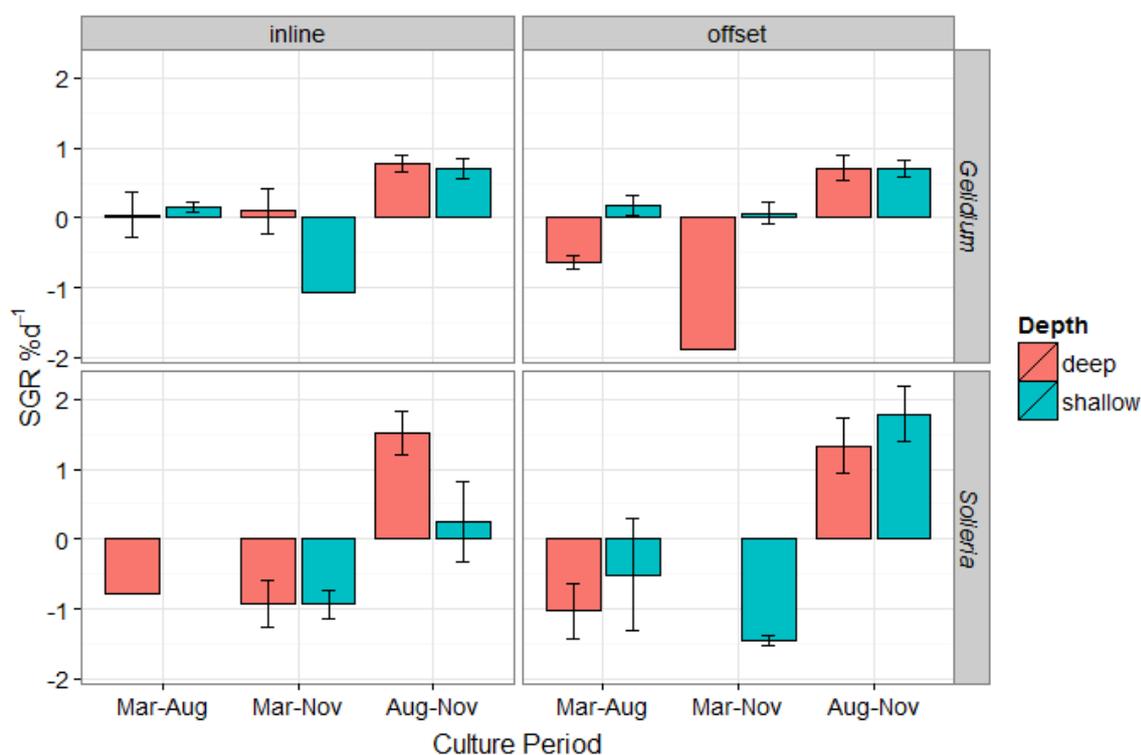
Growth of the red seaweeds during the Boston Island field trial was highly variable (Figure 16). PERMANOVA demonstrated that the variation was not explained by site (in-line or offset), depth, or frame, but there was a significant interaction of deployment x species ($p=0.041$). For specimens of both species that were deployed in March, there was no difference in SGR between 5- and 8-month culture periods (Mar-Aug or Mar-Nov), but SGR was greater for specimens deployed in August (Aug-Nov deployment) than in March. There was no significant difference between species except in the 8-month deployment, where *Solieria* specimens lost more biomass than *Gelidium*. PERMDISP analysis showed that multivariate dispersion (equivalent to variance in the univariate case as applied here) was significantly greater for *Solieria* in Aug-Nov than all other species x deployment groups ($p=0.001$). This was due to some *Solieria* specimens demonstrating SGR up to $3.5\% d^{-1}$ in the August deployment, while maximum SGR of *Gelidium* specimens was $1.5\% d^{-1}$, and biomass losses of other *Solieria* specimens were greater than those of *Gelidium*. Therefore, although differences in mean growth between species were not significant, *Solieria* showed potential for greater maximum growth rates, but was also more severely affected by biomass losses.

Water temperature and insolation decreased from April to July then increased from August to November (Table 8).

PERMANOVA of SGR for the brown seaweeds showed no significant differences, although the interaction of deployment x site x species approached significance ($p=0.056$). The analysis lacked power due to a large number of specimens being lost. Overall growth of the browns was low, but appeared greater for *Ecklonia* than *Cystophora*, with most specimens of the latter losing biomass (Figure 17).

Table 8. Deployment dates and summary of environmental conditions (mean \pm standard error, n= number of days per month) for the field trial.

Deployment	Month	Water Temp ($^{\circ}$ C)	Insolation (MJ)
25 th March 2014	April	19.0 (\pm 0.1)	11.4 (\pm 0.7)
	May	17.2 (\pm 0.1)	9.0 (\pm 0.4)
	June	15.6 (\pm 0.2)	7.4 (\pm 0.3)
	July	13.0 (\pm 0.1)	8.1 (\pm 0.4)
	August	13.2 (\pm 0.1)	12.0 (\pm 0.6)
26 th August 2014	September	15.2 (\pm 0.2)	16.2 (\pm 0.7)
	October	17.9 (\pm 0.1)	20.6 (\pm 0.7)
	November	19.7 (\pm 0.1)	21.1 (\pm 1.3)

**Figure 16.** Mean SGR over three culture periods at Boston Island for the red seaweeds. Error bars show standard error (n = number of retrieved specimens, see Table 9). Note, there were no retrieved specimens for some treatment combinations.

For both red and brown seaweeds, there was a significant effect of deployment \times species on the pattern of losses (reds: $p=0.047$, browns: $p<0.001$). For the reds deployed in March, more *Solieria* specimens were lost over the 5-month trial (Mar-Aug), while more *Gelidium* were lost in the 8-months (Mar-Nov). For the browns, more *Ecklonia* were lost than *Cystophora* over

both the 5- and 8-month trials. There were few losses of the Aug-Nov specimens for any species (Table 9).

Heavy fouling growth occurred on specimens from all deployments, particularly the first (Mar-Aug). Fouling made counting of fluke eggs difficult, so only presence/absence was recorded for that deployment. Approximate counts were made for the Aug-Nov deployment. The number of samples examined and found to have fluke eggs are shown in Table 10.

Table 9. Number of retrieved specimens for each treatment in the Boston Island trial. The total number of bags/ropes retrieved is shown in brackets.

Deployment	Species	n			
		Offset		Inline	
		Shallow	Deep	Shallow	Deep
Mar-Aug	<i>Gelidium</i>	5 (9)	2 (7)	3 (12)	4 (12)
	<i>Solieria</i>	2 (8)	2 (8)	0 (12)	1 (9)
Mar-Nov	<i>Gelidium</i>	2 (9)	0 (9)	1 (4)	5 (6)
	<i>Solieria</i>	5 (10)	1 (9)	2 (5)	5 (6)
Aug-Nov	<i>Gelidium</i>	9 (9)	9 (9)	9 (9)	8 (9)
	<i>Solieria</i>	9 (9)	9 (9)	9 (9)	7 (9)
Mar-Aug	<i>Cystophora</i>	7 (7)	10 (10)	10 (11)	11 (12)
	<i>Ecklonia</i>	3 (10)	3 (7)	2 (9)	2 (9)
Mar-Nov	<i>Cystophora</i>	6 (1)	2 (4)	2 (2)	0 (1)
	<i>Ecklonia</i>	0 (2)	1 (3)	0 (3)	0 (1)
Aug-Nov	<i>Cystophora</i>	5 (7)	7 (8)	5 (5)	4 (8)
	<i>Ecklonia</i>	7 (7)	5 (7)	4 (6)	7 (9)

The majority of samples examined did not have fluke eggs present, so formal statistical analysis could not be performed. It was clear, however, that fluke eggs were found more commonly in Mar-Aug and predominantly on samples from the culture system that was in line with prevailing tidal flow. Increased occurrence of fluke eggs in the Mar-Aug deployment could be associated with fouling type, slower biodegradation of eggs at lower water temperatures over winter, or differences in fluke numbers on fish in the adjacent cages.

Nitrogen content was only analysed for *Solieria* specimens from the Aug-Nov deployment as there were insufficient specimens retrieved of other species and from other culture periods for meaningful analysis. There was no significant difference in nitrogen content with depth or site, with specimens having average nitrogen \pm s.e. (n=33) of $1.60 \pm 0.05\%$. This suggests that specimens were nitrogen limited, despite being cultivated near to fish cages.

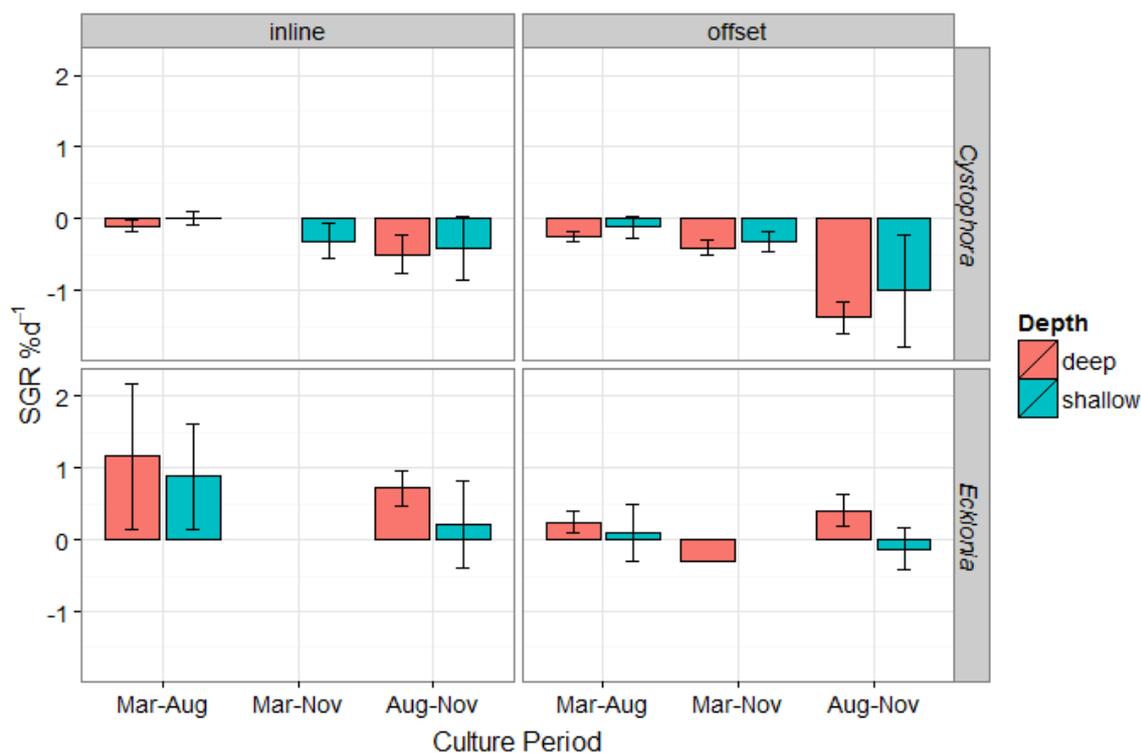


Figure 17. Mean SGR over three culture periods at Boston Island for the brown seaweeds. Error bars show standard error (n = number of retrieved specimens, see Table 9). Note, there were no retrieved specimens for some treatment combinations.

Table 10. Number of samples examined and having fluke eggs present for the two Boston Island deployments. The total number of eggs present is shown in brackets where counted.

		Mar-Aug				Aug-Nov			
		Examined	Flukes present			Examined	Flukes present		
Site	Skin		Gill	Either	Skin		Gill	Either	
bag	offset	18	1	1	2	6	0	0	0
	inline	23	11	12	12	14	0	1 (35)	1
rope	offset	15	3	2	3	6	0	0	0
	inline	23	8	6	9	12	1 (1)	0	1

3.2. Bickers Island trial

3.2.1. Methods

A second on-farm trial was carried out on a yellowtail kingfish lease site located near Bickers Island, Boston Bay in southern Spencer Gulf (34° 44' 52" S, 135° 55' 42" E), 9 km SE of Port Lincoln. This trial used *Solieria* which showed the greatest potential growth rates in the Boston Island trial, plus *Ecklonia* on ropes that had been seeded in the laboratory. The rope seeding was conducted concurrently with the January 2015 seeding reported in section 2.4.1 and using

the same method. Eight seedling collectors containing 1 m each of polyethylene rope wound onto PVC drainpipe were used. These collectors were maintained under the conditions described in section 2.4.1 until use. *Solieria* was held in mesh bags made from nylon mussel netting as per the Boston Island trial.

Two long-lines were set up at the western end of the lease site at a distance of approximately 120 m from fish cages: one on the northern edge of the lease site and one on the southern edge. Eight dropper lines were used on each long-line, comprising four of each species. Specimens were attached to each dropper line at depths of 1, 2, 3, 4 and 5 m below the water surface. The trial ran from 27th February to 22nd April 2015. SGR of *Solieria* was calculated as for the Boston Island trial. Samples for N content analysis were taken at the end of the trial, and N analysed as per initial trials (see section 1.2.1). Temperature and relative light level loggers (Onset HOBO) were placed at 1 m and 5 m deep on four dropper lines per long-line. Daily solar exposure (insolation) data was obtained from the Australian Bureau of Meteorology (www.bom.gov.au/climate/data) for the weather station nearest to the field trial location (Port Lincoln South, station number 018205). Ropes and bags from all samples were examined for the presence of fluke eggs.

Statistical analyses were performed in R (R Core Team, 2013). SGR was analysed with linear mixed models to test the effects of site (long-line) and depth, with dropper rope as a random effect, using “lme” in the “nlme” package (Pinheiro *et al.* 2014). The pattern of nitrogen content was non-linear with depth, so nitrogen data were analysed using a generalised additive mixed model (GAMM) with separate smooth terms for depth fitted per site, and rope as a random effect using the mgcv package (Wood 2006). In all cases an α of 0.05 was used.

3.2.2. Results

No *Ecklonia* was found on seeded ropes in the Bickers Island field trial, although ropes seeded at the same time and maintained in the laboratory did show seedling development (see section 2.4.2). Farm access constraints meant that these ropes were deployed only 4 weeks after seeding, while typically, kelp species are grown in nursery culture for ~2 months or more before out-planting (Hwang *et al.* 2009; Neill *et al.* 2009). Overgrowth by fouling organisms may have occurred in the field before sporophytes could develop, or young sporophytes may have been adversely affected by transport and handling during their deployment. Additionally, development of plants on ropes in the laboratory was sparse, and because sporophytes were not visible at the time of out-planting, we may have inadvertently used sections of rope with no or few *Ecklonia* present. The sparseness of seeding may be due to the plants used for seed production being in the early stages of fertility and so releasing few spores, or simply because the amount of plant material used was too small for the quantity of seedling collectors.

Little growth of *Solieria* was observed (Figure 18), with all specimens suffering heavy fouling and most decreasing in biomass. There was no significant effect of either site or depth on SGR of *Solieria*. Temperature decreased from 21.9 to 17.9°C through the trial, with insolation also decreasing (Table 11). The relative light availability at 5 m deep was 27% of that at 1 m deep.

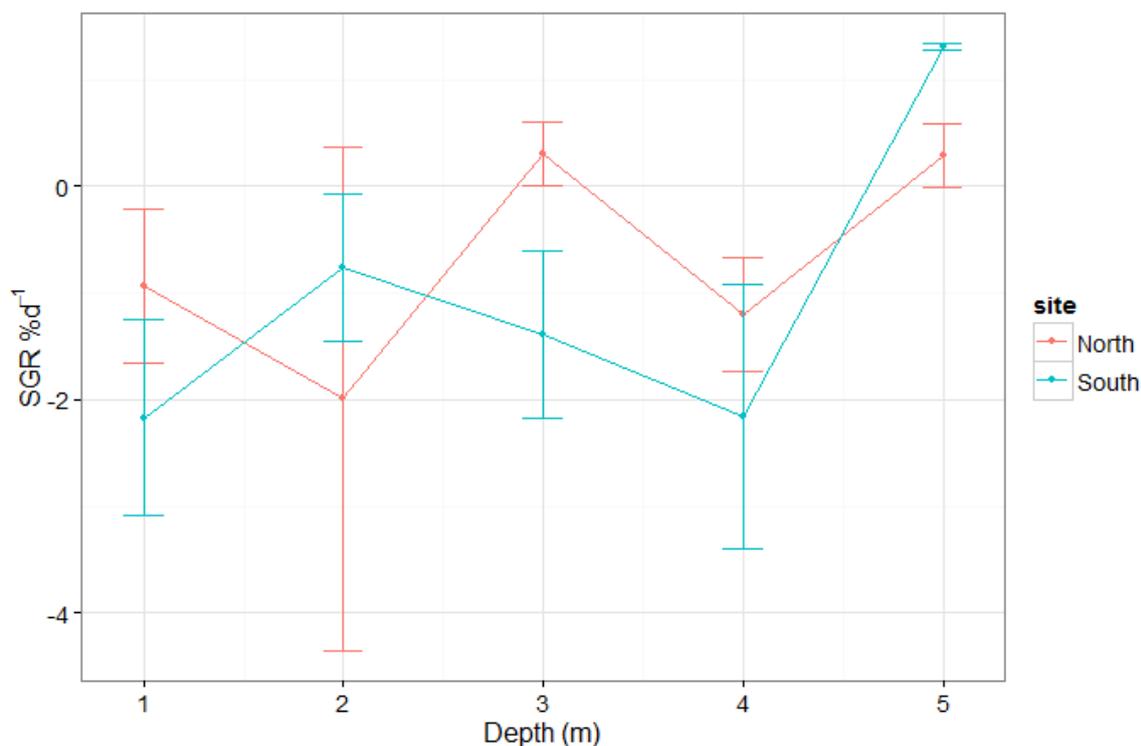


Figure 18. Mean SGR of *Solieria* with depth and site in the Bickers Island trial. Error bars show standard error (n = 4).

Table 11. Deployment dates and summary of environmental conditions (mean \pm standard error, n = 7) for the Bickers Island field trial.

Deployment	Week	Water Temp (°C)	Insolation (MJ)
27 th February– 22 nd April 2015	1	21.9 (\pm 0.1)	17.5 (\pm 2.3)
	2	21.4 (\pm 0.1)	18.3 (\pm 1.5)
	3	21.1 (\pm 0.1)	17.3 (\pm 1.4)
	4	20.5 (\pm 0.2)	14.0 (\pm 1.3)
	5	19.1 (\pm 0.1)	15.0 (\pm 1.7)
	6	18.5 (\pm 0.1)	11.6 (\pm 1.1)
	7	18.1 (\pm 0.1)	10.0 (\pm 1.9)
	8	17.9 (\pm 0.1)	9.9 (\pm 0.7)

The nitrogen content of *Solieria* was significantly different between sites (GAMM: $p < 0.001$), being higher on the southern long-line, and showed a different pattern in depth by site (Figure 19). Specimens on the long-line to the north of the cages did not show any significant difference in nitrogen with depth ($p = 0.657$), but those of the southern long-line decreased with depth ($p = 0.002$), although were always higher in nitrogen than northern specimens. There was insufficient biomass of some specimens for nitrogen analysis, so the number of samples varied between treatments. The number analysed is shown in Table 12.

Table 12. Number of *Solieria* specimens retrieved out of four deployed, and, in brackets, analysed for nitrogen from the Bickers Island trial

Depth (m)	North	South
1	2 (2)	3 (1)
2	2 (1)	3 (3)
3	3 (3)	4 (4)
4	3 (3)	4 (3)
5	4 (4)	3 (3)

Fluke eggs were found on very few samples (Table 13). Where they occurred, they were often entangled in the byssal threads of mussels, one of the major fouling organisms observed. Formal statistical analysis, including testing for an association with mussels, was not possible due to the absence of fluke eggs from most samples.

Table 13. Number of samples examined and having fluke eggs present for the Bickers Island trial. The approximate total number of eggs present is shown in brackets.

	Site	Examined	Flukes present		
			Skin	Gill	Either
bag	north	19	0	0	0
	south	20	1 (2)	3 (20)	3
rope	north	20	2 (3)	1 (15)	2
	south	20	0	1 (25)	1

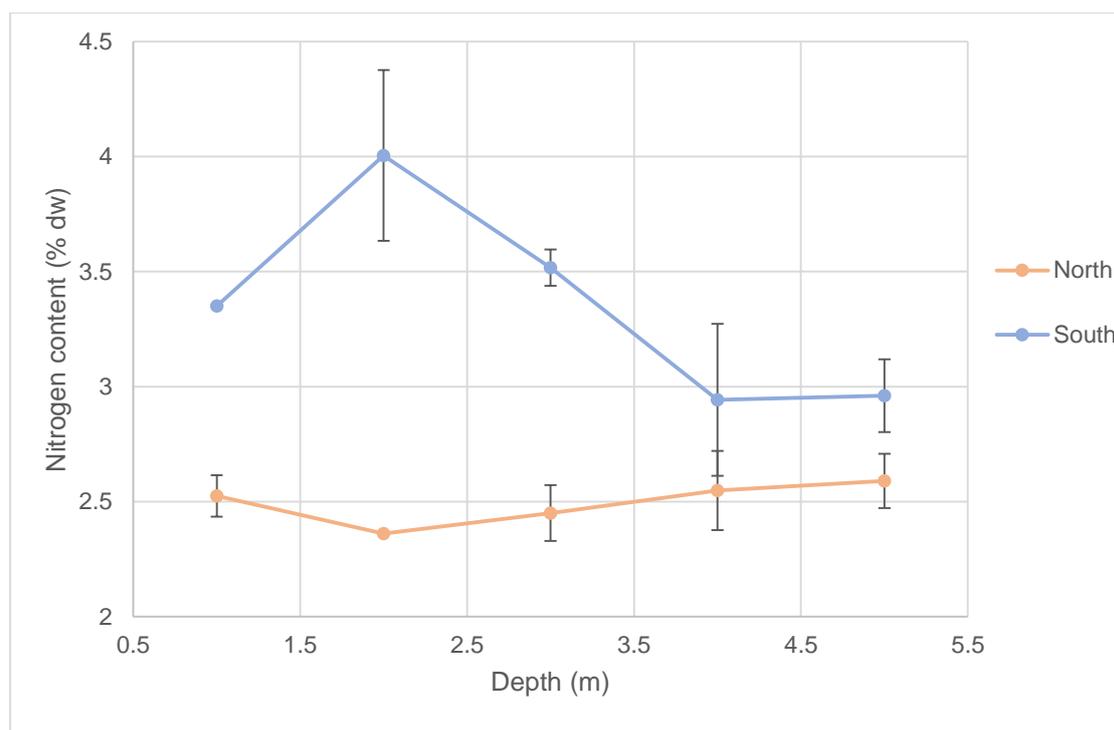


Figure 19. Mean nitrogen content of *Solieria* with depth and site in the Bickers Island trial. Error bars show standard error (n = number analysed as per Table 12).

3.3. Discussion

The Port Lincoln field trials are the first attempt at offshore cultivation of seaweeds in Australia in an IMTA system, and highlight several issues in the establishment of novel species and systems for aquaculture. Seaweed growth in our trials was negatively impacted by heavy fouling growth, a recognised problem with seaweed culture. Performance of large-scale culture is difficult to predict from small-scale trials, such as the current ones reported here, since many aspects of performance vary with stocking density and biomass (Troell *et al.* 2009). A larger trial may prove more successful by providing sufficient initial seaweed biomass to outcompete fouling organisms (Titlyanov and Titlyanova 2010). We were also unable to tend to the farm trial for a prolonged period, whereas seaweed farms are typically attended to regularly to check for fouling and perform preventative maintenance (Ask and Azanza 2002; Troell *et al.* 2009). Careful timing of out-planting is used to minimise fouling of seaweeds grown around salmon farms (Troell *et al.* 2009), and fouling may also vary with location (Abreu *et al.* 2009; Neill *et al.* 2009). Abreu *et al.* (2009) found seaweeds grew better at a distance of 800 m from cages than at 100 m, with the lines at 100 m suffering fouling growth. In a trial of *Ecklonia* culture in New Zealand, sites with high water movement experienced much less fouling than calm-water sites (Neill *et al.* 2009). We were only able to test two farm locations

and could not test further distances from cages due to the requirement to keep infrastructure within the farm lease boundaries.

Despite generally poor growth in the field trials, some specimens of *Solieria* exhibited promising growth rates of up to 3.5 %d⁻¹ during the 3-month spring (August-November) deployment at Boston Island. Spring is generally expected to be the best season for seaweed growth in temperate regions (Titlyanov and Titlyanova 2010), and this was found in both the pilot field trial (section 1.2) and first on-farm trial, although it should be noted that we tested different fish farm sites in different seasons and did not obtain a full 12 month set of on-farm growth data at any one location. These promising growth rates were achieved despite specimens appearing to be nitrogen-limited, though it should be noted that *Solieria* grew well in our initial laboratory trial (section 1.3) and had tissue nitrogen <2% in that case also. It is, however, possible that greater nitrogen availability will further improve growth in this species, as seen in the light and nutrient experiment (section 2.4). The recorded nitrogen levels of <2% in the Boston Island trial are somewhat surprising given the close proximity of seaweed to fish cages. The lease site was, however, stocked minimally during the time of the trial (CleanSeas operations personal communication), so feed and therefore waste nutrient input would have been relatively low. The Bickers Island trial was conducted adjacent to more heavily stocked fish cages, and specimens from that trial showed nitrogen contents of up to ~4%. This indicates favourable nitrogen supply, but growth was clearly compromised by other factors, most likely due to fouling as discussed above. It should also be noted that this second trial took place in summer, which was shown to be the poorest season for growth in the pilot field trial, although the temperature experienced in the Bickers Island trial was within the optimal range for *Solieria* determined in our laboratory experiment.

We found that seaweed aquaculture infrastructure, particularly when located in-line with prevailing tidal currents, can capture eggs of both skin and gill flukes, but numbers observed were very low. Over 60% of fluke eggs produced in aquaculture entangle on the net (SARDI unpublished data); the number of eggs found in this study suggests that while fluke eggs can become entangled on IMTA infrastructure, the effect on overall fluke management is likely to be negligible.

DISCUSSION AND CONCLUSIONS

A shortlist of 14 native SA seaweeds that are potentially suitable for aquaculture, and specifically for IMTA in southern Spencer Gulf, SA, was generated through a literature review and liaison with potential end users and international researchers. Initial surveys and field collections determined that eight of these short-listed species were readily available at sites accessible from Adelaide, and amenable to handling. The suitability for culture of these species was investigated in a pilot field trial, with potential growth and nitrogen removal of the four red candidate seaweeds, and reproduction techniques of the four brown seaweeds, investigated in the laboratory. These initial trials identified two red seaweeds that showed promising growth in the field (*Gelidium australe*) or laboratory (*Solieria robusta*), and two brown seaweeds which could be reproduced in the laboratory and that also showed potential for cultivation in field trials (*Ecklonia radiata* and *Cystophora subfarcinata*). These four species were then further investigated in the laboratory and used in on-farm field trials around yellowtail kingfish farms in Port Lincoln.

Laboratory investigations of the red seaweeds focussed on determining optimal parameters for growth in relation to temperature, light and nutrients. A knowledge of seaweed responses to these factors will help to determine the most suitable sites, depths and seasons for culture, as well as providing parameter estimates of seaweed growth and nutrient uptake for incorporation into biogeochemical models (e.g. Hadley *et al.* 2015). We also investigated tissue culture as a means of producing seed stock of red seaweeds, using *Gelidium australe*. Propagation and string seeding techniques were investigated for the brown seaweeds *Cystophora subfarcinata* and *Ecklonia radiata*.

We demonstrated the feasibility of obtaining red seaweed seeding material through tissue culture, although the method we used did not result in plantlets with rhizoids that could be attached to substrates for out-planting. This method could, however, be used to produce material for bag culture. The information obtained on temperature, light and nutrient responses of red seaweeds showed that summer water temperatures in the SA gulfs may exceed those optimal for growth, particularly for *Gelidium*. *Solieria* was found to benefit from added ammonia, although low levels did not produce significant improvement in growth. *Solieria* was able to grow across a range of light levels, but high irradiance caused some bleaching and loss of photosynthetic performance, as well as encouraging epiphytic growth. The optimal irradiance for growth of this species is therefore likely to be $<250 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Investigation of the light availability in potential culture areas will be needed to determine at what depth this level is achieved, but it is likely to correspond to a depth of ~2-3 m in late spring based on average insolation from Port Lincoln (Bureau of Meteorology climate data)

and the recorded light attenuation in Boston Bay (Middleton *et al.* 2013). The nitrogen content of all red seaweeds tested increased with added nutrient, but in our field trials, growth was not correlated with nitrogen content. This indicates that, while specimens were potentially nitrogen-limited at times (nitrogen content <2%), other factors limited growth when nitrogen was more available.

In addition to knowledge of seaweed biology, information on conditions at potential field sites will be important in determining the best locations for IMTA. We were only able to carry out small-scale trials at limited locations, and seaweed growth in these trials was negatively impacted by fouling growth. These trials were hampered by having only limited material available, as initial attempts to seed brown seaweeds onto rope were unsuccessful, and we did not have facilities available to grow out cultured material of *Gelidium* for out-planting. Larger-scale trials may have greater success through giving seaweeds more chance to outcompete fouling growth, although additional sites and seasons should also be tested to determine the locations, planting times and culture periods that are best for seaweed growth and where fouling is minimised. A range of planting densities should be tested, since planting too densely can lead to competition as seaweed grows, and overall reduced growth (Troell *et al.* 2009). Regular monitoring of field trials would also help to identify potential issues and allow preventative measures to be carried out, as they would on an operational farm. Future research should seek to refine laboratory techniques for the production of seeding biomass to allow larger-scale trials to be performed, and to determine the best conditions for seedling culture prior to out-planting. Spring is likely to be the best season for growth in the field, but further data in different areas is needed to confirm this. Nutrient inputs from tuna peak in autumn-winter, corresponding to the ranching period for fish, whereas yellowtail kingfish inputs are maximal in spring (Tanner and Volkman 2009; Middleton *et al.* 2013), and it is this sector that is most likely to expand in the short term (Econsearch 2015). Seaweed culture in spring would therefore be suitable to mitigate nutrient inputs from yellowtail kingfish farming.

Our pilot field trial tested two cultivation methods for red seaweeds: tied and bagged. There was little difference in growth performance between these systems, but bag culture results in less specimen loss and is less labour intensive (Góes and Reis 2011), as well as providing more opportunity for automation. The bags used in our on-farm trials were made from a readily-available aquaculture product (mussel netting) and had a larger mesh size than the bags used in the pilot trial. These bags appear suitable for use, as evidenced by the promising growth rates obtained for *Solieria*.

Solieria robusta showed the best potential growth rates of the red seaweeds tested across our experiments. Although this species has a low nitrogen content when compared with other red seaweed species, it could potentially remove more nitrogen in IMTA due to faster growth, in

addition to increasing its nitrogen content when grown under higher nutrient conditions. Further work is needed to optimise the growth rate and nitrogen removal of *Solieria* in the field. Knowledge of *Solieria* light, temperature, and nutrient requirements obtained through this project will aid in determining suitable culture depths and locations for this species, although further field trials will be needed to confirm performance. Growth rates were highly variable between *Solieria* specimens. Different growth rates in Solieriaceae have been observed between different genetic strains and also between life history stages (de Paula *et al.* 1999; Ask and Azanza 2002; Hayashi *et al.* 2009). Investigation of these aspects could help to identify the best performing specimens, with strain selection performed via tissue culture or spore production to obtain seed stock. Both tissue culture and spore production techniques have been used on Solieriaceae and other Gigartinales (de Paula *et al.* 1999; Bulboa *et al.* 2007; Hayashi *et al.* 2009) and are likely to be applicable to *Solieria*.

Gelidium australe performed well in our initial field trial and appeared resistant to herbivory. It has a robust morphology and is tolerant of rough conditions so may be a good candidate for culture further offshore, for example, if IMTA is applied around tuna farms. This species also grew better than *Solieria* in cooler temperatures, so is better suited to culture over winter, corresponding to the time when nutrients are released from tuna farms, but improvements in its growth rate through optimisation of culture conditions and strain selection would be needed.

We did not achieve high growth rates in the field for any of the tested brown seaweeds, but all trials were performed with transplanted material threaded onto rope, rather than seeded, except in the very last trial. In this case ropes were out-planted only four weeks after seeding and it is likely that fouling growth developed before seedlings were able to grow. Better growth rates may be achieved with seeded ropes, although suitable site selection and hatchery culture prior to out-planting will clearly be important. Of the brown seaweeds tested, only *Ecklonia radiata* was successfully seeded onto, and grown on, string. This is therefore the best immediate candidate brown seaweed for culture. Gametophyte culture, which is employed for several farmed Laminariales (Zhang *et al.* 2008; Forbord *et al.* 2012), could be used to provide denser seeding of collectors and make seedlings available outside the main reproductive season.

When applied to IMTA, the value of nitrogen removal of seaweeds should be taken into account, but it will also be important to develop markets for species used. *Solieria robusta*, *Gelidium* spp. and *Ecklonia* spp. have a history of human consumption and so may be farmed for food or functional food applications, although food safety aspects will also need to be considered in this case (Holdt and Kraan 2011). These species also produce phycocolloids of value and a range of bioactive compounds, or may be used in production of stock or aquaculture feeds, fertiliser or biofuels. Biorefinery techniques that are being developed for

seaweeds could be applied to obtain multiple products from farmed biomass through sequential extraction (Lorbeer *et al.* 2013; Lorbeer *et al.* 2015).

Based on the growth rates and nitrogen content of *Solieria* recorded in our initial laboratory trial, and assuming a culture period of 90 days, an initial biomass of 900 tonne of seedlings of this species would be needed to completely mitigate the annual nitrogen outputs of 1000 tonnes of kingfish production, or 400 tonnes of tuna (= 200 tonne N, Fernandes *et al.* 2007; Fernandes and Tanner 2008). We used individual bags in our on-farm field trial, but an up-scaled culture system could be based on the method of Góes and Reis (2011), who used parallel lengths of tubular mesh separated by 0.3 m, with seedlings of initial weight 100 g inserted every 0.25 m. A total area of approximately 67 ha would therefore be needed grow sufficient *Solieria* to offset 1000 tonnes of kingfish production using this system, but further research would be needed to determine whether this type of culture system and planting density is suitable for this species, and for the location used. This farm size is approximately the same as that calculated for *Gracilaria* to remove nitrogen from a tonne of salmon production (Abreu *et al.* 2009), although they based their calculations on less dense culture than the above, and the initial biomass of *Gracilaria* needed would be only ~42 tonnes. The difference in the amount of seaweed required is due primarily to the much greater nitrogen released from kingfish than salmon, rather than to differences in the nitrogen removal capacities of the seaweed. The required farm size to mitigate wastes could be reduced by optimisation of seaweed growth, selection of sites where nutrient removal is maximised (Hadley *et al.* 2015), or by the use of multiple extractive species, for example, multiple seaweed species with different depth requirements or seasons for growth (Buschmann *et al.* 2008; Troell *et al.* 2009), or inclusion of filter-feeding species such as mussels (Whitmarsh *et al.* 2006; Barrington *et al.* 2009). Nitrogen loads from tuna and kingfish occur primarily in dissolved form (Fernandes *et al.* 2007; Fernandes and Tanner 2008), meaning that seaweeds, which utilise dissolved inorganic nitrogen, are the most appropriate extractive species for direct mitigation, but filter feeders complement seaweeds by removing particulate wastes and contribute to overall nitrogen reduction (MacDonald *et al.* 2011; Handå *et al.* 2012). Even if complete mitigation of kingfish nitrogen impacts is not feasible, the application of IMTA could allow higher fish stocking densities within an area for the same environmental footprint, improving farm profitability while also providing crop diversification.

Growing demand for seafood is likely to drive expansion of fish aquaculture in Australia. In order for this expansion to be sustainable, a means of nutrient mitigation may be needed. Although development of seaweed aquaculture in Australia requires further research, globally, the benefits of IMTA using seaweeds have been demonstrated, and demand for seaweed products is also increasing, creating further interest in the development of seaweed industries

in Australia. We have obtained important information about the biology of several seaweeds that are candidates for aquaculture and specifically for use in IMTA, which will assist in establishing seaweed aquaculture in southern Australia.

IMPLICATIONS

This project contributes the first information towards development of seaweed aquaculture as a component of IMTA in southern Australia. When implemented on a large scale, improved productivity and decreased environmental impacts for additive aquaculture industries are likely, but further research and investment in development is required to assess the economic viability of seaweed production, identify suitable locations, optimise propagation and culture systems, and manage industries developing in close proximity. IMTA infrastructure does not appear to accumulate substantial loads of fluke eggs and so will not impact fluke management if seaweed culture is implemented close to fish cages. Whilst the conceptual basis for this project revolved around IMTA to offset nutrient inputs associated with finfish, the knowledge gained is also applicable to the development of a stand-alone seaweed aquaculture industry, or seaweed culture in association with species such as oysters in a polyculture context (i.e. farming multiple species together, but without the trophic linkages that are integral to IMTA).

RECOMMENDATIONS

The findings of this project are preliminary, but form the first steps toward development of IMTA in Australia. Continued assessment and further trials to optimise seaweed culture, maximise benefits to communities, and decrease the environmental footprint of additive aquaculture industries are required. Future field trials would probably require regular active involvement of an aquaculture operator to be successful, and would greatly benefit from the contribution of farm staff with previous experience at culturing seaweeds elsewhere.

FURTHER DEVELOPMENT

Development of IMTA in Australia has potential to improve the productivity and profitability of existing aquaculture operations while assisting environmental sustainability. Farming native seaweeds in Australia will help reduce dependence on imported seaweed products and could provide niche products from the local unique flora. Development of IMTA using seaweeds on

a large scale, however, requires further investigation of seaweed biology, culture systems and locations. Propagation and nursery techniques need refining and up-scaling to produce adequate biomass for seed stock for large scale seaweed culture. Offshore culture systems need to be optimised to be deployed in high energy environments and in a country with high labour costs. Sites near existing and future finfish aquaculture development need to be identified and assessed to determine their suitability for seaweed culture. Aquaculture planning, management and licensing systems need to be assessed and developed to manage different types of aquaculture operating in close proximity, and should consider the potential for IMTA to operate at the zone as well as farm scale. Economics of seaweed culture will need to be assessed to determine how to develop a sustainable, profitable IMTA sector. This should include further investigation of potential markets for products from farmed seaweeds, and future culture trials should consider product quantity and quality as well as seaweed growth and nutrient removal. Additional species, including those native to other parts of southern Australia, could be considered if they are identified as having potential markets, or for development of seaweed aquaculture outside of existing fish aquaculture areas.

An alternative approach to establishing a seaweed industry in southern Australia may be to work in association with the oyster aquaculture industry. Smaller-scale operations may be more feasible in this circumstance, and could target the high demand for regular but relatively low quantities of fresh product in the restaurant industry.

EXTENSION AND ADOPTION

A workshop was provided to representatives of end-user industries (including nutraceutical and aquaculture feed companies) in 2011, and discussions were held with CleanSeas Aquaculture in 2013 and at the time of each site visit. Three site visits to CleanSeas farms were undertaken in 2014 and two in 2015. The Australian Southern Bluefin Tuna Industry Association were briefed on project progress in 2013, 2014 and 2015. Staff at PIRSA Fisheries and Aquaculture, SA Department of Environment, Water and Natural Resources, and the Environmental Protection Authority of SA have been regularly briefed on the project throughout its duration. Discussions with potential end-users of seaweed products and research collaborators have also regularly taken place throughout the project.

Copies of the final report will be provided to farmers, managers, veterinarians and researchers, including to Seaweeds Australia.

Sections of this work were presented at the following conferences: 21st International Seaweed symposium, Bali, April 2013, 3rd Algal World conference, Adelaide, August 2013, World

Aquaculture Adelaide, June 2014, 5th International Society for Applied Phycology Congress, Sydney, June 2014, Australian Marine Sciences Association South Australian symposium, Adelaide, October 2014, Australian Marine Sciences Association national conference, Geelong, July 2015. The work has also been presented at seminars hosted by the Flinders University Centre for Marine Bioproducts Development in 2012 and 2015.

PROJECT COVERAGE

The project has received the following media coverage:

- Adelaide Advertiser (11/12/2010): Article on p3 of the Saturday edition, including a half-page colour photograph.
- Port Lincoln Times (14/12/2010): Article on p 5, including colour photograph.
- Adelaide Advertiser (8/6/2011); Articles on p 9 and p 34.
- InDaily online news (8/6/2011): Article.
- West Coast Sentinel (16/6/2011): Article on p 15
- ABC radio 891 Adelaide (15/12/2010): Interview
- ABC radio West Coast SA (10/1/2011): Interview
- ABC radio North & West SA (10/1/2011): Interview
- Channel 7 (5/7/2011): Segment filmed for Escape with ET broadcast October 2011
- FRDC's FISH Magazine (March 2011): 2 page article
- ABC radio North & West SA (13/6/13): Interviews with J. Tanner and Minister Gago
- ABC online (13/6/13): Article (<http://www.abc.net.au/news/2013-06-13/seaweed-trials-may-help-fishing-industry-expansion/4751076>)
- MIX FM radio Adelaide (13/6/13): News report
- 891 ABC Adelaide (13/6/13): News report
- Cruise radio Adelaide (13/6/13): News report and interview with Minister Gago
- ABC Rural (13/6/13): Podcast (<http://www.abc.net.au/news/2013-06-13/rural-sa-seaweed-farming-trial-1306/4751240>)
- Farming Ahead Online (13/6/13): Article (<http://www.farmingahead.com.au/News/agricultural/13/06/2013/190711/seaweed-farm-trial-for-sa>)
- ABC radio Country Hour (13/6/13): Interview
- ABC Eyre Peninsula & West Coast (13/6/13): News report
- Port Lincoln Times (4/9/2014): Article on seaweed culture trials in Port Lincoln.

- Australian Rural Communications Network (16/9/2014): Interview with K. Wiltshire.
- Radio Adelaide (17/9/2014): Interview with K. Wiltshire (replayed 14/1/15).
- ABC regional radio (19/9/2014): Interview with K. Wiltshire.
- Channel 7 (22/11/2014): Article on seaweed farming including interviews with K. Wiltshire and a representative from Cleanseas Aquaculture.
- Well-Being magazine (27/1/2015): Interview with K. Wiltshire for use in an article on the future of farming, published 1/7/2015.
- Landline (16/4/15): Contact with Kathryn Wiltshire regarding potential feature on seaweed

APPENDIX I: REFERENCES

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APPENDIX III: INTELLECTUAL PROPERTY

No intellectual property has been generated by this project.

APPENDIX IV: LITERATURE REVIEW FOR SEAWEED AQUACULTURE, FOCUSING ON OFFSHORE FARMING

M. G. K. Loo, J.E. Tanner, and S. Clarke

Introduction

With worldwide demand for seafood increasing rapidly, and global fisheries catch static or declining, aquaculture is a rapidly growing industry. In Australia, like other western countries, aquaculture is generally undertaken on a single-species basis. So finfish are farmed in one location, and shellfish in another. One consequence of this is that wastes from species that are fed are released into the environment, and thus the industry is restricted in terms of its stocking rates so as not to cause undue environmental impacts. In many Asian countries, however, multiple species from different trophic levels are often farmed in close proximity. So in a single small area, you might see finfish (which are fed), shellfish filter feeders (which remove particulate wastes from the finfish, as well as phytoplankton that grow on the nutrients released), macroalgae (which also grow on the waste nutrients from finfish), herbivores (which feed on the algae), and detritivores (which feed on wastes deposited on the seafloor). This approach, termed Integrated Multitrophic Aquaculture (IMTA), results in a much higher proportion of the nutrients fed to the finfish being recovered, which leads to a lower environmental impact, more efficient use of resources (especially space), and potentially better economic returns.

With aquaculture production increasing rapidly in South Australia, as well as several other states including Tasmania, there is an increasing level of concern about the trade-offs between economic returns and environmental sustainability. In Australia, most aquaculture, particularly in-sea aquaculture, is undertaken on a single species basis. For finfish, which are fed a diet based on either baitfish or pelleted feeds; this means that a considerable amount of nutrients are released into the environment. For example, for the two main species farmed in Spencer Gulf in South Australia, southern bluefin tuna and yellowtail kingfish, it has been calculated that for every tonne of production, as much as 500 and 200 kg respectively of nitrogen is released into the environment (Fernandes *et al.* 2007, Fernandes and Tanner 2008). In areas of high production, these wastes have the potential to stimulate plankton blooms and/or smother the benthos. As a consequence, stocking levels are closely regulated to reduce the potential for environmental harm, and there is considerable interest in methods for removing wastes and/or mitigating their impacts.

PIRSA Fisheries and Aquaculture are currently using simple biogeochemical models to help set limits on stocking in each aquaculture zone in South Australia, based on potential impacts

on water quality, in particular levels of dissolved nutrients, with the goal being to limit nutrient inputs to what can be sustained by the environment. At the same time, industry is keen to maximise the use of their infrastructure by increasing stocking densities, and to reduce costs by moving to automatic feeding. These two agendas are potentially in conflict. There are two main pathways by which this conflict can be avoided while attaining both goals. The first is to improve feed conversion ratios, and thus reduce the amount of nutrients being delivered to the environment per unit of production. The second is to remove nutrients from the environment in some way. As filter feeders and macroalgae are nutrient extractors, culturing them in close proximity is one way of removing nutrients, while producing secondary benefits associated with diversification of income streams. Thus, IMTA is gaining increasing attention in the western world, with pilot projects being undertaken in North America, Scotland, Chile, and New Zealand, among others. However, the majority of these projects are undertaken in cold temperate waters, and are focussed on salmonids. While there are important lessons to be learned from this work for warm temperate aquaculture, it is generally not possible to directly transfer the results, as different species would be required in warmer waters. While several groups are looking at IMTA in Australia (e.g. DeNys at James Cook University and Winberg at Wollongong University), to our knowledge it is only being studied in on-shore systems, and not offshore, which will require different culture techniques and possibly species.

A recent FAO review of IMTA in an international context summarises a number of studies that have examined both the economic and social aspects of IMTA, primarily associated with salmon aquaculture in Canada (Soto 2009). The review concludes that public perception of IMTA is substantially higher than that of monospecific aquaculture, although it should be noted that this result is for an area where aquaculture is widely perceived to have caused a number of environmental problems, and where it is thus more contentious than in Australia. A number of studies documented also show that IMTA has the potential to be more profitable, and to reduce risks, compared to monospecific aquaculture (e.g. Ridler *et al.* 2007). Indeed, Whitmarsh *et al.* 2006) showed that the net present value of IMTA was higher (by up to 24%) than that for monospecific salmon aquaculture under the full range of scenarios that they investigated.

To investigate the potential of farming seaweeds in association with current finfish aquaculture in southern Australia as part of an IMTA approach to reducing the ecological impacts of increased finfish production, FRDC have funded SARDI to lead a project titled *Feasibility study of integrated multitrophic aquaculture in southern Australia* (FRDC 2010/201). The first objective of this project is to:

Review available published and unpublished literature and databases, and liaise with international research teams, to assess potentially suitable species and farming techniques for use in IMTA.

This document covers the literature review component of the above objective. The component on liaising with international research teams is documented elsewhere (see Appendix 1). As the project is restricted to an examination of farming seaweeds in association with existing finfish aquaculture operations, this literature review is restricted to the farming of seaweeds, both in IMTA systems and in single species systems. Other components of IMTA systems, including filter feeders, detritivores and finfish, are not reviewed.

When selecting species to use in an IMTA system, the suitability of the species must be carefully considered for the particular habitat or culture systems. As such, to ensure successful growth and economic value, the following considerations have been recommended (Soto 2009):

- Use local species that are well within their normal geographic range and for which technology is available. The risk of invasive species causing harm to the local environment, and harm to other economic activities can thus be avoided.
- Use species that will complement each other on different trophic levels. One species being cultured together with another should be able to feed on the other species' waste such that water quality is improved, and the species can grow efficiently. In choosing the farm site, particulate organic matter and dissolved inorganic nutrients should be considered.
- Select species that are capable of growing to a significant biomass as this is important if the organisms are to act as a biofilter for excess nutrients. Alternatively, species with a very high value may be selected with the trade-off of reduced biomitigation.
- Select species that are established or have a perceived market value as farmers must be able to sell the alternative species to increase economic gains.
- Use species that are approved by regulators and policy makers to facilitate the exploration of new markets without regulatory impediments.

The cultivation/aquaculture of macroalgae (seaweeds) has a long history, spanning several hundred years and is now a multi-billion dollar industry with an estimated total value of US\$7.3 billion from the production of 15.7 million tonnes in 2008 (FAO 2010). Seaweed culture is dominated by countries in east and southeast Asia with China accounting for 62.8% of the world's production by quantity. Other major producers include Indonesia (13.7%), Philippines (10.6%), Republic of Korea (5.9%), Japan (2.9%) and Democratic People's Republic of Korea (2.8%) (FAO 2010). Outside of Asia, Chile is the most important seaweed producing country, harvesting 21,700 tonnes of farmed seaweeds in 2008 while the United Republic of Tanzania,

South Africa and Madagascar together produced 14,700 tonnes of farmed seaweeds in 2008 (FAO 2010).

Australia imports almost all of its requirements for seaweed products, whether fresh, dried or frozen. About 5,000 tonnes of seaweed products worth over \$17 million were imported from Japan, China, Korea and Ireland in 2008/09 (Lee 2010). There are only five major seaweed industries in Australia (Lee 2010). Kelp Industries Pty Ltd³ in Tasmania collects beach cast bull kelp *Durvillea* sp., which are then dried before being sent to Scotland for the production of alginates. South Pacific Seaweeds⁴, a cooperative venture between the Brian Russell Group and Townsville's James Cook University via its Department of Marine and Tropical Biology, produces Green Caviar (*Caulerpa lentillifera*), a tropical aquatic vegetable most popular in Japan (called umidudo) where it is considered a delicacy. In many parts of the world where Green Caviar is grown, it is only available at certain times of the year, but South Pacific Seaweeds has managed to produce the alga all year round. Along coastal areas around Australia, various industry groups also collect *Durvillea* sp. and other kelps such as *Ecklonia radiata* for processing into fertilizers for horticulture and animal feed for livestock. Marinova Pty Ltd⁵ in Tasmania processes *Undaria pinnatifida* (an invasive species now found in Tasmania and Victoria) harvested from Tasmanian waters, along with a variety of imported seaweeds, as a source of fucoidan bioactive compounds. The only commercially cultured alga is *Dunaliella salina* (a microalgae) at Whyalla, South Australia, where some 400 hectares of hypersaline lakes grow the salt tolerant alga from which Cognis⁶ produces natural betacarotene and key dietary carotenoids. Similarly at Hutt Lagoon, 600 km north of Perth near Port Gregory, *Dunaliella salina* are harvested from 400 hectares of salt lakes and natural betacarotene and other carotenoids are recovered.

Uses of Seaweeds

Seaweeds are grown and/or wild harvested for a wide diversity of uses. Until the end of the last decade, at least 221 species of seaweed were used worldwide (Zemke-White and Ohno 1999). Of these, over 145 species were used for food, 41 species for alginates, 33 for agar and 27 for carrageenan. At least 25 species of seaweeds are also used in animal feed and fertiliser. Furthermore, the discovery of metabolites with biological activities from macroalgae

³ <http://www.kelpind.com.au/>

⁴ <http://www.southpacificseaweeds.com.au/>

⁵ <http://www.marinova.com.au/>

⁶ <http://www.cognis.com/countries/Australia/en/Company+Profile/>

has increased in the last three decades with work on biological activities carried out using crude aqueous extracts and fractions of these extracts (Smit 2004).

As Food

The use of seaweed as food can be traced back for centuries in Japan and China. These two countries and the Republic of Korea are today the largest consumers of seaweeds (McHugh 2003). Table gives the list of algae commonly used as food.

Table 1. List of algae commonly used as food

Brown Algae	Red Algae	Green Algae
<i>Laminaria</i> spp. - <i>L. longissima</i> - <i>L. japonica</i> - <i>L. angustata</i> - <i>L. coriacea</i> - <i>L. ochotensis</i>	<i>Porphyra</i>	<i>Enteromorpha</i> (now synonym of <i>Ulva</i>) - <i>Ulva</i>
<i>Undaria pinnatifida</i>	<i>Palmaria palmata</i> (formerly <i>Rhodymenia palmata</i>)	<i>Monostroma</i> spp.
<i>Hizikia fusiformis</i> (now synonym of <i>Sargassum</i> <i>fusiforme</i>)	<i>Gracilaria</i> spp.	<i>Caulerpa</i> spp. - <i>C. lentillifera</i> - <i>C. racemosa</i>
<i>Cladosiphon okamuranus</i>		
<i>Alaria esculenta</i>		

Food from brown algae comes mostly from the genera *Laminaria*, *Undaria* and *Hizikia*. Originally harvested from natural sources, the demand has increased in the last 50 years, and thus methods of cultivation have been developed to mass produce harvestable seaweed to meet demands.

The genus *Laminaria* is eaten in Japan (called kombu) and is derived from a mixture of species that include *L. longissima*, *L. japonica*, *L. angustata*, *L. coriacea* and *L. ochotensis*. They are native to Japan and are mostly harvested from natural sources. In Korea, *Laminaria* grows naturally and although local consumption is not high, they are cultivated for export to China. In China, *Laminaria japonica*, called haidai, is consumed in large quantities as food. It is not native to China, but was accidentally introduced from Japan through shipping. All requirements for *Laminaria japonica* were initially imported from Japan and Korea, but it is now cultivated on a large scale in China to meet increased demands (Tseng 2001).

The brown macroalgae *Undaria pinnatifida* is native to Japan, China and Korea, occurring on rocky shores and bays. The species has now spread to France, Mediterranean, Atlantic coast of Spain, England, North America, Mexico, New Zealand and Australia (Pérez *et al.* 1981,

Boudouresque *et al.* 1985, Hay and Luckens 1987, Sanderson 1990, Castric-Fey *et al.* 1993, Fletcher and Manfredi 1995, Salinas *et al.* 1996, Campbell and Burridge 1998, Silva *et al.* 2002). The consumption of *Undaria pinnatifida* (called wakame in Japan and Korea and quandai-cai in China) is highest in Korea. The cultivation of this brown macroalga started in the 1960s in Japan and Korea, followed by China in the mid-1980s (Tseng 2001). As the consumption of this alga increased in Europe, cultivation trials have been carried out off the French Brittany coast and the Spanish Galician coast (Peteiro and Freire 2011). Currently, commercial cultivation is being developed in Northwest Spain (Peteiro and Freire 2011).

Hizikia fusiformis (now a taxonomic synonym of *Sargassum fusiforme* (Harvey) Setchell) is another brown macroalga that is popularly consumed in Japan (called hiziki) and Korea (called nongmichae). Harvest of this seaweed is from natural beds, which are found at the bottom of the eulittoral and top of the sublittoral zones (i.e. lower intertidal and upper subtidal). Wild harvest in Korea continued until 1984, when cultivation commenced to meet demand, and a large proportion of the production is now exported to Japan.

Cladosiphon okamuranus is a brown macroalga that is consumed as a fresh vegetable in Japan (called mozuku). This macroalga grows in the sublittoral at depths of 1-3 m, preferring reef flats in calm water with some moderate water movement to supply sufficient nutrients. Due to increasing demands, cultivation of this macroalga was started in Japan in the 1980s. Another large brown macroalga eaten either fresh or cooked in Ireland, Scotland and Iceland is the winged kelp, *Alaria esculenta*. It grows in the upper limit of the sublittoral zone in temperatures below 16°C and is therefore widely distributed in Ireland, Scotland, Iceland, Brittany (France), Norway, Nova Scotia (Canada), Sakhalin (Russia) and northern Hokkaido (Japan). Local people usually collect the alga from the wild and the cultivation of this kelp is successful but is not carried out on a commercial scale.

Porphyra species are the largest source of food from red seaweeds, growing in most temperate intertidal zones around the world. It is known commonly as nori or purple laver and used after it is dried and processed into sheets. The cultivation of *Porphyra* was started in Japan and Korea centuries ago as demand for the seaweed was higher than the natural stocks even then (Oohusa 1993). Today, *Porphyra* is also cultivated in China, and has the highest value of any cultivated seaweed.

Another red macroalga that is used as food is dulse or *Palmaria palmata* (formerly *Rhododymenia palmata*). Collected by coastal people from the wild, they are consumed locally after drying as whole pieces or as a powder used as a condiment. In Canada and Iceland, dried dulse is served as a salty snack while in Ireland, it is often eaten raw or cooked with potatoes, in soups and fish dishes (Guiry and Guiry 2011, McHugh 2003). In Ireland and

Spain, cultivation of this alga is on ropes (Martínez *et al.* 2006, Pang and Lüning 2004) while in Germany, the alga is grown in tanks⁷ (Pang and Lüning 2006).

Gracilaria species are red macroalgae used mainly as sources of agar (discussed below) but fresh *Gracilaria* are also collected and used as a salad vegetable (McHugh 2003). *Gracilaria* is widely distributed, ranging from tropical countries such as Indonesia to colder waters in southern Chile and the Atlantic coast of Canada. In Hawaii, the mix of ethnic groups (Hawaiians, Filipinos, Koreans, Japanese and Chinese) has created an increasing demand for *Gracilaria* as food over the last decades (McHugh 2003). Consequently, several species (*G. coronopifolia*, *G. parvisipora*, *G. salicornia* and *G. tikvahiae*) are grown commercially in aerated tank systems or harvested from the oceans for food (Paull and Chen 2008). In Indonesia, Malaysia, the Philippines and Vietnam, various species of *Gracilaria* are collected by coastal people for food (McHugh 2003). The major species of *Gracilaria* that is commonly cultivated and consumed in Taiwan is *Gracilaria tenuistipitata* (Hsu *et al.* 2008). In the West Indies, *Gracilaria* is marketed as sea moss, used as a base for a non-alcoholic drink and is cultivated successfully in St Lucia and adjacent islands (Smith *et al.* 1984). *Gracilaria*, known as jiangli in China, is cultivated as food but more importantly it is used as a source of agar (Tseng 2001). Cultivation of this genus is also widespread in Chile.

Green seaweed used as food includes the genera *Monostroma* and *Enteromorpha* (now *Ulva*), which are commonly called aonori or green laver. *Monostroma* spp. occurs naturally in the upper eulittoral zone of bays and gulfs in Japan. It is now cultivated in a similar way to *Porphyra*. Around Japan, *Enteromorpha* spp. (*E. prolifera* and *E. intestinalis*) are found in bays and river mouths, thriving in both salt and brackish waters. These macroalgae can also be found in other countries including North America and Europe. Both genera are cultivated in Japan and Korea. Another green alga used popularly in salads is two species of *Caulerpa*. *C. lentillifera* (commonly called sea grapes or green caviar) and *C. racemosa* are successfully cultivated in ponds in the Philippines (Trono 1999, Horstmann 1983).

As a source of alginate

The cell walls of brown macroalgae contain a range of different polysaccharides including alginic acids (alginates). The chemical structure of the alginate varies between the various genera of brown macroalgae, and therefore the extracted alginates can have different properties. The main applications of alginate are in thickening aqueous solutions and forming gels. Therefore, alginates are used as emulsifiers, thickeners, binding and gel forming agents in food, cosmetic, textile, construction and pharmaceutical/biomedical industries. Species of

⁷ <http://www.algenfarm.de/>

brown macroalgae with alginate include *Ascophyllum*, *Durvillaea*, *Ecklonia*, *Laminaria*, *Lessonia*, *Macrocystis* and *Sargassum*.

Ascophyllum grows as distinct bands of dark brown, branched plants of 1-4 m long in sheltered areas of the eulittoral zone (Guiry and Guiry 2011). *Durvillaea* is only found in the Southern Hemisphere. It grows to lengths of 2-3 m on rocky shores or offshore reefs and thrives best in rough waters of the sublittoral zone (Guiry and Guiry 2011). *Ecklonia* are found on rocky substrates of the upper sublittoral zone. *Laminaria* plants grow in depths of 2-15 m and are found on rocks and reefs in the sublittoral zone, in calm waters with temperatures between 3 and 20°C (Guiry and Guiry 2011). The two species of *Lessonia* used for alginate extraction are mainly collected in Chile (McHugh 2003). *Lessonia nigrescens* grows in the rocky lower eulittoral zone in fairly rough waters while *Lessonia trabeculata* grows in the sublittoral zone from 1-20 m depths (Guiry and Guiry 2011). *Macrocystis pyrifera* are found in temperatures not more than 15°C in calm, deep waters, growing to massive lengths of 20 m on rocky bottoms (Guiry and Guiry 2011). *Sargassum* species are found worldwide, growing in eulittoral and upper sublittoral zones in a wide variety of shape and form (Guiry and Guiry 2011). The alginate content of *Sargassum* species is low compared to the other species, and it is therefore rarely used for this purpose.

As a source of carrageenan

The main uses of carrageenan extracted from red algae are in the food industry, in particular in dairy products. There are several types of carrageenans (iota, kappa and lambda), depending on their chemical structure and properties. The various types are related to the formation of thick solutions or gels. Iota is a clear, elastic gel formed with calcium salts, with no bleeding of liquids, and remains stable through freezing and thawing. Kappa is a strong, rigid gel formed with potassium salts but brittle with calcium salts. It is opaque, becoming clear with sugar and there is some bleeding of liquid. Lambda forms high viscosity solutions and does not become a gel (McHugh 2003). Carrageenan is now mostly extracted from *Kappaphycus alvarezii* and *Eucheuma denticulatum* but was originally extracted from *Chondrus crispus*. Other sources of carrageenan include *Betaphycus gelatinum*, *Gigartina skottsbergii*, *Sarcothalia crispata* and *Mazzaella laminaroides* and *Hypnea musciformis*. Each of these red algae has a unique carrageenan composition.

Kappaphycus alvarezii is found on sandy/coral to rocky substrate of reef areas in the upper part of the sublittoral zone, where water flow is slow to moderate and water temperatures are 21°C or higher in bright light. *Chondrus crispus* can be found from the fringe of the littoral zone to a depth of 20 m, growing on stable rock ledges and large boulders. *Gigartina skottsbergii* grows to a depth of 10 m from the eulittoral to the sublittoral zones. *Mazzaella laminaroides*

also grows in the eulittoral zone on wave-exposed sites, but can also be found in estuaries (Guiry and Guiry 2011).

As a source of agar

Agar is commercially produced from the red algae *Gelidium* and *Gracilaria*. *Gelidium* grows on rocky areas in the eulittoral and sublittoral zones where there is fast water movement, to depths of 2-20 m with temperatures of 15-20°C. *Gracilaria* grows on sandy or muddy substrates that are protected from waves in the eulittoral zone or at the start of the sublittoral zone. They can also be found free-floating in tidal lakes with salt or brackish waters (Guiry and Guiry 2011).

For bioremediation

In wastewater treatment, algae are used to reduce nitrogen and phosphorus compounds before release to prevent eutrophication of receiving waters (e.g. Chung *et al.* 2002). Algae are also used for the removal of toxic metals from industrial wastewater (e.g. Suzuki *et al.* 2005). As seaweeds take up ammonium as the form of nitrogen for their growth, and ammonium is the form of nitrogen in domestic and agricultural effluents, they are suited for reducing nutrients in wastewater.

Algae used for bioremediation include *Ulva* (e.g. Liu *et al.* 2010), *Gracilaria* (e.g. Naldi and Viaroli 2002, Suzuki *et al.* 2005, Hernández *et al.* 2006, Huo *et al.* 2011), *Porphyra* (e.g. Pereira *et al.* 2006) and *Laminaria* (e.g. Feng *et al.* 2004, Neori *et al.* 2004).

As livestock and aquaculture feed

Livestock farmed along coastal areas have traditionally eaten seaweeds that have been washed ashore. However, seaweed is now used in animal feed as seaweed meal (e.g. Neori 2008). Species in seaweed meal include *Ascophyllum nodosum* (Norway and Iceland) and *Laminaria digitata* (France, Iceland and United Kingdom).

Wet feed used in fish farming consists of meat waste and fish waste mixed with dry additives containing extra nutrients. A binder which is usually a technical grade of alginate is used to ensure the feed does not disintegrate in the water and an even cheaper option is the use of finely ground seaweed meal from brown seaweeds. The move to dry feed meant that this market is not expected to expand (McHugh 2003). However, there is increasing research on the direct use of fresh seaweed as food for aquaculture species, in particular for abalone. For example, in Chile, cultivation techniques were developed to produce significant quantities of *Lessonia trabeculata* in long-line culture as food for tank-cultured *Haliotis rufescens* and *Haliotis discus-hannae* Ino (Edding and Tala 2003). In Korea, the mass cultivation of *Ecklonia stolonifera* was studied to assess the feasibility of using this alga as a summer feed for the

abalone (*Haliotis discus-hannae*) industry between August and November when *Undaria* and *Laminaria* (the preferred locally cultured brown seaweeds by Korean farmers for feeding the abalone) are not available (Hwang *et al.* 2009).

A comprehensive review on the use and production of algae and manufactured diets as feed for sea-based abalone aquaculture in Victoria was carried out in 2010 (Kirkendale *et al.* 2010). The review included what is known from existing diets and feeding trials of abalone in cultivation worldwide, what seaweed species might be suitable for the culture of species or hybrids of the two most commonly farmed abalone in Victoria (blacklip abalone, *Haliotis rubra* and greenlip abalone, *Haliotis laevigata*), the technology options for cultivation of suitable seaweed species and cost-benefit considerations from existing abalone cultivation enterprises.

The benefits of seaweed inclusion in abalone diets were unclear, as results from different studies were not directly comparable and appeared contradictory due to the complexity of feeding trials. Different species of seaweeds were used in different trials, with varying environmental and animal husbandry factors affecting the growth and health of cultivated abalone. However, comparable feed trials indicated that seaweed diets (mixed seaweeds, feed fortified with seaweed or protein-enhanced seaweeds) yielded better growth rates when compared to artificial diets (see references in Kirkendale *et al.* 2010).

A list of endemic and non-endemic algal species consumed by both the blacklip and greenlip abalone was given (Kirkendale *et al.* 2010). Australian algal species that were good or very good food sources for the abalone and responded well to cultivation included the brown alga *Scytothalia dorycarpa*, red algae *Lomentaria* sp., *Mychodea hamata*, *Plocamium augustum* and *Plocamium preissianum*. The review also gave a list of algal species that were potentially suitable for cultivation in Australia and usable as feed for abalone aquaculture. The list included two brown algae (*Ecklonia radiata* and *Macrocystis angustifolia*), three red algae (*Gracilaria* sp., *Asparagopsis armata* and Gelidiaceae) and one green alga (*Ulva* sp.).

In nutraceuticals, medicinal and pharmaceutical

Algae have been included in cosmetics formulations for many years in emollients, skin conditioners and viscosity-controlling ingredients, primarily in the form of alginate or carrageenan. The use of seaweeds themselves is more limited, although products such as milled seaweed have been used as additives to bath water and for algotherapy (therapeutic use of seaweed in spa treatments), and are gaining popularity (references in McHugh 2003). A review by Smit 2004) on medicinal and pharmaceutical uses of seaweed natural products showed that substances from algae receiving most attention are sulphated polysaccharides for use as antiviral substances, halogenated furanones for antifouling compounds, and

kahalalide F as a possible treatment for lung cancer, tumours and AIDS. Fucoidans are known for their ability to inhibit tumours and for lowering serum cholesterol through their ability to block absorption of acids, and have anti-inflammatory and anti-thrombotic properties (Smit 2004). Lectins, kainoids and aplysiatoxins are other macroalgal substances being investigated for potential biological activities (Smit 2004).

Cultivation of Seaweed

Commercial cultivation of seaweed has a long history, especially in Asia. Chile is the most important country outside Asia, in culturing seaweed. The Japanese kelp, *Laminaria japonica* is the most important seaweed, being cultivated mainly in China (Lüning and Pang 2003). In 2008, the world production of cultured seaweed was 4.8 million tonnes of *Laminaria japonica* (Japanese kelp), 3.8 million tonnes of *Eucheuma* seaweeds (*Kappaphycus alvarezii* and *Eucheuma* spp.), 1.8 million tonnes of *Undaria pinnatifida* (wakame), 1.4 million tonnes of *Gracilaria* spp. and 1.4 million tonnes of *Porphyra* spp. (nori) (FAO 2010).

Some seaweed can be cultivated vegetatively while others have to go through a reproductive cycle that involves alternation of generations. In vegetative cultivation, small pieces of the seaweed are grown in a suitable environment until they are ready for harvest. When whole plants are completely removed, pieces are cut from the plants and re-established for further cultivation. Another method is to cut most of the plant leaving a small piece to grow again. Cultivation techniques vary with the morphology of the algae and environmental factors (Santelices 1999). Farming methods range from laying algae on nets in surface waters, bottom or mid water planting of algae, to suspending algae on rafts in deep water (McHugh 2003).

Many species cannot be reproduced vegetatively, and need to be produced through sexual reproduction. This is the case with most brown macroalgae, and therefore a good understanding of the life cycle of these species is needed before they can be cultivated successfully. The reproductive cycle of many brown algae has an alternation between two stages; the large sporophyte stage and the microscopic gametophyte stage. Mature sporophytes are collected and induced to release spores in the hatchery, where they settle and grow into the gametophytes. The gametophytes, when fertile, release sperm and eggs that join to form an embryonic sporophyte, which settles out onto the substrate provided. Once established, the young sporophytes are often then moved into open water for grow out and eventual harvest once they reach a suitable size. The difficulties with this cultivation method often revolve around the transition stages from spore to gametophyte to embryonic sporophyte. Much work has been carried out to manage these stages, mostly growing them in land-based facilities with controlled water temperature, light and nutrients. A recent review on seaweed cultivation described the approaches and cultivation methods used globally and

discussed the problems resulting from the application of these methods (Titlyanov and Titlyanova 2010). The following in this report will review some work that has been carried out on cultivation of macroalgal species that have been shortlisted for this project where available and also related species of the same genera.

***Ecklonia* spp.**

Limited work has been carried out on the culture of this species, and other species of *Ecklonia* are also not commonly farmed (Neill *et al.* 2009). In New Zealand, experimental culture of *Ecklonia radiata* was carried out in 2006-2008 (Neill *et al.* 2009). The study investigated best-practice methods, laboratory culture and out-planting, and field culture. Water current speeds and key water nutrients were also measured during the field culture. Results indicated that the culturing of *E. radiata* is achievable in the laboratory, from release of spores, to the gametophyte stage, then to the sporophyte stage. The grow-out trials produced mixed results, with success contingent upon selecting sites with good water flow, and matching the timing to natural recruitment from late autumn to spring. Nutrient loading also needed to be considered to prevent potential growth from being nutrient limited. At the best site, *E. radiata* reached a maximum length of 0.41 m with average biomass of 2.3 kg m⁻¹ of rope in seven months, which was considered to be comparable to wild *Ecklonia* biomass (10 kg m⁻²) in Western Australia.

E. maxima, although not present in Australia, is an important food source for the abalone *Haliotis midae* and is wild harvested widely in South Africa. As the harvest is reaching maximum sustainable levels, non-lethal harvesting methods are being tested commercially, involving cutting the distal parts of the kelp and leaving the basal meristems intact (Troell *et al.* 2006). The success of this technique suggests that cultured *E. maxima* plants may be able to produce several harvests before new sexually produced sporophytes are required.

E. stolonifera, also not present in Australia, was mass cultivated as a trial to form a stable supply of summer feed for the abalone industry in Korea (Hwang *et al.* 2009). This trial was undertaken as cultured *Laminaria japonica* and *Undaria pinnatifida*, the preferred feeds used by local farmers of abalone, are unavailable during the summer months. As cultivation of *Laminaria* and *Undaria* had been successful in Korea, the artificial seeding and cultivation techniques for *E. stolonifera* were based on those used for these algae. Zoospores were collected from mature thalli and the seedlings that developed from these spores were reared in indoor tanks. Grow-out used a horizontal cultivation system of ropes. The results of this trial showed that the growth cycle of *E. stolonifera* is seasonal and dependent on the age of the plant, with growth rates of the thalli averaging 4.8 mm day⁻¹ in the first year and 5.3 mm day⁻¹ in the second year. Production was also higher in the second year (24,800 kg fresh wt. ha⁻¹) compared to 6,400 kg fresh wt. ha⁻¹ in the first year. This higher productivity was attributed to

the perennial nature of the algae, with regeneration of thalli developing from the holdfasts of first year algae. Other results from the trial showed that the greatest growth rate was achieved at a depth of 2 m during nursery stage cultivation (thalli < 1.0 cm) and a depth of 1.5 m for adult stage cultivation (thalli > 50cm). The optimum irradiance for cultivation was therefore 671 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for the nursery stage (71% of surface irradiance) and 825 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for the adult stage (79% of surface irradiance).

***Sargassum* spp.**

Species of *Sargassum*, including *S. horneri*, *S. thunbergii*, *S. fulvellum*, *S. ilicifolium* and *Hizikia* (formerly *Sargassum*) *fusiformis*, which have been investigated or cultivated, are not found in South Australia. However, much of the work carried out on these species can form the basis for developing cultivation techniques for local species.

The complex life cycle of *Sargassum* has made the commercial cultivation of species in this genus difficult, in particular for economically important species such as *Hizikia fusiformis* (Pang *et al.* 2005). However, Pang *et al.* 2008) trialled a solution by controlling the synchronisation of receptacle development, to produce simultaneous discharge of male and female gametes. The outcome was a greatly improved fertilisation rate of *H. fusiformis*. A total of 550 million embryos were obtained from 100 kg of female sporophytes, the seedlings grew to 3.5 mm in greenhouse tanks in one month, and were further grown in the open sea for over three months. The mass production of seedlings of this species suggested that commercial cultivation is viable (Pang *et al.* 2008).

Sargassum fulvellum is commonly used in seaweed salads or soups in Korea (Hwang *et al.* 2006), and has also been extracted for compounds of biomedical importance (e.g. Kang *et al.* 2008). In Korea, the demand for this alga is high, and the resultant likelihood of an unsustainable wild harvest has led to the necessity to develop mass cultivation techniques. Artificial seeding and cultivation of *S. fulvellum*, growth and maturation were investigated in 2002/03 (Hwang *et al.* 2006). Culture experiments for maturation induction were conducted indoors at various temperatures and irradiances with 16 hours light and 8 hours dark photoperiod. The results showed that higher temperature and irradiance levels favoured the maturation of receptacles in *S. fulvellum*. Artificial seed production could be induced a month earlier than in nature by controlling the temperature and irradiance. To artificially seed a 100 m length of string, 200 g wet wt. of mature thalli was required. The immature thallus grew between 3 and 6 mm in one month. Germling survival in the indoor culture system was high, with a density of 13 to 20 individuals per cm on the seed string after one month of culture. The mean production obtained from this artificial seeding technique *in situ* was 3.0 kg wet wt. m^{-1} of culture rope during the 6 month cultivation period (Hwang *et al.* 2006), and in 2005 with

commercial cultivation, production levels reached 704 t (Hwang *et al.* 2007). Further work in 2004/05 determined optimal depth and photon irradiance for growth (Hwang *et al.* 2007). During the nursery cultivation stage, growth in length was greatest at a depth of 1.5 m with a midday irradiance of 466 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. At the next stage before the main cultivation, greatest length increase occurred at 1 m depth with average irradiance of 845 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. During the main cultivation stage, the thalli attained maximum growth in length during March and early April at depths of 1 - 2 m and 3 m. These results suggest that the cultivation of *S. fulvellum* can be controlled by adjusting the depth of cultivation ropes (Hwang *et al.* 2007).

Sargassum thunbergii is of great economic importance, being widely used in alginate production, extraction of natural bioactive products, biosorption of heavy metal ions and as a source of feed for holothurian aquaculture in China (see references in Zhao *et al.* 2008). The increasing demand for this alga in China has resulted in the depletion of natural populations. Therefore the need arose to develop techniques for breeding and cultivation of *S. thunbergii* (Zhao *et al.* 2008). Culture studies were carried out under controlled laboratory conditions using a range of temperatures (10 to 25°C) and irradiances (9 to 88 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The use of blue and white light was also trialled. Germlings had a broad tolerance to temperature and irradiance conditions. Optimal growth occurred at 25°C and 44 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Growth was inhibited by blue light when compared to white light, but this may be a species specific response and highlights the importance of light quality. This study suggested the potential for using zygote derived germlings as a source of seedlings for the cultivation of *S. thunbergii* in the field (Zhao *et al.* 2008). Another study focusing on optimizing the indoor culture conditions for growth and synchronous reproduction of *S. thunbergii* used young seedlings at different temperatures, light intensities and nutrient ratios (Wang *et al.* 2011). The results indicated different combinations of factors affected length and mass growth rates. The length growth rate was affected by temperature, nutrient ratio and then illumination intensity in order of importance, while mass growth rate was affected by temperature, illumination intensity and then nutrient ratio. The emergence of receptacles, and zygote formation and release, were also regulated by temperature, with higher temperatures slowing the growth rate of receptacles and delaying the time of egg fertilisation. Nutrients affected the formation of branchlets, with a higher proportion of KH_2PO_4 resulting in later emergence. Increased illumination intensity reduced pigment content. Using a combination of culture conditions, the indoor culture of *S. thunbergii* could be carried out 3 to 4 months earlier than the maturing of thalli naturally. The early development of receptacles and synchronisation of fertilisation could therefore be used for large scale cultivation of *S. thunbergia* (Wang *et al.* 2011). The vegetative propagation of *S. thunbergia* has also been studied in China to meet demands of

this species (Li *et al.* 2010). Cauline leaves were used under laboratory conditions and there was survival of 45.75% of the excised leaves with several new individuals produced by each leaf. After three months of culture, adventitious burgeons grew into branches of 2 mm in length. These new individuals were then cut off and used as seedlings for raft culture or seabed restoration.

Recent disappearance of *S. horneri* along the coast of the East China Sea due to deterioration of the coastal environment from rapid economic development and increased industrial pollution, has led to the need to develop artificial cultivation techniques to rebuild the population (Pang *et al.* 2009). In 2007/08, a series of culture experiments were carried out in indoor raceways and rectangular tanks under reduced solar irradiance at ambient temperature to produce seedlings. With higher temperature and light climates, sexual reproduction of *S. horneri* could be induced up to 3 months earlier than in the wild. The eggs produced had a 48-hour fertilization potential, giving the species a greater number of surviving zygotes, and the life cycle could be shortened to 4.5 months. These results also suggested that both suspension and fixed culture methods were effective for the growing of seedlings to the stage of cultivation on long lines. The effects of temperature, irradiance and daylength on the growth of *S. horneri* were investigated in Korea (Choi *et al.* 2008). The results showed that the germlings and adults of this alga grew over a wide range of temperatures, irradiances and daylengths. The germlings grew at temperatures ranging from 10 to 25°C and irradiances from 20 to 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Growth of germlings was inhibited between 25 and 30°C, with most dying at 30°C, regardless of irradiance levels. The germlings also grew well in a wide range of daylengths (from 8 to 24 hours) reaching 1.02 to 1.28 mm after 12 days. The relative growth rate of *S. horneri* was maximum at 21% day^{-1} with optimal conditions at a temperature of 25°C, irradiance of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and a daylength of 8 hours. For adults, the relative growth rate for blade length was inhibited at high irradiance levels (80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high water temperatures (25°C) with optimal growth at 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Relative growth rate for blade weight was greater than blade length, indicating that growth at this life stage is in the blade width rather than length. The optimal temperature and irradiance for both blade length and width was 15°C and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a daylength of 12 hours. This study showed that *S. horneri* germlings grew faster than adults, implying that germlings will be better for transplantation to the field. However, germlings are more vulnerable to invertebrate grazers (Choi *et al.* 2003). Transplantation of live adults might be a better option because of their resistance to grazing, ability to provide immediate habitat to marine animals, and ability to act as a seed bank for producing embryos, but is difficult because of cost and labour intensiveness (Choi *et al.* 2008).

***Gracilaria* spp.**

The methods employed at the commercial level for *Gracilaria* cultivation are based on vegetative propagation of the seaweed. Several methods have been developed of which the direct method and the plastic tube method are most commonly used. In the direct method, the thalli are directly buried into the sandy bottom with the use of different tools while the plastic tube method involves the fastening of bundles of thalli to sand-filled plastic tubes, which anchor the algae to the sea bottom.

Many studies on *Gracilaria* include investigating the potential of the algae for bioremediation. One such study investigated the productivity of *G. chilensis* near salmon farms in Chile and assessed its nitrogen removal and photosynthetic performance (Abreu *et al.* 2009). Floating long-lines and bottom cultivation methods were also evaluated. The study was conducted in 2007 when three long-line cultivation units were set at different distances from a salmon farm, including one away from any influence from salmon farming. A similar cultivation unit was also set as a traditional bottom culture. The results indicated that *Gracilaria* growth performance was higher on the suspended cultures near the salmon cages with daily mean growth rates increasing over 4% and a mean biomass production over 1680 g m⁻² month⁻¹. The productivity of bottom cultured *Gracilaria* was highly variable (400 to 2000 g m⁻² month⁻¹) with biomass losses due to the unstable sandy bottom. The study further showed that these differences in productivity could be attributed to N removal and photosynthetic performance. The long-line cultivation unit was most efficient in nutrient removal with monthly removal of up to 9.3 g N m⁻¹ of long-line at a distance of 800 m rather than 100 m from the salmon farm, probably due to higher epiphytic growth on the algae at 100 m. The study suggested that a 100 ha *G. chilensis* long-line system will effectively (ca. 100%) reduce the N inputs of a 1500 tonne salmon farm.

Another study in Chile investigated the production and performance of two suspended *Gracilaria* cultivation methods in open water, spore inoculated ropes and ropes with twined field collected seaweed (Halling *et al.* 2005). *Gracilaria chilensis* was used and the results showed that production from both methods was comparable for the first month of culture, but productivity thereafter was higher on the twined ropes. The production on the twined ropes reached 1.34 kg m⁻² and was significantly higher than bottom cultures. Fish farm wastes did not have any significant fertilizing effect on the alga's growth rate. Comparisons of spore-originated thalli and field collected thalli were also carried out under both laboratory conditions and in suspended culture using the same cultivation techniques. The spore-originated thalli grew 50% slower than the field collected thalli under laboratory conditions but there were no differences in the field. However, thalli from spore cultivation techniques appear to have a higher level of polymorphism compared to vegetative thalli, probably a result of different

genotypes. These results indicate the potential for the generation of a wide variety of characteristics which will allow greater adaptability of the alga to environmental variations.

In Brazil, experimental open water vertical cultivation of *Gracilaria domingensis* was carried out (Salles *et al.* 2010). Cutting attachment methods on the cultivation rope, cutting density, cultivation period, and cystocarpic versus infertile thallus performance were evaluated. The feasibility of cultivation with and without cages was evaluated before further experiments. The cultivation of the alga within cages in order to exclude macrograzers showed good yields with potential relative growth rates (excluding negative relative growth rates) ranging from 1.5 to 4.6 % \cdot day⁻¹. Subsequent experiments were carried out using cages. The comparison of attachment methods indicated that tying the cuttings (thalli) with soft nylon tape to the rope yielded better relative growth rates than inserting the thalli between the rope strands. The evaluation of cutting density was not significant with potential relative growth rates ranging from 2.1% to 2.7%.day⁻¹. Comparison of cultivation periods found that the two-week period had higher loss of cuttings but results were not significantly different from the one-week period. This study did not find differences between the cystocarpic and infertile thalli although another study (referenced in Salles *et al.* 2010) did observe higher relative growth rate in infertile thalli.

A study in China evaluated the bioremediation potential of *Gracilaria lamaneiformis* integrated with fed fish culture in coastal waters (Zhou *et al.* 2006). The growth and nutrient removal from fish culture water were investigated in laboratory conditions and the feasibility of integrated seaweed cultivation and fish-cage aquaculture were also investigated in the field. The laboratory experiments indicated that *G. lamaneiformis* is efficient in removing nutrients from the system with more than 90% of N removed. The field cultivation trials showed that the alga attained maximum growth rate of 11.03%.day⁻¹ and uptake rates of N and P for the thalli were estimated at 10.64 and 0.38 μ mol.g⁻¹ dry weight.h⁻¹ respectively. These results indicated that potentially a 1-ha cultivation of *G. lamaneiformis* in fish farming waters would give an annual harvest of >70 t of fresh seaweed (9 t of dry material), a production of 2.5 t C. At the same time, the seaweed would be sequestering 0.22 t N and 0.03 t P from the seawater.

***Gelidium* spp. and *Pterocladia* spp.**

Gelidium spp. are important as sources of agar and closely related to this alga is *Pterocladia* spp. from the same order Gelidiales. Currently *Gelidium* and *Pterocladia* are collected or harvested only from the sea and despite attempts to develop cultivation technologies, no successful techniques have yet been developed. Two cultivation techniques have been tested; one involving the attachment of *Gelidium* fragments to concrete cylinders floating in the sea and the other involves free-floating pond cultivation technique. A review was carried out by Friedlander (2008) to determine why there is no commercial cultivation of Gelidiales species.

The review indicated that both cultivation techniques can be optimised by controlling physical, chemical and biological growth factors. As such, the pond cultivation technique is a more controllable option. However, due to the low growth rate of the algae, cultivation of *Gelidium* or *Pterocladia* is not considered economically viable at this stage of development (McHugh 2003, Friedlander 2008). There is need of genetic improvement through selection or genetic engineering to obtain high yield strains (Friedlander 2008).

***Hypnea* spp.**

Hypnea spp. have been irregularly harvested in many countries (e.g. Senegal, Vietnam, USA, Philippines, India, Brazil, etc) as a source of carrageenan, in particular the tropical/subtropical species *H. musciformis* (Ganesan *et al.* 2006). *H. musciformis* has kappa carrageenan, which is an important resource in the phycocolloidal industry, and experiments on the cultivation of this alga has been trialled in several countries. In India, *H. musciformis* is the only indigenous source of carrageenan. This species is tolerant to a wide range of water temperatures, salinities and light intensities (Dawes *et al.* 1976). A study to optimise culture conditions for commercial cultivation of *H. musciformis* showed that the biomass yield was lowest in January (8 g fresh weight m⁻¹) and highest in August (403 g fresh weight m⁻¹), and increased with increasing seedling density. Maximum growth rate (7% day⁻¹) and biomass yield (130 g fresh weight m⁻¹) were obtained at the surface and the lowest were observed at 120cm. Higher biomass yield was obtained in 75 and 150 days after planting and was always higher on coir rope (biomass 50 g fresh weight m⁻¹ and growth rate 3.8% day⁻¹) when compared to polypropylene rope. These results suggested that commercial cultivation is feasible throughout the year along the southeast coast of India and could be implemented by local fishers.

***Cystophora* spp. and *Scytothalia* spp.**

No published works on experimental or commercial cultivation of any species of the brown alga *Cystophora* were found. Substances such as phlorotannins, phlorethols, fucophlorethols, terpenoids have been extracted from species of this alga and many are being investigated for potential bioactivity (e.g. Glombitza *et al.* 1997, Hauperich and Glombitza 1993, Reddy and Urban 2008). No published work on cultivation of *Scytothalia* could be found.

Species for potential cultivation in open waters in South Australia

In choosing candidate species for trialling seaweed aquaculture in southern Australia, there are a number of important considerations:

- The species should be native to the area where it is to be cultivated (in the case of this project, Spencer Gulf)

- Ideally the species would already be in cultivation, thus avoiding the need to develop culture systems and learn about its biology. However, no species of macroalgae native to South Australia are currently commercially cultivated. The next best option is to choose species that are closely related to those in cultivation elsewhere, so that knowledge for those species can be transferred and adapted to the local species.
- There should be an existing market for the species, or it should be easily substitutable for a species with an existing market. This will avoid the need to create a market.
- For bioremediation, the species should be fast growing and allow a high biomass harvest.
- The species must be adapted to growing in the environment in which it is to be farmed. In this case, seaweeds will be farmed in association with finfish farms which are located offshore primarily in southern Spencer Gulf. Thus the seaweed species chosen must be capable of withstanding the high levels of water movement typical of these areas.

Below we discuss a range of genera and species that may be suitable for aquaculture, targeting those that could be used for IMTA in the relatively open waters of Spencer Gulf. These taxa were selected on the basis of several sources of information:

- 1: The outcomes of this literature review.
- 2: Discussion with potential end-users of algal biomass (e.g. Marinova are particularly interested in *Ecklonia radiata* and the fucales species for extraction of fucoidans, AIL are particularly interested in some of the Rhodophyta for abalone food).
- 3: Discussion with research teams working on algal aquaculture in New Zealand and China.
- 4: Systematic evaluation of the entire list of algal species known to occur in South Australian waters. To this end, the list of 1168 taxa identified by HBS Womersley (http://www.flora.sa.gov.au/algae_flora/The_Marine_Benthic_Flora_of_SA_static_index.shtml) was downloaded, and each species assessed on the characteristics detailed in the above table, as well as its distribution within SA. Species were retained for further consideration if they routinely grow to > 20 cm, occur in the vicinity of southern Spencer Gulf, were not listed as rare or uncommon, were not listed as only coming from calm conditions or did not have a morphology that suggested they would only flourish in calm conditions, and that were listed as occurring in depths < 10 m. The resulting list of 89 species was then examined by members of the project team, and further reduced based on their knowledge of these characteristics. When multiple species from a single genus were still retained, an attempt was then made to choose the two that were considered the most likely candidates for further examination.

Brown Algae (Phylum: Ochrophyta, Class: Phaeophyceae)

***Ecklonia* Hornemann**

Order: Laminariales

Family: Alariaceae

There are currently nine species of *Ecklonia* accepted taxonomically (Guiry and Guiry 2011). Two species have been recorded in Australia: *Ecklonia brevipes* J.Agardh from Western Australia and *Ecklonia radiata* (C.Agardh) J.Agardh from Kalbarri and the Albrohos Island in Western Australia, South Australia, Tasmania, Victoria and north at Caloundra in Queensland (Edgar 2000, Womersley 1987). The following will focus on *Ecklonia radiata* as it fulfils the above criteria. *Ecklonia radiata* has been recorded in Spencer Gulf (Womersley 1987) and there has been trial cultivation of the species in New Zealand (Neill *et al.* 2009). Other *Ecklonia* species have also been cultivated overseas (Troell *et al.* 2006, Hwang *et al.* 2009) and it is used as a source of alginate or as feed for abalone. As a big brown alga, *Ecklonia* has a high biomass and the work by Neill *et al.* (2009) showed that the biomass attained by the alga under cultivation is comparable to wild biomass.

Ecklonia radiata is common and abundant along the temperate coasts of Australia and New Zealand (Connell and Irving 2008). It is often dominant in the upper sublittoral under moderate wave action, and can grow to deeper depths (to 44 m) on rough-water coasts (Womersley 1987). It forms dense forests on subtidal rocky coasts, which are important habitats for a diversity of fish, invertebrate and other algal taxa (e.g. Kennelly and Underwood 1993, Smith *et al.* 1996, Salter *et al.* 2010).

***Sargassum* J.Agardh**

Order: Fucales

Family: Sargassaceae

There are 338 species of *Sargassum* currently accepted taxonomically of which 63 species are found in Australia and 14 species are specifically in South Australia (Guiry and Guiry 2011). The distribution of the 14 species of *Sargassum* found in South Australia (Womersley 1987, Guiry and Guiry 2011) are:

- *Sargassum decipiens* (R.Brown ex Turner) J.Agardh with distribution from Cape Naturaliste, Western Australia to Westernport Bay, Victoria and around Tasmania.
- *Sargassum distichum* Sonder with distribution from Champion Bay, Western Australia, around southern Australia to Port Phillip, Victoria.

- *Sargassum fallax* Sonder with distribution from Houtman Abrolhos, Western Australia, around southern Australia, to Ballina, New South Wales and around Tasmania.
- *Sargassum hetermorphum* J.Agardh with distribution from Rottnest Island, Western Australia, to San Remo, Victoria and northern Tasmania.
- *Sargassum lacerifolium* (Turner) C.Agardh with distribution from Pearson Island, South Australia, to Pebbly Beach, north of Batemans Bay, New South Wales and around Tasmania.
- *Sargassum linearifolium* (Turner) C.Agardh with distribution from Port Denison, Western Australia, around southern Australia to New South Wales.
- *Sargassum paradoxum* (R.Brown ex Turner) Gaillon with distribution Arno Bay, South Australia, to Westernport Bay, Victoria and around Tasmania.
- *Sargassum podacanthum* Sonder with distribution from Point Peron, Western Australia, to Port Noarlunga, South Australia as isolated records.
- *Sargassum sonderi* (J.Agardh) J.Agardh with distribution from Cowaramup Bay, Western Australia to Wilsons Promontory, Victoria and around Tasmania.
- *Sargassum spinuligerum* Sonder with distribution from Houtman Abrolhos, Western Australia, around southern Australia to Westernport Bay, Victoria and the north coast of Tasmania.
- *Sargassum tristichum* Sonder with distribution Rottnest Island, Western Australia to Port Noarlunga, South Australia.
- *Sargassum varians* Sonder with distribution from Cottesloe, Western Australia to Wilsons Promontory, Victoria and northern Tasmania.
- *Sargassum verruculosum* C.Agardh with distribution from Cape Leeuwin, Western Australia, around southern Australia, to Maroubra, New South Wales and around Tasmania.
- *Sargassum vestitum* (R.Brown ex Turner) C.Agardh with distribution from Robe, South Australia, to Mallacoota Point, Victoria, to Sydney, New South Wales and around Tasmania.

Of these 14 species, three species were considered to be potential candidates for open water cultivation in southern Australia. *S. fallax*, *S. linearifolium* and *S. paradoxum* were selected as they are widely distributed and common along southern Australian coasts while the distribution of the other species are more patchy and frequently found as drift algae (Womersley 1987). Economically, *Sargassum* spp. are sources of alginate, although it has one of the lowest yields of alginates among the brown macroalgae. However, being from the order Fucales,

Sargassum spp. has potential in medicinal uses as fucoidans are known for their ability to inhibit tumours, with anti-inflammatory and anti-thrombotic properties (Smit 2004).

Sargassum fallax is a common species on southern Australian coasts, found in rock pools or the uppermost sublittoral but can extend to 48 m depth.

Sargassum linearifolium is the most widely distributed southern Australian species and is commonly found in rock pools or the uppermost sublittoral on coasts of moderate to strong water movement.

Sargassum paradoxum is probably the largest species of *Sargassum* in southern Australia.

Cystophora J.Agardh

Order: Fucales

Family: Sargassaceae

There are 26 species of *Cystophora* that are currently accepted taxonomically with 23 species recorded in Australia and 18 species in South Australia (Guiry and Guiry 2011). *Cystophora* is the largest genus of Fucales on southern Australian coasts and is endemic to Australasia (Womersley 1987). The 18 species found in South Australia and their distribution (Womersley 1987, Guiry and Guiry 2011) are as follows:

- *Cystophora botryocystis* Sonder with distribution from Cottesloe, Western Australia, and Cape Donington, east of Port Lincoln, through the Gulf region of South Australia to Port Phillip, Victoria, and the north coast of Tasmania.
- *Cystophora brownii* (Turner) J.Agardh with distribution from Port Denison, Western Australia, around southern Australia to Victor Harbour and around Kangaroo Island, South Australia and northeast Tasmania.
- *Cystophora congesta* Womersley & Nizamuddin ex Womersley with distribution from Elliston, South Australia to Wilsons Promontory, Victoria and around Tasmania.
- *Cystophora cuspidata* J.Agardh with distribution from Point Sinclair, South Australia to Port Phillip, Victoria and northeast Tasmania.
- *Cystophora expansa* Womersley with distribution from Yallingup, Western Australia to Long Bay, New South Wales and the north coast of Tasmania.
- *Cystophora gracilis* Womersley with distribution from Coqarramup Bay, Western Australia to Wanna, South Australia and Seal Beach, Kangaroo Island, South Australia.
- *Cystophora grevillei* (C.Agardh ex Sonder) J.Agardh with distribution from 7 mile beach, Dongara, Western Australia, around southern Australia to Wilsons Promontory, Victoria, and around Tasmania.

- *Cystophora intermedia* J.Agardh with distribution from Point Sinclair, SA, to Hogan Island and Bass Strait.
- *Cystophora monilifera* J.Agardh with distribution from Nickol Bay, Western Australia, around southern Australia and the north coast of Tasmania to Long Bay, New South Wales.
- *Cystophora moniliformis* (Esper) Womersley & Nizamuddin with distribution from Cape Naturaliste, WA, around southern Australian and Tasmania to Port Stephens.
- *Cystophora pectinata* (Greville & C.Agardh ex Sonder) J.Agardh with distribution from Waterman Bay, Western Australia to Gulf St Vincent and Kangaroo Island, South Australia, and Walkerville, Victoria.
- *Cystophora platylobium* (Mertens) J.Agardh with distribution from east of Eucla, WA, around south-eastern Australia and Tasmania to Bondi.
- *Cystophora polycystidea* Areschoug ex Agardh with distribution from Albany, Western Australia to Long Bay, New South Wales and the north coast of Tasmania.
- *Cystophora racemosa* (Harvey ex Kützing) J.Agardh with distribution from Geographe Bay, Western Australia, around southern Australia to Kangaroo Island, South Australia, and Queenscliff, Victoria.
- *Cystophora retorta* (Mertens) J.Agardh with distribution from Nickol Bay, Western Australia to Wilsons Promontory, Victoria and around Tasmania.
- *Cystophora retroflexa* (Labillardière) J.Agardh with distribution from Victor Harbour and Kangaroo Island, South Australia, around Victoria and Tasmania to Bondi, New South Wales.
- *Cystophora siliquosa* J. Agardh with distribution from Geographe Bay, Western Australia to Wilsons Promontory, Victoria, and north coast of Tasmania
- *Cystophora subfarcinata* (Mertens) J.Agardh with distribution from Nickol Bay, Western Australia to Wilsons Promontory, Victoria and around Tasmania.

Of the 18 species of *Cystophora* found in South Australia, ten species were considered as potential candidates for open water cultivation: *C. botryocystis*, *C. browni*, *C. intermedia*, *C. monilifera*, *C. moniliformis*, *C. platylobium*, *C. polycystidea*, *C. retorta*, *C. siliquosa* and *C. subfarcinata*, and they have been variously studied. For example, the life history of three common species, *C. expansa*, *C. monilifera* and *C. subfarcinata* was examined by Hotchkiss (1999) and recruitment was examined by Emmerson and Collings (1998). Chemical investigation has also been carried out on *Cystophora* such as the isolation of terpenoids (used extensively for their aromatic qualities) from *C. moniliformis* (Reddy and Urban 2008). Furthermore, being from the order Fucales, *Cystophora* spp. are potential sources of

fucoidans, which have medicinal properties (Smit 2004). In the first instance, *C. platylobium* and *C. subfarcinata* are considered the most likely candidates.

Cystophora intermedia is characteristically dominant as a sublittoral fringe species, found along rough-water coasts in South Australia.

Cystophora moniliformis is one of the largest and most distinctive species, often common (1-4 m depth) with moderate to strong water movement. On rough-water coasts, it is confined to rock pools or areas with slight shelter and can extend to a depth of 28 m.

Cystophora platylobium is commonly confined to deep water in depths of 10-48 m, along rough-water coasts in south-eastern Australia but may be found in shallower water in south-east Tasmania.

Cystophora botryocystis is a deep water species on coasts of moderate to slight wave action.

Cystophora polycystidea is commonly found in rock pools and moderately sheltered areas in South Australia. In Victorian and Tasmanian coasts, they are found from low tide level to depths of 3-5 m.

Cystophora monilifera is a widespread species found on coasts with moderate water movement and to depths of 2-42 m, but may be found as shallow as 0.5 m.

Cystophora browni can be abundant in the upper sublittoral and deeper pools on coasts with moderate wave action, extending to a few metres deep.

Cystophora retorta is a common species in larger pools and the upper sublittoral to depths of 21 m, able to withstand moderate water movement.

Cystophora siliquosa is common in pools and the upper sublittoral areas subjected to rough water.

Cystophora subfarcinata is the most common species of *Cystophora* on southern Australian coasts. It is usually found in shallow water to a depth of 5 m that are subjected to moderate to strong wave action.

Scytothalia Greville

Order: Fucales

Family: Seirococcaceae

There are currently two species of the genus *Scytothalia* accepted taxonomically and *Scytothalia dorycarpa* (Turner) Greville is endemic to Australia. The distribution range of this alga is from Geraldton in Western Australia to Point Lonsdale in Victoria and the north coast

of Tasmania (Womersley 1987). *Scytothalia dorycarpa* from the order Fucales has potential medicinal uses as a fucoidan (Smit 2004).

Scytothalia dorycarpa can be found growing along rough water coasts from low water mark to depths of 44 m (Womersley 1987).

***Seirococcus* Greville**

Order: Fucales

Family: Seirococcaceae

The sole species in this genus is *S. axillaris*, which occurs on the east coast of Australia from Fishery Bay (south of Port Lincoln) to Walkerville (Victoria) and around Tasmania. Being from the order Fucales, it has potential medicinal uses as a fucoidan (Smit 2004).

Seirococcus occurs mostly in deep water (3-40 m).

Red Algae (Phylum Rhodophyta, Class Florideophyceae)

***Gracilaria* Greville**

Order: Gracilariales

Family: Gracilariaceae

There are 167 species that are currently accepted taxonomically of which 25 are found in Australia and four in South Australia (Guiry and Guiry 2011). The three species of *Gracilaria* recorded in South Australia and their distribution (Womersley 1996) are as follows:

- *Gracilaria chilensis* C.J.Bird, McLachlan & E.C.Oliveira with distribution from Cowell, Eyre Peninsular, South Australia to Hobsons Bay, Victoria and Port Arthur, Tasmania
- *Gracilaria ramulosa* Withell, J. Agardh with distribution from Cottesloe, Western Australia to Walkerville, Victoria and around Tasmania.
- *Gracilaria secundata* Harvey with distribution from Bales Beach, Kangaroo Island, South Australia to southeast Tasmania and Newcastle, New South Wales.

Gracilaria is among the major edible red algae (Norziah and Ching 2000) and it is being cultivated in many parts of the world, mainly for use in agar production (Troell *et al.* 1997, Marinho-Soriano and Bourret 2003). The use of *Gracilaria* for bioremediation has also been evaluated and results indicate efficient removal of nutrients (e.g. Abreu *et al.* 2009). *Gracilaria secundata* is not considered for cultivation, as the only records in SA are from a single location on Kangaroo Island.

Abundant in regions where mean water temperatures are 25°C, growing in the eulittoral zone or at the beginning of the sublittoral zone. They are usually found on sandy or muddy

substrates that are protected from waves, but may be free-floating in brackish water (Guiry and Guiry 2011).

Pterocladia J.Agardh

Order: Gelidiales

Family: Pterocladaceae

There are five species of *Pterocladia* that are currently accepted taxonomically and three are found in Australia, of which two have been recorded in South Australia (Guiry and Guiry 2011). The two species recorded in South Australia are *Pterocladia lucida* (R.Brown ex Turner) J.Agardh and *Pterocladia rectangularis* (Lucas) Womersley & Guiry. The distribution range of *Pterocladia lucida* is from Murchison River mouth (Kalbarri), Western Australia, around southern Australia and Tasmania to Coffs Harbour, New South Wales while that of *Pterocladia rectangularis* is from Safety Bay, Western Australia, to the Isles of St. Francis, South Australia (Womersley and Guiry 1994). *Pterocladia* is closely related to *Gelidium*, which is an important source of agar, collected and harvested worldwide (McHugh 2003). These algae can be cultivated in ponds and tanks but because it is slow growing, commercial cultivation is not generally considered economically viable (Friedlander 2008).

P. lucida is a common subtidal species on rough water coasts. However, it has not been observed in southern Australia in quantities sufficient for harvesting as a source of agar (Womersley 1994). *P. rectangularis* is usually found in deep water or shaded pools (Womersley and Guiry 1994).

Gelidium J.Agardh

Order: Gelidiales

Family: Gelidiaceae

Numerous species of *Gelidium* occur worldwide, with four in southern Australia. Of these, two are only a few cm long, and therefore considered unsuitable, while one only occurs in the south-east of SA. This leaves *Gelidium australe* as the only candidate species. *Gelidium* is an important source of agar, collected and harvested worldwide (McHugh 2003). These algae can be cultivated in ponds and tanks but because they are slow growing, commercial cultivation is not generally considered economically viable (Friedlander 2008).

An intertidal to deep subtidal genus of cold to tropical waters worldwide (Guiry and Guiry 2011).

Solieria J.Agardh

Order: Gigartinales

Family: Areschougiaceae

Nine species of *Solieria* occur worldwide, with two in southern Australia, although only *S. robusta* occurs in South Australia. This is a common species which is a good food for abalone (S. Clarke pers. obs.). Given its taxonomic position, it is also likely to be a source of agar. However, nothing is known about its cultivation.

A common alga under moderate to fairly turbulent water movement, extending from sheltered areas with strong current flow to shallow to deep water on rough-water coasts (Womersley 1994).

Plocamium Lamouroux

Order: Gigartinales

Family: Plocamiaceae

Some 40+ species of *Plocamium* occur worldwide, with eight in southern Australia. Some species are commonly found as drift, and they are a good food for abalone (S. Clarke pers. obs.). However, nothing is known about their cultivation. The two species selected for further consideration, based on size and abundance, are *P. mertensii* and *P. preissianum*.

Common on coasts with moderate to strong wave action, from shallow water down to 50 m depth (Womersley 1994).

Asparagopsis Montagne

Order: Bonnemaisoniales

Family: Bonnemaisoniaceae

Three species of *Asparagopsis* are currently recognised occur worldwide, with two in southern Australia. *Asparagopsis armata* is rare in South Australia, however *A. taxiformis* is abundant. They are a good food for abalone, but relatively fragile so might not be good for open waters (S. Clarke pers. obs.). Nothing is known about their cultivation.

Asparagopsis taxiformis is primarily a tropical/subtropical species, but also occurs in and around the South Australian gulfs in water depths from 4-19 m (Womersley 1996).

Hypnea J.V.Lamouroux

Order: Gigartinales

Family: Cystocloniaceae

There are 53 species that are currently accepted taxonomically of which 17 have been recorded in Australia and three are in South Australia (Guiry and Guiry 2011). The three species of *Hypnea* recorded in South Australia and their distribution (Womersley 1994) are as follows:

- *Hypnea charoides* J.V.Lamouroux with distribution from Port Denison, Western Australia to Cape Jaffa, South Australia, and northern Tasmania.
- *Hypnea filiformis* (Harvey) Womersley with distribution from Port Denison, Western Australia to Nora Creina, South Australia.
- *Hypnea ramentacea* (C.Agardh) J.Agardh with distribution from Port Denison, Western Australia to Walkerville, Victoria and the north coast of Tasmania.

They generally attach subtidally to coral, stones or shells in depths of 6 to 13 m (Womersley 1994, Guiry and Guiry 2011).

Conclusion

A short-list of candidate seaweed species for use in IMTA in Spencer Gulf (Table 2) has been developed based on the information presented above.

While it is recognised that some of these species are likely to be less than ideal (e.g. *Gelidium* and *Pterocladia* are considered to be slow growing), and will thus probably be eliminated as candidate species fairly quickly, each of the species in Table 1 will initially be trialled using one or more of three techniques:

- 1: Seeding of sexual propagules onto ropes
- 2: Attaching vegetative propagules onto ropes
- 3: Placing vegetative propagules into some form of net bag (e.g. lantern net as used in scallop culture)

The techniques chosen will depend on the species life-history and morphology.

Table 2: Short-list of candidate species for IMTA in Spencer Gulf, South Australia, along with key characteristics.

Order	Family	Sp	Size	Abundance in SA	Exposure	Depth	Genus farmed	Bioactives etc	Food	Ab feed	Agar etc
		<i>Characteristics for retention</i>	> 20 cm	Not rare/ uncommon	mod-rough	< 10 m					
Phylum Phaeophyta											
Fucales	Cystoseiraceae	<i>Cystophora platylobium</i>	1-2 m	Common		0-48 m					
		<i>C. subfarinata</i>	20-80 cm	Common	rough	0-5 m		Y			
Fucales	Sargassaceae	<i>Sargassum fallax</i>	20-100 cm	Common		0-48 m	Y	Y	Possible		
		<i>S. linearifolium</i>	10-50 cm	Common	rough	0-5 m	Y	Y	Possible		
Fucales	Seirococcaceae	<i>Scytothalia dorycarpa</i>	0.5-2 m	Common	rough	0-44 m		Y		Y	
		<i>Seirococcus axillaris</i>	0.5-2 m	Common	rough	0-44 m		Y			
Laminariales	Alariaceae	<i>Ecklonia radiata</i>	0.3-2 m	Common	rough	0-38 m	Y	Y			
Phylum Rhodophyta											
Gelidiales	Gelidiaceae	<i>Gelidium australe</i>	10-25 cm			3-13 m	Y	N		Y	Y
	Pterocladaceae	<i>Pterocladia lucida</i>	8-40 cm	Common	rough	3-38 m	Y	N			Y
Gigartinales	Areschougiaceae	<i>Solieria robusta</i>	10-30 cm	Common	rough	0-23 m	Y	N		Y	Y
Gigartinales	Hypneaceae	<i>Hypnea ramentacea</i>	10-25 cm	Common		1-25 m	Y	N	Y	Y	Y
Plocamiales	Plocamiaceae	<i>Plocamium mertensii</i>	10-50 cm	Common	rough	0-50 m		Possible		Y	N
		<i>P. preissianum</i>	20-50 cm	Common	rough	2-50 m		Possible		Y	N
	Bonnemaisoniaceae	<i>Asparagopsis taxiformis</i>	10-30 cm	Common		4-19 m		Y		Y	N
Gracilariales	Gracilariaceae	<i>Gracilaria chilensis</i>	10-60 cm	Common	Sheltered	0-6 m	Y	N	Y	Y	Y
		<i>G. ramulosa</i>	5-25 cm	Common		1-30 m	Y	N	Y	Y	Y

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