Application of seaweeds to integrated multi-trophic aquaculture in southern Australia: identifying and investigating suitable native species

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Cover image: Seaweed longline adjacent to fish cages (top), and specimens of *Solieria robusta* (bottom left) and *Ecklonia radiata* (bottom right) during field trials of seaweed aquaculture. Photographs by Kathryn H. Wiltshire.

Thesis abstract

Integrated multi-trophic aquaculture (IMTA) involves strategic co-culture of organisms so that wastes from one species are used to grow another. Seaweeds can be used in IMTA systems to remove and utilise dissolved inorganic nutrients from fish aquaculture, improving environmental performance and providing economic benefits through diversification and increased productivity.

IMTA with seaweed could be applied to assist sustainable expansion of fish farming in South Australia (SA), where dissolved nitrogen (N) wastes limit environmental carrying capacity. Seaweed farming is also of interest in Australia to meet increasing demand for seaweed products, of which Australia is a net importer. Several native seaweeds have been identified as potential candidates for aquaculture in SA based on general knowledge of their biology and potential economic value, but specific knowledge of their suitability for cultivation was lacking. I investigated eight candidate seaweeds, comprising four red (*Solieria robusta, Gelidium australe, Pterocladia lucida, Plocamium angustum*) and four brown (*Ecklonia radiata, Cystophora subfarcinata, Sargassum linearifolium, Scytothalia dorycarpa*) species, to determine which species were most suitable for farming, with specific emphasis on application to IMTA in SA. I assessed feasibility of cultivation and potential for nutrient remediation of the eight species in two field trials and in laboratory experiments, and applied species distribution modelling (SDM) to identify the most suitable candidate species for aquaculture in the vicinity of current SA fish farms.

My research identified the red seaweed *Solieria robusta* and the brown seaweed *Ecklonia radiata* as the most suitable species for aquaculture. The red *Gelidium australe* showed promising growth in a pilot field trial and removed the most N in a 4-week laboratory trial, but *S. robusta* grew best in laboratory trials and would remove more N over time due to its faster growth. *Solieria robusta* tolerated a wider temperature range and grew better at higher temperatures than *G. australe.* SDM results demonstrated that *S. robusta* has high environmental suitability in aquaculture zones throughout Spencer Gulf, where all SA finfish farming currently occurs, while *G. australe* was poorly suited to most existing aquaculture zones. *Pterocladia lucida* and *Plocamium angustum* had slower growth rates, and SDMs indicated low suitability in aquaculture zones. There was little difference in field performance of the brown seaweeds, apart from *Scytothalia dorycarpa*, which performed poorly, but *Ecklonia radiata* was most amenable to hatchery reproduction and cultivation. SDM showed that several aquaculture zones in southern Spencer Gulf had good suitability for *E. radiata.*

Seedstock production methods used for commercially farmed relatives were successfully applied to *S. robusta* and *E. radiata,* and I developed protocols that can be employed to up-scale production of these seaweeds. *Solieria robusta* and *E. radiata* demonstrated the ability to accumulate tissue N, and N uptake rates comparable to other IMTA seaweeds, supporting the suitability of these species for IMTA. Data from my experiments help to inform suitable depths, locations and seasons for cultivation of these seaweeds, and to incorporate N removal by seaweeds into biogeochemical models. These experiments provide the foundation for developing seaweed aquaculture in southern Australia, including IMTA.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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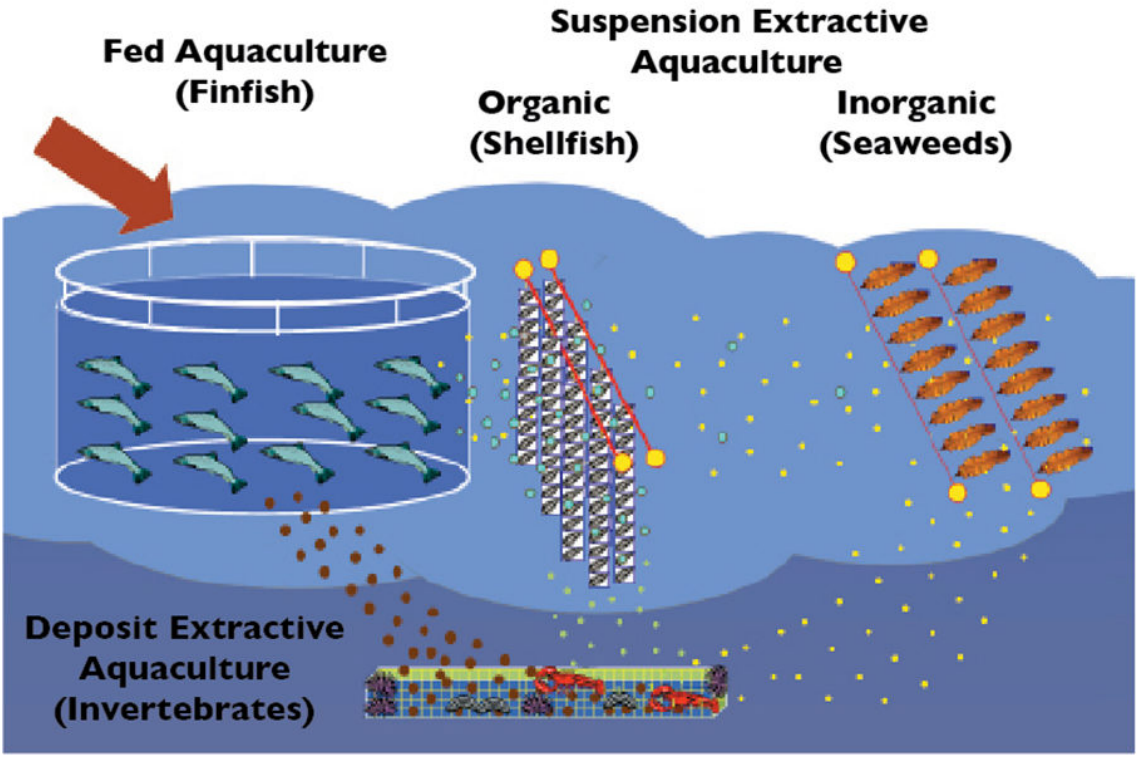
Chapter 1. General Introduction



Schematic diagram of an integrated multi-trophic aquaculture system illustrating the co-cultivation of complementary trophic levels so that organic and inorganic nutrients are recycled and utilised to produce additional crops. Figure reproduced from Chopin, et al. (2010).

Reference:

Chopin T, Troell M, Reid GK, Knowler D, Robinson SMC, Neori A, Buschmann AH, Pang S (2010) Integrated multi-trophic aquaculture, part 1 Accessed: 21/5/2020. URL: https://www.aquaculturealliance.org/advocate/integrated-multi-trophic-aquaculture-part-1/

1 Introduction

1.1 Integrated multi-trophic aquaculture and the South Australian context

Global demand for seafood is increasing due to both population growth and increasing per capita consumption (Kobayashi, et al., 2015). Because supply from wild fisheries is limited, aquaculture production is expanding worldwide to meet demand (FAO, 2018; Kobayashi, et al., 2015). Increased aquaculture production, however, is accompanied by concerns about environmental impacts, such as eutrophication and benthic enrichment (Buchholz, et al., 2012; Froehlich, et al., 2017; Silvert, 1992). Best management practices can alleviate many local aquaculture impacts, but dissolved nutrients released from fish farms can have broad reaching impacts, including through changes to phytoplankton productivity and community composition (Buck, et al., 2018; Hadley, et al., 2015).

One option to improve sustainability of aquaculture is to apply integrated multi-trophic aquaculture (IMTA). IMTA is a system involving strategic co-culture of organisms at complimentary trophic levels, such that wastes from one or more fed species are recycled and utilised by others, such as filter-feeders and deposit feeders, which consume particulate wastes, and autotrophs, which remove dissolved inorganic nutrients (Buchholz, et al., 2012; Buck, et al., 2018; Chopin, et al., 2001; Kim, et al., 2017; Neori, et al., 2004; Soto, 2009). The extractive species used in IMTA are also crops of commercial value, providing additional income and reducing economic risk through diversification of farmed products (Barrington, et al., 2009; Ridler, et al., 2007; Soto, 2009). Extractive species in IMTA systems grow faster than in monoculture, hence IMTA farms can provide greater overall profitability (Handå, et

al., 2012; Petrell and Alie, 1996; Sanderson, et al., 2012; Sarà, et al., 2009; Troell, et al., 2003; Whitmarsh, et al., 2006). Nutrient and carbon sequestration by extractive species also provide economic value in jurisdictions with relevant trading schemes (Abreu, et al., 2011; Barrington, et al., 2009; Kim, et al., 2015). IMTA can also lead to greater social acceptance of aquaculture activity (Martínez-Espiñeira, et al., 2015; Ridler, et al., 2007), and IMTA seafood can be marketed at a premium price (Martínez-Espiñeira, et al., 2015; Whitmarsh and Wattage, 2006). These combined environmental and economic benefits are driving increasing global interest in IMTA systems (Buck, et al., 2018).

Several fish species are farmed in sea cages in Australia, and production is increasing to meet growing demand for seafood both nationally and internationally (Department of Agriculture and Water Resources, 2016; Mobsby and Koduah, 2017). There is a strong emphasis on environmentally sustainable management of Australian aquaculture, but community concerns about environmental impacts remain (Department of Agriculture and Water Resources, 2016; Rimmer and Ponia, 2007). Advances in production technology and implementation of IMTA are two pathways identified to improve the environmental performance of Australian aquaculture, allowing environmentally sustainable expansion while enhancing public perception of the industry (Department of Agriculture and Water Resources, 2017).

Fish aquaculture in South Australia (SA) involves off-shore farming in Spencer Gulf of Southern Bluefin Tuna (tuna), *Thunnus maccoyii*, and Yellowtail Kingfish (kingfish), *Seriola lalandi*, with > 7 000 tonnes annual production of tuna since 1999/2000, and 579 – 3,757 tonnes annual

production of kingfish since 2007 (BDO Econsearch, 2019). Tuna production is projected to increase by ~ 11 % from 2018/19 to 2020/21, while kingfish production is projected to increase by 51 % over the same period (BDO Econsearch, 2019). For every tonne of production, tuna release up to 500 kg of nitrogen (N), with ~ 90 % in dissolved form (Fernandes, et al., 2007), and kingfish release up to 200 kg N, 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 farmed salmonids, which release 42 – 57 kg N per tonne of production (Fernandes and Tanner, 2008; Fernandes, et al., 2007).

Aquaculture activities in SA are governed by a variety of legislation and regulated by Primary Industries and Regions SA (PIRSA). To maintain environmental quality, PIRSA use biogeochemical models (e.g. Collings, et al., 2007; Middleton, et al., 2013; Tanner, et al., 2007) to set limits on stock biomass. These models demonstrate that dissolved N wastes limit the environmental carrying capacity for fish in current aquaculture regions. Industry is keen to increase stocking densities and to reduce costs by employing automated feeding, but these are likely to increase localised nutrient inputs. There is also interest from both industry and government in expanding production and opening up new areas to aquaculture. To avoid increased nutrient loading, FCRs would need to be improved, or nutrients removed, potentially by growing seaweed in an IMTA system (Neori, 2008; PIRSA, 2013).

Seaweed farming is not an established industry in Australia, but is also of interest to meet increasing demand for seaweed products, of which Australia is a net importer (Lee, 2010;

Roos, et al., 2018). Total imports of seaweed products into Australia in 2008-9 were ~ 5 000 tonnes, with a value over AUD$17 million, and are increasing by almost 30 % per annum (Lee, 2010). Globally, seaweeds are widely utilised for food and for their extracts, including hydrocolloids and bioactive compounds (Holdt and Kraan, 2011; Lorbeer, et al., 2013; Smit, 2004; Thomas and Kim, 2011; White and Wilson, 2015). Seaweed consumption has a long history in Asia, but with increasing globalisation and recognition of the sustainability and health benefits of eating seaweeds, they are increasingly used as, or incorporated in, food in many parts of the world (Buschmann, et al., 2017; McHugh, 2003; Skrzypczyk, et al., 2018; White and Wilson, 2015). Aquaculture production of edible seaweeds is therefore increasing rapidly to meet this growing demand (Buschmann, et al., 2017).

The species predominantly used for human consumption include several brown seaweeds of the order Laminariales (kelps), especially the genera *Laminaria* and *Saccharina* (known as kombu), and *Undaria pinnatifida* (wakame), plus red seaweeds, primarily of the order Bangiales, genera *Pyropia* and *Porphyra* (nori) (see Buschmann, et al., 2017; FAO, 2018; White and Wilson, 2015). Seaweed hydrocolloids are used as gelling agents in many food products, and in a range of industrial and biomedical applications (Bixler and Porse, 2011; Buschmann, et al., 2017; Holdt and Kraan, 2011; McHugh, 2003; White and Wilson, 2015). These hydrocolloids include: agar, produced by red seaweeds of the orders Gracilariales and Gelidiales; carrageenan, produced by the red order Gigartinales; and alginates, which are primarily sourced from the brown orders Laminariales and Fucales (Bixler and Porse, 2011; White and Wilson, 2015). Seaweeds are also used in fertilisers, stock feed and for biofuel production (Buschmann, et al., 2017; Craigie, 2011; Dworjanyn, et al., 2007; Evans and

Critchley, 2014; Forbord, et al., 2012; Hwang, et al., 2009; Wei, et al., 2013; White and Wilson, 2015).

The aforementioned applications are typically high-volume but low-value uses of seaweed biomass, but seaweeds are increasingly being utilised for high-value products. Many seaweeds produce bioactive compounds, some of which exhibit anti-ageing, anti-tumor, anti-viral, anti-bacterial, and anti-fungal activities, that are of use in functional foods, cosmetics, medicines, and pesticides (Buschmann, et al., 2017; Gupta and Abu-Ghannam, 2011; Holdt and Kraan, 2011; Lorbeer, et al., 2013; Smit, 2004; Thomas and Kim, 2011). Seaweed extracts are also used to produce plant growth regulators for agriculture (Briceño-Domínguez, et al., 2014; Craigie, 2011; Panda, et al., 2012). Sequential extraction, or biorefinery, technologies are being developed to obtain multiple products from seaweed biomass (Balina, et al., 2017; Buschmann, et al., 2017).

Australia, and in particular southern Australia, has a highly diverse seaweed flora with high endemism (Phillips, 2001). Australia's seaweed flora has the potential to yield novel bioactive compounds, and to be utilised as a source of hydrocolloids, stock feed, including in aquaculture, fertiliser or biofuel (Kirkendale, et al., 2010; Lee, 2010; Lorbeer, et al., 2013; Roos, et al., 2018; Winberg, et al., 2011). Several native Australian seaweeds are palatable and nutritious, having good fatty acid profiles, antioxidant and gut health promoting properties (Charoensiddhi, et al., 2015; Charoensiddhi, et al., 2017; Skrzypczyk, et al., 2018). Bioactives, including terpenoids, polyphenols and halogenated compounds, with promising anti-cancer, anti-viral, anti-bacterial and anti-fouling properties are found in many Australian

species (Lorbeer, et al., 2013). While the potential value of Australia's seaweed resources has been recognised, commercial utilisation to date has been minimal, comprising limited harvest of beach-cast and wild biomass (Lee, 2010; Lorbeer, et al., 2013; Roos, et al., 2018). Regulatory frameworks are unlikely to permit expansion of these activities and aquaculture is therefore essential for commercialisation of Australia's seaweed resources (Roos, et al., 2018).

1.2 Potentially suitable native seaweeds

Farming local species is desirable for IMTA applications to ensure suitability for local conditions and to avoid risks associated with introducing non-native, and potentially invasive, species for farming (Soto, 2009). Few Australian seaweeds have been commercially cultivated, therefore seaweed industry development in Australia, including IMTA, requires development of methods for growing novel species in aquaculture. The 1168 species described in the "Marine Benthic Flora of Southern Australia" series (Womersley, 1984; 1987; 1994; 1996; 1998; 2003) were systematically reviewed by experts from the South Australian Research and Development Institute (SARDI) Aquatic Sciences and the State Herbarium of SA in 2011 to assess their suitability for aquaculture (see appendix to Wiltshire, et al., 2015).

Fish farming in SA occurs primarily in moderately to relatively exposed regions of Spencer Gulf (Figure 1), 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 in the review. The review considered desirable characteristics of IMTA seaweeds, which include: an established or potential market value, available cultivation technology, and ability to

achieve adequate nutrient mitigation through tissue nutrient and biomass accumulation (Kang, et al., 2013; Neori, et al., 2004; Soto, 2009). High value species that are slower growing could also be suitable, with the trade-off of reduced bio-mitigation (Soto, 2009).

There is no established market for the majority of southern Australian seaweeds, so the review assessed commercial uses of seaweeds globally to determine which Australian species may have market value. Further to having a potential market value, species were retained for consideration only 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 cultivated using existing technologies (e.g. as used for related farmed species). The resulting list of 89 species was further reduced based on expert knowledge of their characteristics and consultation with industries that utilise seaweed products. When multiple species from a single genus were retained, an attempt was then made to choose two that were considered the most likely candidates. It was noted, however, that especially for the brown seaweed genera *Cystophora, Sargassum* and the red *Plocamium*, related species were also likely to be suitable. Seven species of brown seaweeds (Ochrophyta: Phaeophyceae) and nine species of red seaweeds (Rhodophyta: Florideophyceae) were regarded as worthy of further investigation (Table 1).

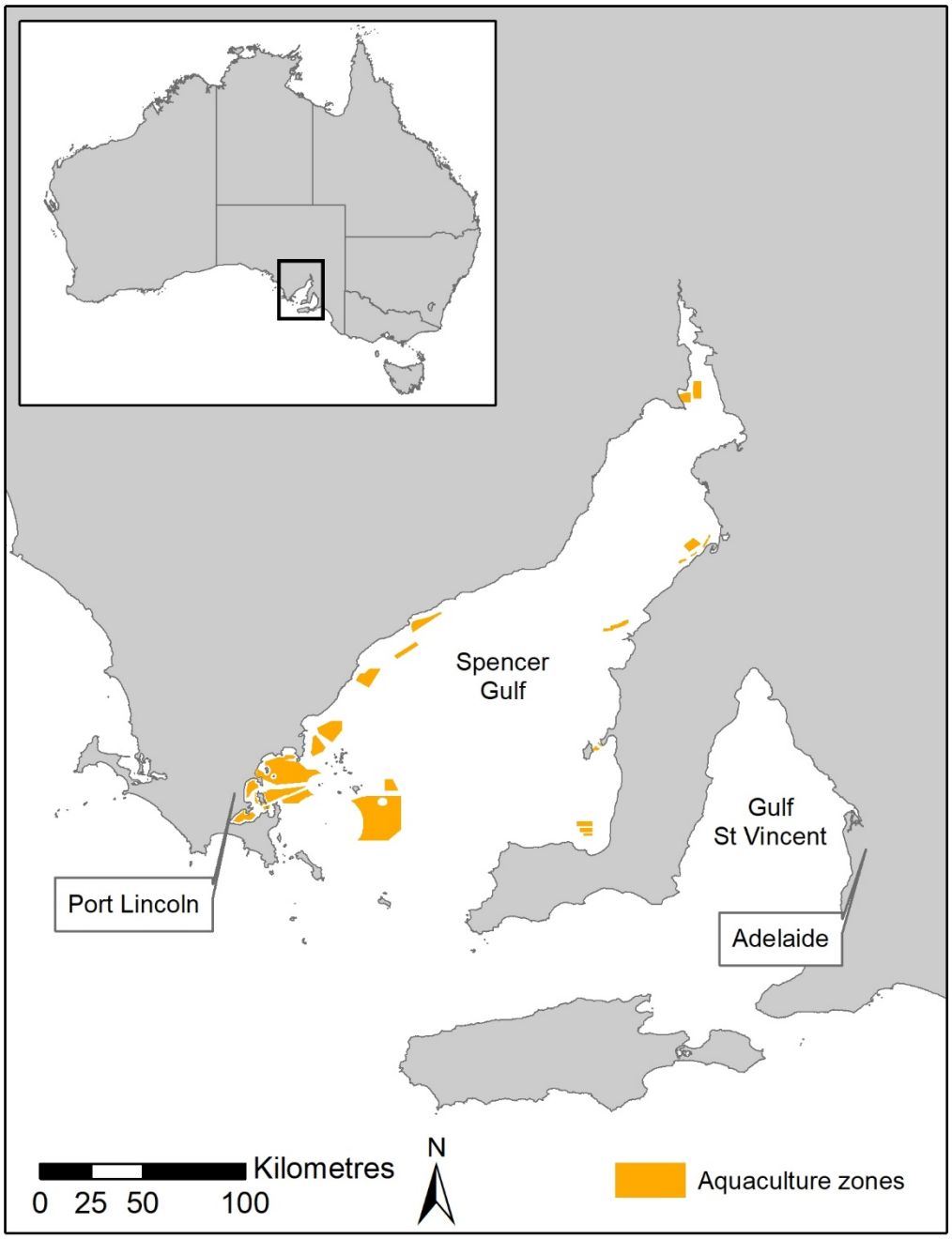


Figure 1. Map of Spencer Gulf, South Australia, showing current aquaculture zones and locations of Adelaide and Port Lincoln.

Table 1. Shortlist of South Australian seaweed species with potential for IMTA developed from a literature review (see Wiltshire, et al., 2015)

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

Note: Taxonomic classifications and scientific names in this table have been updated from those shown in the appendix to Wiltshire, et al. (2015) to those currently accepted according to AlgaeBase (Guiry and Guiry, 2020).

1.3 Selection of candidate species for research

The shortlist of candidate species generated by the literature review was refined based on the accessibility of seaweeds for collection, availability of sufficient biomass for experiments, and amenability to transport and handling.

To identify potential collecting sites for each short-listed species, I compiled occurrence data from published literature, SARDI databases, and herbarium records. The SARDI data was from surveys of temperate reefs in SA (Collings, et al., 2008; Turner, et al., 2007) and biodiversity surveys (Rowling, et al., 2009). Herbarium records were accessed from Australia's Virtual Herbarium[1]. Literature searches used Scopus and Google Scholar with each species name and known synonyms plus appropriate geographical area names as search terms.

Between April and September 2012, 23 locations around South Australia were visited and material of shortlist species and other commonly encountered seaweeds was collected and brought to the South Australian Aquatic Sciences Centre (SAASC) at West Beach, Adelaide, where it was housed in outdoor tanks. Tanks were supplied with sand-filtered, untreated flow-through seawater sourced from the adjacent Gulf St Vincent at ambient temperature and salinity. Specimens that could not readily be identified were examined at the State Herbarium of SA to confirm their identity. Where multiple representatives of a genus of interest were found, their

[1] http://avh.ala.org.au

relative abundance and ease of collection were noted, and their survival in holding tanks assessed as a measure of their amenability to handling and maintenance in tanks.

Several *Plocamium* species were collected, but *P. angustum* showed the best survival when held in outdoor tanks and was selected as the best candidate of this genus. Of the Fucales, three shortlisted species were common in the field: *Cystophora subfarcinata* and *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 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 common, although none were as abundant as *C. subfarcinata.* Most of these species also did not appear as amendable 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 (Hotchkiss, 1999; Klemm, 1988), further supporting 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 red seaweeds: *Gelidium australe, Pterocladia*

lucida (Gelidiales), *Solieria robusta* (Gigartinales), and *Plocamium angustum* (Plocamiales); and the brown seaweeds: *Ecklonia radiata* (Laminariales), *Cystophora subfarcinata, Sargassum linearifolium,* and *Scytothalia dorycarpa* (Fucales).

Pterocladia lucida is an agarophyte that is 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 *Eucheuma* and *Kappaphycus* spp., 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 (Khanzada, 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).

Ecklonia radiata belongs to the same brown algal order (Laminariales, commonly known as kelps) as several major aquaculture species such as *Saccharina japonica* and *Undaria pinnatifida*, which are farmed primarily for human consumption and as a source of alginates (FAO, 2018; McHugh, 2003; White and Wilson, 2015). *Ecklonia* species are also utilised as food globally (White and Wilson, 2015), and *Ecklonia radiata* has a good nutritional profile and palatability for human

consumption (Charoensiddhi, et al., 2015; Skrzypczyk, et al., 2018). *Scytothalia dorycarpa* (family Seirococcaceae), *Cystophora 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.) (see White and Wilson, 2015). These 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 (Holdt and Kraan, 2011; Lorbeer, et al., 2013; Smit, 2004; Thomas and Kim, 2011). Laminariales and Fucales are also used to produce plant growth stimulators (Briceño-Domínguez, et al., 2014; Craigie, 2011; Panda, et al., 2012), animal and aquaculture feeds (Dworjanyn, et al., 2007; Evans and Critchley, 2014; Hwang, et al., 2009), and are a potential source of biomass for biofuel production (Buchholz, et al., 2012; Forbord, et al., 2012; Wei, et al., 2013).

1.4 Assessing suitability for IMTA

The over-arching aim of the work presented in this thesis is the assessment of suitability for IMTA of the eight candidate species selected. Specific aspects of suitability considered were feasibility of propagation and cultivation, including suitable conditions for growth relative to those in SA aquaculture areas, and nutrient removal and storage ability.

Relevant literature used to refine aims of the research and inform choice of methods applied is reviewed below. Specific aims and an outline of the work performed for each thesis chapter are provided in section 2.

1.4.1 Feasibility of propagation and cultivation

Cultivation methods, reproduction, and growth patterns differ between red and brown seaweeds. Most red seaweeds such as the commonly cultivated Gigartinales (which include Solieriaceae) and Gracilariales can be grown vegetatively from fragments, while most brown seaweeds of the main farmed 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). Restrictions on harvesting wild seaweeds in Australia mean that seedstock production is a critical step in developing a species for aquaculture (Roos, et al., 2018). For the brown seaweeds, therefore, the ability to obtain spores and gametes is essential for aquaculture development, while, for red seaweeds, an ability to regrow from cuttings is required.

1.4.1.1 Reproduction 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, also known as the true kelps, show distinct differences between alternate generations, with a large conspicuous sporophyte and a 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 (sporophylls) 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).

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 experimental purposes (Bolton and Levitt, 1985; Jennings, 1967; Novaczek, 1984a; Papenfuss, 1942). Both Neill et al. (2009) and Hwang et al. (2009) used typical methods applied for farmed Laminariales to obtain *Ecklonia* spores. Sori of *E. radiata* are located mainly on the central blade but extend onto laterals, and are often extensive but relatively inconspicuous (Womersley, 1987). The fertile season of this species is unclear; peak fertility is reported in winter-spring in New Zealand (Novaczek, 1984b) but in summer-autumn in southern Australia (Mohring, et al., 2013).

Fucales produce male and female gametes directly and do not have a gametophyte stage (Womersley, 1987). *Sargassum* is the most speciose genus in the Fucales (Guiry and Guiry, 2020) and several *Sargassum* species are farmed, including *S. fusiforme* (known as Hijiki), *S. horneri, S. thunbergii* and *S. fulvellum* (see Hwang, et al., 2007; Li, et al., 2010; Pang, et al., 2007; Pang, et al., 2009; Zou, et al., 2012). Most of these species are dioecious, and synchronisation of reproduction in male and female plants is an important consideration in their culture (Pang, et al., 2006; Pang, et al., 2005). 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

(Hwang, et al., 2007; Pang, et al., 2005; Zhao, et al., 2008). Seedling development is facilitated over a period of nursery culture prior to planting in the sea (Hwang, et al., 2007; Pang, et al., 2008).

Although related fucalean species are farmed, there is no history of cultivation for *Sargassum linearifolium, Cystophora subfarcinata* or *Scytothalia dorycarpa.* In contrast to many farmed Fucales, these three species are monoecious (Womersley, 1987). No published information on reproduction in *Sargassum linearifolium* is available, but several accounts exist for closely related southern Australian species, including S. *spinuligerum, S. podacanthum* and *S. distichum* (e.g. Kendrick and Walker, 1991; 1994). These species are fertile from September to January, with peak reproductive biomass in November; mature plants have visible zygotes attached to the reproductive structures, which are developed in fertile branches (Kendrick and Walker, 1991; 1994). *Cystophora* species are not farmed, but have been reproduced in laboratories for research using manipulation of light and temperature to stimulate gamete release from fertile plants (Klemm, 1988; Klemm and Hallam, 1987; Taylor and Schiel, 2003). *Cystophora subfarcinata* is fertile from July to December in southern Australia, with peak fertility in October – November (Klemm, 1988). Fertile structures in *C. subfarcinata* develop on upper branches (Womersley, 1987). There are few published studies on reproduction in Seirococcaceae, with most on *Phyllospora comosa,* but fertile structures of *Scytothalia dorycarpa*, located in branch axes*,* occur year-round (Womersley, 1987). Gametes have been obtained from *Phyllospora comosa* using light and temperature manipulation similar to that used in *Cystophora* spp. (e.g. Burridge and Hallam, 1993; Burridge, et al., 1993; Schoenwaelder and Clayton, 2000).

1.4.1.2 Applicable farming technologies

Cultivation methods for farmed red seaweeds, including Gelidiales and Solieriaceae, typically involve using algal fragments tied to substrates, e.g. ropes, shells, stones, concrete cylinders, or contained in mesh bags or tubes (Ask and Azanza, 2002; Friedlander, 2008; Ganesan, et al., 2011; Góes and Reis, 2011; Kim, et al., 2017). Farming of Laminariales and Fucales involves spores or zygotes, respectively, being seeded onto string or rope, or nursery-grown seedlings are manually inserted onto culture ropes for out-planting in the sea (Kim, et al., 2017; Sahoo and Yarish, 2005; Titlyanov and Titlyanova, 2010).

1.4.1.3 Implications of IMTA for farm management

Co-cultivation of species in IMTA systems may have implications for transmission of parasites and disease between cultivated species, especially where one species is an intermediate host or reservoir of a disease or parasite affecting the other (Skar and Mortensen, 2007; Troell, et al., 2003), but this aspect of IMTA is rarely studied (Soto, 2009). External parasitic flatworms (flukes) are an ongoing health issue for culture of kingfish (Chambers and Ernst, 2005; Ernst, et al., 2002). Skin (*Benedenia seriolae*) and gill (*Zeuxapta seriolae*) flukes occur in wild kingfish populations and proliferate on farmed fish due to the parasites' direct lifecycles and host fish density (Whittington, 2012). Flukes are controlled by in-feed or immersion treatments, but reinfection occurs from eggs, which are resistant to treatment and attach to fish cage infrastructure, or from wild fish (Chambers and Ernst, 2005; Ernst, et al., 2002). Although seaweed is not a host for flukes, placing additional aquaculture infrastructure in the vicinity of fish cages, such as in an IMTA system, could result in more fluke eggs being retained, with the seaweed cultivation system acting as an additional reservoir for infection.

1.4.2 Suitable areas and conditions for cultivation

As part of the management of aquaculture activities in SA, PIRSA develops aquaculture zone policies that prescribe what classes of aquaculture, in terms of types of organism and farming methods, are permitted, and maximum biomass limits for farmed species in spatially defined areas (PIRSA, 2013; 2017a). These zone policies consider biogeochemical models (e.g. Middleton, et al., 2013; Tanner, et al., 2007) and aim to achieve environmental sustainability in setting production limits for fed species, such as fish (PIRSA, 2013). Although seaweed farming is not an established industry in Australia, the potential for seaweed to be used to offset nutrient inputs from other aquaculture is recognised (PIRSA, 2013), and several current aquaculture zone policies (PIRSA, 2017b) list seaweed farming as a permitted class of aquaculture.

Aquaculture zones in SA are assessed with regard to ecologically sustainable development principles, hence zones are located to avoid potential impacts on high-value habitats, marine protected areas, and threatened species, in addition to avoiding spatial conflict with other marine users including shipping (PIRSA, 2013; 2017a). Habitat suitability for growth and productivity of farmed organisms is also an important consideration in aquaculture site selection (Kapetsky, et al., 2013; Ross, et al., 2013). Suitability for production is also considered by PIRSA in determining zone locations, permitted classes of aquaculture, and biomass limits, but the zone policies acknowledge that factors determining environmental suitability for cultivation of seaweeds and some invertebrates in SA are not well understood (PIRSA, 2013).

Understanding species responses to environmental conditions provides information on optimal conditions for growth, helping to inform the best locations, depths, and times of year for

cultivation. For seaweeds, temperature, light and nutrient availability are important for growth (Hurd, et al., 2014). For existing farmed or well-studied species, where suitable environmental conditions for growth are known, habitat suitability indices can be developed and combined with spatial data to identify potential sites for aquaculture (e.g. Falconer, et al., 2016; Radiarta, et al., 2011; Silva, et al., 2011; Snyder, et al., 2017; Zhang, et al., 2017). Where this biological knowledge is lacking, an alternative approach is to use correlative models that link species occurrence data to environmental conditions and spatially predict suitability for cultivation across a region (Castelar, et al., 2015; Falconer, et al., 2016; Linhoss, et al., 2016; Oyinlola, et al., 2018; Vincenzi, et al., 2007; Vincenzi, et al., 2011). Such correlative models are typically referred to as species distribution models (SDMs), but may also be called habitat suitability models or environmental niche models, with terminology often reflecting the aim of the modelling, rather than the methodology used (Elith and Leathwick, 2009; Marcelino and Verbruggen, 2015).

Maximum entropy (maxent) modelling is widely used for developing correlative SDMs, particularly where available occurrence data are presence-only, but default maxent methods sometimes produce overly complex models (Halvorsen, et al., 2015; Radosavljevic and Anderson, 2014; Syfert, et al., 2013; Verbruggen, et al., 2013; Warren and Seifert, 2011). More parsimonious models may be produced by increasing regularisation (Anderson and Gonzalez Jr, 2011; Muscarella, et al., 2014; Radosavljevic and Anderson, 2014), limiting the complexity of response curves by using only linear and quadratic feature types (Elith, et al., 2010; Merow, et al., 2013; Syfert, et al., 2013), or applying forward step-wise variable selection using likelihood ratio or *F*-tests under a maximum likelihood (ML) interpretation of maxent (Halvorsen, 2013; Halvorsen, et al., 2015; Halvorsen, et al., 2016; Mazzoni, et al., 2015; Vollering, et al., 2019).

1.4.3 Nutrient removal and storage

For seaweeds to be useful for IMTA the farmed seaweed needs to achieve adequate nutrient mitigation through tissue nutrient and biomass accumulation (Kang, et al., 2013; Neori, et al., 2004; Soto, 2009). For open ocean IMTA, the total N incorporated into seaweed tissue demonstrates the effective N removal by seaweed over the cultivation period (Buschmann, et al., 2008; Kang, et al., 2013; Kim, et al., 2014; 2015; Neori, 2008; Ribeiro, et al., 2012). Seaweed nutrient uptake dynamics, however, influence the effectiveness of seaweeds at intercepting and removing nutrients over finer spatial and temporal scales (Chopin, et al., 2001; Kang, et al., 2013; Neori, et al., 2004). Seaweed growth rate and tissue N data can be used to estimate the biomass of farmed seaweed needed to offset a given N input (e.g. Abreu, et al., 2009; Kim, et al., 2014; 2015), but to incorporate seaweed N removal into dynamic biogeochemical models (e.g. Broch, et al., 2013; Hadley, et al., 2015), data on uptake rates is also required.

2 Research aims and thesis outline

My research aimed to determine the most suitable species for aquaculture from the candidate species, especially with respect to IMTA application in southern Australia. To assess suitability, the following characteristics were considered: feasibility of propagation and cultivation using existing technology; growth rate and N storage ability; and likely environmental suitability of existing aquaculture zones for each of the eight candidate species. Further investigation of relevant aspects of the biology of the best performing candidate species was then carried out.

Specific aims of the research were:

1. Assess feasability of seed stock production for each species

2. Determine which species were most feasible for IMTA including:
    a. Assess ability of each species to be cultivated using farming systems adapted from existing farmed seaweeds, and
    b. Compare growth rates and nitrogen storage ability
3. Compare relative environmental suitability of farming areas for each species
4. Further assess the best potential candidates to:
    a. Assess growth responses to temperature, light and nutrient
    b. Improve seed stock production methods
    c. Obtain data on N uptake kinetics

This thesis comprises six chapters, the introduction, four data chapters (Chapters 2 – 5), and a general discussion (Chapter 6). The data chapters are published, or are manuscripts prepared for journal submission. These chapters are each written in plural, reflecting that they are co-authored manuscripts. One or more of my supervisors is a co-author on each paper given their contribution to formulation of research aims, development of methodology, oversight and mentorship, as well as assistance with manuscript preparation (review and editing) and funding acquisition. For chapter 5, Mr. Quentin Point, a Masters student at Université du littoral, France, conducted the investigation of gametophyte vegetative cultivation under my supervision while completing a work placement at SARDI, and contributed to writing the original draft for that section of the manuscript, while I performed the balance of the experimental work, original draft manuscript preparation and other roles as described below.

The role of co-authors for each paper is described below using CRediT (Contributor Roles Taxonomy) statements, as per https://www.elsevier.com/authors/journal-authors/policies-and-

ethics/credit-author-statement. Further detail is included in the Statement of Authorship for each manuscript; these statements are presented at the start of each data chapter. Because all data chapters are prepared as papers for publication, there is some repetition within the text, particularly in the introductions to each paper and in some of the methods.

Chapter 2. Field trials for aquaculture of native southern Australian seaweeds.

This is a co-authored manuscript formatted for submission to *Aquaculture.* It describes investigation of propagation in brown seaweeds (aim 1) and the initial assessment and fish farm cultivation field trials used to assess feasibility of cultivation and suitability for IMTA of the eight candidate species (aims 1 and 2).

To address aim 1, I reviewed potential propagation methods for the candidate brown seaweeds (see section 1.4.1), and then attempted reproduction using the identified methods. For the red seaweeds, growth from cuttings (aim 1) was assessed in two field trials, with the first of these also comparing potential field cultivation methods (aim 2). The field trials also assessed feasibility of cultivation (aim 2) for the brown seaweeds.

The field trials comprised an initial field trial in Adelaide using all eight candidate species; and a fish farm field trial on a kingfish lease site in Port Lincoln using the species that showed greatest aquaculture potential from initial investigations. Species for the fish farm trial were selected based on their performance in the initial field trial and additionally, for the brown seaweeds, the feasibility of seedstock production, and, for the red seaweeds, growth and N storage ability, which was assessed in the field trials and also in the laboratory (Chapter 4).

To address industry concerns about co-locating seaweed with fish aquaculture, the potential impact of seaweed cultivation aquaculture on skin and gill flukes, two important parasites of kingfish, was also assessed in the fish farm trial.

Cultivation methods for each trial were adapted from those of related farmed species (see section 1.4.1). For the four red seaweeds, the initial field trial assessed growth performance of cuttings grown in the field using methods based on the 'tie-tie' and 'bag net' methods used for farmed Solieriaceae (Ask and Azanza, 2002). The method for the fish farm trial was chosen based on results from the initial trial. For the brown seaweeds, growth in the field was assessed using seedlings threaded onto ropes for both initial and fish farm trials because the initial field trial was conducted concurrently with investigations of seedstock production, and there was insufficient time to perform seeding of ropes with the selected species prior to the fish farm field trial commencing. Both field trials used adaptations of the floating raft method (Sahoo and Yarish, 2005) to suspend specimens at appropriate depth. A single depth was used for the initial trial, while the fish farm trial compared growth of each species at two depths and in two locations within the lease site, one being in-line with the prevailing tidal currents, and one offset.

I assessed N removal and storage ability by comparing growth rates of each species during the two field trials, and by measuring tissue N for the best performing species in the initial trial.

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Kathryn Wiltshire: Methodology, Investigation, Formal Analysis, Visualization, Writing – original draft

Marty Deveney: Conceptualization, Methodology, Supervision, Writing – Review and Editing

Fred Gurgel: Conceptualization, Supervision, Writing – Review and Editing

Jason Tanner: Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Writing – Review and Editing

Chapter 3. Comparing maximum entropy modelling methods to inform aquaculture site selection for novel seaweed species.

This co-authored manuscript has been published in *Ecological Modelling.* It describes the species distribution modelling that was used to determine the relative suitability of Spencer Gulf aquaculture zones for each candidate species (aim 3).

I applied species distribution modelling (SDM) to assess relative environmental suitability for the eight candidate species of existing Spencer Gulf aquaculture zones where seaweed farming is permitted under current legislation (see section 3.1.4).

For the purpose of aquaculture site selection, models with good transferability are required, therefore more parsimonious models are preferred. I compared the performance of default maxent models to that of models applying each of the proposed strategies to avoid over-fitting: increased regularisation, restricted feature types, and the forward selection approach, using a range of performance metrics. Using the most parsimonious models, I then examined predictions of suitability of existing aquaculture zones for each of the eight candidate species.

I focused on biological suitability for the candidate seaweeds in developing these SDMs because other aspects of site suitability for aquaculture are already considered in PIRSA's zone policies.

In addition to providing information on relative habitat suitability for the candidate seaweeds, the SDMs can assist in improving zone policies, underpinning successful development and sustainability of aquaculture in SA.

Authors: Kathryn Wiltshire, Jason Tanner

Kathryn Wiltshire: Conceptualization, Methodology, Software, Formal Analysis, Data Curation, Writing – Original Draft, Visualization

Jason Tanner: Conceptualization, Methodology, Writing – Review and Editing, Supervision, Project Administration

Chapter 4. Exploring novel Rhodophyta species for aquaculture and nutrient remediation.

This is a co-authored manuscript formatted for submission to *Aquaculture.* It describes the laboratory experiments used to select the best candidate species of the red seaweeds for aquaculture (aims 1 and 2), and further investigations of *Gelidium australe* and *Solieria robusta* including: growth responses to temperature, light and N; N uptake dynamics; and micropropagation by explant production (aim 4).

To obtain additional data on the ability of the red seaweeds to grow from cuttings and to accumulate tissue N, I conducted a laboratory experiment in which nutrients were added to simulate conditions around SA fish farms. In this laboratory experiment, the ability of each species to remove and store N was assessed based on changes in tissue N content over the experiment and growth (biomass accumulation).

I carried out further investigation of relevant aspects of the biology of the species that demonstrated greatest aquaculture potential in initial experiments. These investigations focused on: growth responses to temperature light and nutrients, refinement of seedstock production methods, and assessing nutrient uptake rates. Specific aims and methods applied for these investigations were developed based on results of the earlier experiments and tailored to provide relevant data for the species that showed greatest aquaculture potential.

Of the red seaweeds, *Gelidium australe* and *Solieria robusta* grew best in the initial field trial (Chapter 2) and laboratory experiments (Chapter 4) respectively. Data on the growth responses of these two species to light, nutrient and temperature were lacking.

Of the important factors for seaweed growth, temperature is typically the most important for determining broad-scale environmental suitability and seasonal growth responses (Bearham, et al., 2013; Hurd, et al., 2014; Martínez, et al., 2018). Northern Spencer Gulf experiences warmer temperatures and a greater annual variation in temperature than southern Spencer Gulf (Nunes and Lennon, 1986; Petrusevics, 1993). Species temperature responses will therefore provide further information on suitability of cultivation in aquaculture zones throughout Spencer Gulf, and optimal times of year for cultivation and seasonal growth patterns. I therefore investigated temperature responses of *Gelidium australe* and *Solieria robusta* in a laboratory experiment. Based on the results of this experiment and the SDM investigations (Chapter 3), *Solieria robusta* showed the best potential for aquaculture over a wide area of Spencer Gulf, and was the focus of additional investigation of light and nutrient responses, and of refinement of seedstock production.

I conducted a laboratory experiment to investigate growth responses of *Solieria robusta* to light and ammonium. Ammonium was used as the nutrient source because this is the primary form of N produced by fish farms. Light levels were chosen to be representative of those that would be experienced by seaweeds cultivated in fish farming regions of SA.

To provide data to incorporate seaweed N removal into dynamic biogeochemical models (e.g. Broch, et al., 2013; Hadley, et al., 2015), I investigated N uptake rates of *Solieria robusta* using both ammonia and nitrate as N sources.

While red seaweeds can be grown from cuttings, micropropagation methods allow production of more seedstock from the best performing plants, providing a larger number of propagules with desirable traits than simple vegetative reproduction (Reddy, et al., 2008; Yong, et al., 2014). Micropropagation of *Solieria robusta* was therefore investigated using methods that have been applied for related farmed species (Solieriaceae).

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Kathryn Wiltshire: Conceptualization, Methodology, Investigation, Formal Analysis, Visualization, Writing – original draft, Funding acquisition

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Marty Deveney: Conceptualization, Supervision, Writing – Review and Editing

Jason Tanner: Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Writing – Review and Editing

Chapter 5. Hatchery production and nutrient remediation potential of the common kelp *Ecklonia radiata*.

This is a co-authored manuscript formatted for submission to *Algal Research.* It describes the investigations into feasibility of *Ecklonia radiata* string seeding and vegetative gametophyte cultivation, plus N uptake dynamics and N responses of *Ecklonia radiata* sporophytes in hatchery cultivation (aim 4).

Ecklonia radiata grew best of the brown species in the initial field trial, and spore release and string seeding were successful, demonstrating the feasibility of propagating this species (Chapter 2). I therefore further investigated important aspects of propagation and cultivation for this species.

To assess string seeding, I seeded *Ecklonia radiata* spores onto three types of string that are used in cultivation of Laminariales and assessed seedling growth.

For Laminariales, vegetative cultivation of gametophytes may be performed to ensure year-round supply of seedstock and to facilitate strain selection or improved string seeding (Flavin, et al., 2013; Sahoo and Yarish, 2005). In the absence of blue light, gametophytes do not become reproductive, therefore cultivating gametophytes under red light maintains them in a vegetative state, and reproduction can be triggered when required by exposure to blue or full spectrum light (Edwards and Watson, 2011; Flavin, et al., 2013; Redmond, et al., 2014).

I therefore investigated the feasibility of vegetative gametophyte cultivation for *Ecklonia radiata* using methods applied to other Laminariales.

Ecklonia radiata is one of the best studied Australian seaweeds, and its light and temperature responses are characterised (Bearham, et al., 2013; Mabin, et al., 2013; Staehr and Wernberg, 2009), but responses of this species to nutrient enrichment have not been studied, and fertilising Laminariales seedlings during the hatchery grow-out stage can improve at-sea performance (Rößner, et al., 2014). I therefore investigated optimum nutrient addition for hatchery cultivation using young seedlings of *Ecklonia radiata*.

To provide data to incorporate seaweed N removal into dynamic biogeochemical models (e.g. Broch, et al., 2013; Hadley, et al., 2015), I investigated N uptake rates for *Ecklonia radiata*, using both ammonia and nitrate as N sources.

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Quentin Point: Investigation, Writing – original draft

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Marty Deveney: Conceptualization, Supervision, Writing – Review and Editing

Jason Tanner: Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Writing – Review and Editing

Chapter 6. General discussion

In this chapter, I discuss the overall findings of the research presented in the data chapters, and suggest future research directions for development of seaweed farming and integrated multi-trophic aquaculture in southern Australia.

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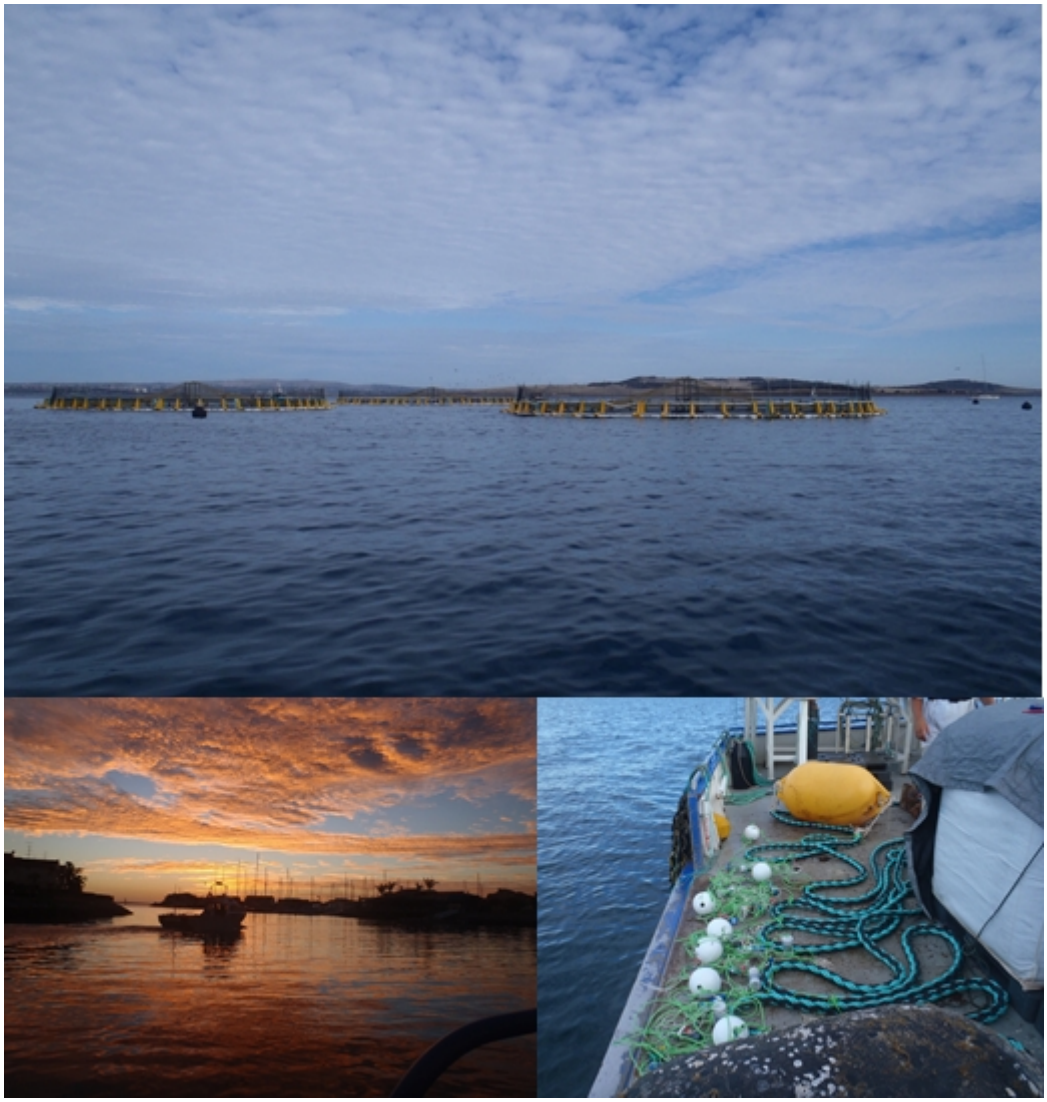
Chapter 2. Field trials for aquaculture of native southern Australian seaweeds



Preparing to deploy the fish farm trial: Yellowtail Kingfish cages on the fish farm lease site, an early departure from Port Lincoln marina, and seaweed aquaculture longline ready for deployment.

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Kathryn. H. Wiltshire		
Contribution to the Paper	Designed experiments, carried out field and laboratory work, designed cultivation system for fish farm trial, analysed data and prepared the manuscript		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	C. Frederico D. Gurgel		
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Contribution to the Paper	Assisted with assessment of fluke egg occurrence for the fish farm trial, provided advice on experimental design and assisted with review and editing of the manuscript		
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Contribution to the Paper	Obtained funding for the project, assisted with design of cultivation systems and with field work for pilot field trial, provided advice on experimental design and statistical analysis, assisted with review and editing of the manuscript		
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Field trials for aquaculture of native southern Australian seaweeds

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Abstract

Native seaweeds have not been cultivated in southern Australia, but there is interest in applying seaweed aquaculture to mitigate nutrients from fish farming. We investigated aquaculture potential of eight seaweeds native to temperate Australia, comprising four red (*Solieria robusta, Gelidium australe, Pterocladia lucida, Plocamium angustum*) and four brown (*Ecklonia radiata, Cystophora subfarcinata, Sargassum linearifolium, Scytothalia dorycarpa*) species, during two field trials. *Gelidium australe* and *Ecklonia radiata* were the best performing species of the red and brown seaweeds respectively during an initial 12-month assessment trial in Adelaide, South Australia (SA), where all species grew best over spring. These species, along with the red *Solieria robusta* and the brown *Cystophora subfarcinata,* which both performed acceptably in the initial trial and showed suitable characteristics for aquaculture in other work, were used in a fish farm field trial. The fish farm trial investigated seaweed performance on a fish farm lease near Port Lincoln, SA, to determine the potential application of these species to integrated multi-trophic aquaculture. This trial was impacted by fouling of the seaweeds and associated ropes and bags used for cultivation, but *Solieria robusta* nonetheless showed promising growth over spring. To address industry concerns about co-location of seaweed and fish farming, during the fish farm trial we examined the bags and ropes used to attach seaweeds to long lines for eggs of commercially important fish parasites (skin and gill flukes). Fluke eggs were found, but numbers were low in comparison to the typical numbers that occur on fish cages, suggesting seaweed aquaculture in the vicinity of fish cages should not negatively impact fluke infections or management.

1 Introduction

Seaweed aquaculture is not an established industry in Australia, but is of interest due to increasing demand for seaweed products, of which Australia is a net importer (Lee, 2010), and also for nutrient mitigation. Several finfish species are farmed in Australia, and there is a strong emphasis on management of the aquaculture industry to ensure environmental sustainability (Rimmer and Ponia, 2007). In South Australia (SA), two marine fish: Southern Bluefin Tuna, *Thunnus maccoyii* (Castelnau, 1872), and Yellowtail Kingfish, *Seriola lalandi* (Valenciennes 1833), are farmed, primarily in southern Spencer Gulf. These are both predatory fish with high food conversion ratios, particularly tuna, which are fed baitfish rather than manufactured pellet feed. Each ton of production releases 200 kg (kingfish) to 500 kg (tuna) of nitrogen (N), with 50 – 70 % in dissolved form (Fernandes and Tanner, 2008; Fernandes, et al., 2007). Dissolved N is the nutrient limiting the environmental carrying capacity of fish aquaculture in southern Spencer Gulf (Collings, et al., 2007; Middleton, et al., 2013; Tanner, et al., 2007) and seaweed aquaculture could be applied to extract dissolved nutrient (Kim, et al., 2017; Neori, 2008), improving sustainability of the finfish aquaculture industry (PIRSA, 2013).

This type of strategic co-culture is termed integrated multi-trophic aquaculture (IMTA), and provides economic and environmental benefits by removing and recycling dissolved nutrient waste from fish aquaculture into valuable biomass (Barrington, et al., 2009; Neori, et al., 2004; Troell, et al., 2003). IMTA systems also reduce economic risks for farmers through crop diversification (Barrington, et al., 2009; Ridler, et al., 2007; Soto, 2009), and can achieve greater productivity and profitability than monoculture systems (Abreu, et al., 2009; Petrell and Alie, 1996; Troell, et al., 2003; Whitmarsh, et al., 2006).

Farming native species is clearly desirable to ensure they are appropriate for the habitat and to avoid the risks involved with introduced species (Barrington, et al., 2009; Williams and Smith, 2007). Few seaweeds with established farming technology are native to Australia; therefore, local seaweed species that have not been previously cultivated will need to be used to develop a seaweed farming industry in Australia. We investigated the aquaculture suitability of four red (Rhodophyta: Florideophyceae) and four brown (Ochrophyta: Phaeophyceae) seaweed species that occur naturally in southern Spencer Gulf. Candidate seaweed species were chosen based on desirable characteristics for aquaculture such as suitable size and likely economic value (Wiltshire, et al., 2015), but relatively little is known about the biology of any of these species making it difficult to determine which are most suitable for aquaculture in this region.

The red species used were: *Pterocladia lucida* (R. Brown ex Turner) J. Agardh (Pterocladiaceae), *Gelidium australe* J. Agardh (Gelidiaceae), *Solieria robusta* (Greville) Kylin (Solieriaceae), and *Plocamium angustum* (J. Agardh) J.D. Hooker & Harvey (Plocamiaceae). *Pterocladia lucida* and *Gelidium australe* are agar producers (Brasch, et al., 1984; Gordon-Mills, et al., 1990), while *Solieria robusta* produces ι-carrageenan (Chiovitti, et al., 1999). *Plocamium angustum* is of potential commercial interest as a feed for farmed abalone (Kirkendale, et al., 2010), and as a source of bioactives (Timmers, et al., 2012).

The brown seaweeds comprised one kelp species (Laminariales): *Ecklonia radiata* (C. Agardh.) J. Agardh (Lessoniaceae), and three Fucales: *Scytothalia dorycarpa* (Turner) Greville (Seirococcaceae), *Cystophora subfarcinata* (Mertens) J. Agardh, and *Sargassum linearifolium* (Turner) C. Agardh (both Sargassaceae). These Phaeophyceae have potential uses as food, and as a source for alginates and secondary metabolites, including several bioactive

polyphenols (Charoensiddhi, et al., 2015; Charoensiddhi, et al., 2017; Holdt and Kraan, 2011; Lorbeer, et al., 2013; Skrzypczyk, et al., 2018; Smit, 2004; Thomas and Kim, 2011; White and Wilson, 2015).

The majority of the seaweed species we considered have not been farmed, but were assessed as likely to be able to be grown using existing technology, e.g. methods adapted from those used for farmed relatives (Wiltshire, et al., 2015). We reviewed offshore cultivation methods successfully implemented for species related to those used in this study, including in the same genera or families where possible. These related species include: *Ecklonia* spp. (e.g. Hwang, et al., 2009) and other Laminariales, e.g. commercially cultivated *Laminaria* and *Saccharina* spp. (see Kim, et al., 2017; Sahoo and Yarish, 2005; Titlyanov and Titlyanova, 2010); *Sargassum* spp. (e.g. Hwang, et al., 2007; Li, et al., 2010; Pang, et al., 2007; Pang, et al., 2009; Zou, et al., 2012); Gelidiales, including *Gelidium* spp. (e.g. Boulus, et al., 2007; Fei and Huang, 1991; Friedlander, 2008; Rojas, et al., 1996; Seoane-Camba, 1997); and Solieriaceae, including the commercially farmed *Kappaphycus* and *Eucheuma* spp. (e.g. Ask and Azanza, 2002; Góes and Reis, 2011; Neish and SuriaLink Seaplants, 2003; Sahoo and Yarish, 2005), and *Solieria* spp. (e.g. Caamal-Fuentes, et al., 2017; Fournet, et al., 1999; Goulard, et al., 2001; Penuela, et al., 2018; Zepeda, et al., 2020).

Laminariales and Fucales typically do not regrow from cuttings, making controlled reproduction a critical step and a determinant of the aquaculture feasibility of these taxa. We therefore considered available protocols for reproduction of the brown seaweeds in addition to methods for field cultivation. Farming of Laminariales and Fucales typically involves settling spores or zygotes, respectively, directly onto rope substrates, or threading nursery cultivated

seedlings onto rope for out-planting (Kim, et al., 2017; Sahoo and Yarish, 2005; Titlyanov and Titlyanova, 2010).

Laminariales have heteromorphic alternating generations with a typically large conspicuous sporophyte and microscopic filamentous gametophyte. Motile spores are produced in sori located on the central blade and/or laterals of the sporophyte (Sahoo and Yarish, 2005; Womersley, 1987). 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). Experimental cultivation of *E. radiata* in New Zealand has been carried out using spores settled onto rope, following the typical methods applied for Laminariales (Neill, et al., 2009).

Fucales do not show alternating generations and have no gametophyte stage, with adult plants producing male and female gametes directly (Womersley, 1987). Many *Sargassum* species, including the majority of farmed representatives, are dioecious, hence synchronization of reproduction in male and female plants is an important consideration in their cultivation (Hwang, et al., 2007; Li, et al., 2010; Pang, et al., 2007; Pang, et al., 2009; Zou, et al., 2012). 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 (De Wreede, 1978; Deysher and Norton, 1981; Monteiro, et al., 2009). For cultivation, zygotes are collected by rubbing or washing them from the female reproductive structures (Hwang, et al., 2007; Kim, et al., 2017; Pang, et al., 2005; Zhao, et al., 2008). No *Cystophora* spp. or Seirococcaceae have been cultivated, but light and temperature manipulation can be applied to stimulate gamete release in a range of fucalean species, including these taxa (e.g. Burridge, et al., 1993; Klemm, 1988; Klemm and Hallam, 1987; Taylor and Schiel, 2003). In contrast to many farmed Fucales, the three species we considered are all monoecious (Womersley, 1987).

Cultivation methods for Gelidiales and Solieriaceae include using cuttings tied to ropes, shells, stones or concrete cylinders, or contained in mesh bags or tubes (Ask and Azanza, 2002; Friedlander, 2008; Ganesan, et al., 2011; Góes and Reis, 2011; Kim, et al., 2017). Cuttings are typically taken from the best performing specimens in each cultivation cycle to seed the next cycle (Ask and Azanza, 2002; Neish and SuriaLink Seaplants, 2003), or partial harvesting is used to leave fragments from which the seaweed regrows (Ganesan, et al., 2011). Plocamiales have not been farmed but are likely to be amenable to cultivation using existing red seaweed protocols (Kirkendale, et al., 2010).

To assess the suitability for aquaculture of the eight species and investigate their seasonal growth patterns, we performed an initial 12-month field trial in Adelaide, SA, during which two attachment methods (bag and tie), selected from the reviewed methods, were trialed to grow the red seaweeds, and a single method (threaded) for the browns. The feasibility of sexual reproduction, i.e. obtaining spores for *Ecklonia*, or gametes for Fucalean species, was investigated in the laboratory concurrently with field trials. Based on results of the initial field trial, results of our laboratory investigations on propagule production in the brown seaweeds, and data from separate laboratory investigations of the candidate red seaweeds (Wiltshire, et al., 2015, Chapter 4), four species were selected for further study on a Yellowtail Kingfish farm off Port Lincoln, SA. The Port Lincoln fish farm trial was used to assess the performance of the selected species in an IMTA system.

Co-cultivation of species in IMTA systems may have implications for transmission of parasites and disease between cultivated species, especially where one species is an intermediate host or reservoir of a disease or parasite affecting the other (Skar and Mortensen, 2007; Troell, et al., 2003), but this aspect of IMTA is rarely studied (Soto, 2009). External parasitic flatworms

(flukes) are an ongoing health issue for cultivation of yellowtail kingfish (Chambers and Ernst, 2005; Ernst, et al., 2002). Skin (*Benedenia seriolae*) and gill (*Zeuxapta seriolae*) flukes occur naturally in wild populations of Yellowtail Kingfish and can proliferate on farmed fish due to the parasites' direct lifecycles and high host fish density (Whittington, 2012). Flukes are controlled by in-feed or immersion treatments of fish, strategic cage placement and hygiene, but reinfection occurs from fluke eggs, which are resistant to treatment and attach to fish cage infrastructure, or from wild fish (Chambers and Ernst, 2005; Ernst, et al., 2002). Although seaweed is not a host for flukes, placing additional cultivation 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. We therefore investigated the potential of seaweed aquaculture infrastructure to harbor eggs of skin and gill flukes during the fish farm trial.

2 Materials and Methods

2.1. Seaweed material

Due to lack of existing seedstock or established seedling production techniques for the species considered, all field trials were conducted using wild-collected specimens. Specimens of all red species, except *So. robusta*, were collected at 3-8m depth from Granite Island, SA (35° 33′ 59″ S, 138° 37′ 41″ E). *Solieria robusta* was collected at ~ 3 m depth from Outer Harbor (34° 48′ 14″ S, 138° 28′ 24″ E). Additional specimens of *G. australe* and *Pt. lucida* were obtained at 2 –5 m depth from Chinamans Hat (35° 17′ 19″ S, 136° 55′ 5″ E). *Ecklonia radiata* was collected at Outer Harbor, *Sc. dorycarpa* at Granite Island and Chinaman's Hat, *C. subfarcinata* at Rapid Bay (35° 31′ 18″ S, 138° 11′ 09″ E) in ~ 2 m depth, and *Sa. linearifolium* at Hallet Cove (35°04′ 25″ S, 138° 29′ 40″ E) in ~ 5 m depth. Specimens were held in outdoor tanks at the South Australian Aquatic Science Centre (SAASC) in West Beach, Adelaide, SA, for

one to four weeks before use in field trials. These tanks were supplied with flow-through sand-filtered natural seawater, sourced via pipeline from Gulf St Vincent, at ambient temperature and with no additional nutrient supplied.

2.2. Feasibility of reproduction

We explored sexual reproduction for the brown seaweeds since Laminariales and Fucales tend not to regrow from cuttings. Feasibility of cultivation is therefore dependent on sexual reproduction to obtain seedstock. For red seaweeds we assessed ability to grow from cuttings.

During each collection of seaweed material for field deployment, surplus stock of each species was collected where sufficient biomass was available. Collected specimens of *E. radiata, Sc. dorycarpa, C. subfarcinata*, and *Sa. linearifolium* were examined at the time of collection using descriptions from Womersley (1987) to identify fertile structures. Seaweed material that was surplus to field trial requirements was maintained in the outdoor tanks at SAASC and examined monthly for fertility. Where fertile material was found, reproduction was attempted as described below.

Ecklonia radiata: Based on methods applied by Neill, et al. (2009) and Hwang, et al. (2009), clean sections of the central blade with fertile tissue (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 a shallow tray. Gentle agitation by hand was applied periodically over a period of four hours. Water samples were examined under a compound microscope to assess if spores were present.

Sargassum linearifolium: Based on methods described for other *Sargassum* spp. (Hwang, et al., 2007; Pang, et al., 2005; Zhao, et al., 2008), fertile branches were excised and placed in glass aquaria with filtered seawater and aeration provided to keep branches in constant motion. Unlike several farmed *Sargassum* spp., *S linearifolium* is monoecious, therefore we did not need to obtain separate male and female specimens, but the branches used in the experiments were from several individuals in each case because it is unclear if *S. linearifolium* is self-fertile. Fertile structures were examined daily under a dissecting microscope to assess if zygotes were present.

Cystophora subfarcinata and *Sc. dorycarpa*: Following published methods for obtaining gametes from *Cystophora* spp. (e.g. Klemm, 1988; Klemm and Hallam, 1987; Taylor and Schiel, 2003) and Seirococcaceae (e.g. Burridge and Hallam, 1993; Burridge, et al., 1993; Schoenwaelder and Clayton, 2000), 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 light and allowed to warm slightly to stimulate gamete release. Water samples were examined under a dissecting microscope to assess if zygotes were present.

2.3. Initial field experiment

The initial field experiment to compare the eight species and assess cultivation methods was carried out from October 3rd 2012 to October 4th 2013 in Gulf St Vincent, Adelaide, SA (34° 54’ 14” S, 138° 28’ 16” E), and consisted of six deployments of approximately 2 months each (Table 1). Each deployment is referred to in the text 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.

Attachment methods tested for the red seaweeds were tie and bag, 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. Specimens were suspended at approximately 5 m low tide water depth on anchored PVC frames. PVC frames were 3 m long and 1.5 m wide, with four ropes strung across the width of each frame at 0.5 m intervals. Ropes with bags, tied specimens or brown seaweeds attached were clipped to these ropes with a spacing of 0.5 m between specimens.

The number of replicate specimens used for each treatment ranged from four to six depending on specimen availability, with specimens randomly assigned to treatments and positions, and new specimens used for each deployment. Due to limited biomass availability and the use of wild-collected, rather than hatchery produced specimens, we monitored performance of discrete seaweeds. The PVC frames we used were therefore designed to maintain specimens in bags or on ropes at appropriate depth while allowing identification and individual monitoring of replicates, and were not intended to emulate a potential commercial set-up.

Algal fresh weights were obtained the day before each deployment and within 24 hours after retrieval for each specimen. Specimens were kept cool and in a small amount of seawater to prevent desiccation between collection and weighing. Fresh weights were measured after gently patting specimens dry 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 not retrieved from the field and for these lost specimens SGR is undefined as FW_t is unknown. 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 the SGR analysis. SGR cut-offs were chosen based on initial visualisation and assessment of SGR data that showed values below these cutoffs to be outliers, and exclusion of these points resulted in datasets that fulfilled ANOVA assumptions. The cut-off values were further supported by examination of retrieved specimens; the specimens regarded as functionally lost comprised only small residual fragments in the case of red seaweeds, and holdfasts or denuded stems only in the case of browns. The chosen cut-off varied between reds and browns due to the fact that the calculation of SGR includes initial weight, and the average initial weight of the brown seaweeds was much greater than that of red seaweeds (mean ± s.e. 52.2 ± 5.7 g for browns [n=106] and 13.5 ± 0.8 g for reds [n = 182]). The number of retrieved specimens, excluding total and functional losses, is shown in Table 3.

Table 2. Deployment dates and summary of environmental conditions (mean ± standard error, n = number of days) for the initial field trial of eight seaweeds in Adelaide, South Australia from October 2012- October 2013.

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

To investigate nutritional status during each cultivation period, samples for tissue N content (N%) 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) were obtained from the Australian Bureau of Meteorology (www.bom.gov.au/climate/data) for the weather station nearest to the location to the initial field trial (Adelaide Airport, station number 023034). Insolation recorded at Adelaide airport is well correlated with subsurface photosynthetically active radiation 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 ms^{-1}) westerly winds was therefore used as a proxy for the relative likelihood of rough sea conditions in each deployment.

2.4. Fish farm field trial

A fish farm field trial consisting of three cultivation periods, over two deployment and collection times, was located on a Yellowtail Kingfish farm lease near Boston Island, Boston Bay, Southern Spencer Gulf (34° 42’ 27” S, 135° 55’ 53” E), offshore (east) from Port Lincoln. The three cultivation periods assessed were: 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). The initial experimental design was planned to consist of three seasonal deployments of ~ 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.

Two long-lines were set up in the south-eastern corner of the farm site, each approximately 150 m from a stocked cage, with one being located south of the cage, in line with the prevailing tidal movement, and the other east, offset from tidal flow. Six PVC cultivation frames were attached to each long-line, with three at each of two depths: approximately 2 m (shallow) and 5 m (deep) below the water surface. PVC frames were 4 m long and 2 m wide, with four ropes strung along the length of each frame at 0.4 m intervals. Bags containing red seaweed specimens or ropes with brown seaweeds attached were clipped to these ropes with a spacing of 0.4 m between specimens. As per the initial trial, these PVC frames were designed to maintain replicates at each experimental depth, rather than to emulate all aspects of a commercial cultivation system, for which we did not have sufficient biomass available.

Three specimens of each species were attached to each frame. The species used were the reds: *G. australe* and *So. robusta*, chosen based on growth performance in the initial field experiments (section 3.3) and concurrent laboratory experiments (Wiltshire, et al., 2015; Chapter 4); and the browns: *E. radiata* and *C. subfarcinata,* which were those that showed greatest potential for seedstock production in laboratory experiments (see section 3.2), given minor differences in field growth during the initial trial (see section 3.3). 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 as per the initial trial.

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 described in section 2.2. Samples for N% were taken from each collected specimen after weighing.

For fluke egg examination, two of the three replicates of each treatment were randomly selected from two cultivation periods: Mar-Aug, and Aug-Nov. The bags used to house red seaweed specimens and the ropes used for browns of the selected replicates 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 were obtained from the Australian Bureau of Meteorology (www.bom.gov.au/climate/data) for the weather station nearest to the fish farm trial location (Port Lincoln South, station number 018205).

2.5. Chemical analyses

Samples for N% were frozen, freeze-dried overnight, and then ground to a fine powder using a Fritsch stainless steel ball mill. A 100 mg aliquot was analysed on a LECO Truspec CNS Elemental Analyser (LECO, St Joseph, MI, USA).

2.6. Statistics

Analyses were performed in *R* (R Core Team, 2019) except as otherwise noted, and an α of 0.05 was used in all cases. Due to the different cultivation methods applied to red and brown seaweeds, these groups were analysed separately for each trial.

The frequency of specimen loss from both trials was analysed by logistic regression, using the *glm* routine and binomial (logit) link function, with nested models compared using likelihood ratio tests.

SGR and N% from the initial field trial were analysed using the *lm* routine and the *car* package (Fox and Weisberg, 2011), with Type III sums of squares, after confirming normality by QQ plots and homoscedasticity by Levene's test. A three-way ANOVA was used for the reds to test effects of species, deployment, and attachment method, with two-way ANOVA used to test the effect of species and deployment for the browns. N% data from the fish farm trial 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., 2019).

For factors having more than two levels, pairwise post-hoc tests were performed using *glht* in the *multcomp* package (Hothorn, et al., 2008) where main effects were significant, with control of false discovery rate (Benjamini, et al., 2006; Verhoeven, et al., 2005). Where significant interaction terms were found, pair-wise tests were performed between factors within levels of the interacting factor.

SGR data from the fish farm trial were highly heteroscedastic even after attempted transformation. In contrast to data from the initial trial, there were no clear outliers in the SGR data from the fish farm trial. Univariate permutational ANOVA (with the PERMANOVA routine) was therefore utilised 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 effects were found, PERMDISP was used to assess if significant differences in multivariate dispersion were present between groups. Euclidean distance was used as the dissimilarity measure, with 9999 permutations; Monte-Carlo p-values were used if less than 1000 unique permutations occurred.

Patterns of fluke egg occurrence with line position (offset or inline) and cultivation period (Mar-Aug or Aug-Nov) from the fish farm trial were analysed by *glm* with binomial (logit) link function and likelihood ratio tests of nested models.

3 Results

3.1. Field trial environmental conditions

During the initial 12-month Adelaide trial over October 2012 – October 2013, average water temperature ranged from a minimum of 14.2°C in the Jul deployment to a maximum of 22.2°C in the Jan deployment. Peak average insolation per deployment occurred in Nov (29.4 MJ), with the lowest average in May (8.9 MJ). Nov had the most frequent strong westerly winds, followed by Oct, while May had the lowest. During the 8-month Port Lincoln fish farm trial in 2014, monthly average water temperature ranged from 13.0 (July) – 19.7°C (November), and insolation from 7.4 (May) – 21.1 MJ (November). Environmental data are summarised in Table 1 for the initial trial and Table 2 for the fish farm trial.

3.2. Brown seaweed reproduction

Freshly collected *E. radiata* with sori were observed in March 2013. Sori were also observed on collected specimens 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 specimens of *Sa. linearifolium* were found in October and November 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 *C. subfarcinata* was found in September 2012 and zygotes were successfully obtained. *Sc. dorycarpa* specimens with apparently mature fertile structures were collected in September and October 2012. This species, however, showed poor survival in holding tanks and poor growth performance in the field (see section 3.3), hence reproduction was not attempted for this species.

Table 3. Deployment dates and summary of environmental conditions (mean ± standard error, n= number of days per month) for the 2014 fish farm field trial.

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

3.3. initial field experiment results

In the initial (2012-13) trial, 149 of 176 specimens of the red seaweeds were retrieved across the deployments, but seven of these were regarded as functionally lost, while for the browns, 90 of 95 specimens were retrieved, with 10 of these considered functionally lost (Table 3). For the retrieved red seaweeds, there were significant differences in SGR between both species and attachment methods contingent upon the deployment period, as shown by significant deployment x species and deployment x attachment interaction terms in the ANOVA (Table 4). Greatest growth was achieved in the Oct deployment for all four reds, while many specimens lost biomass in Nov, Jan and Mar deployments (Figure 1). *Plocamium angustum*

was only used in the first three deployments due to difficulty in obtaining enough biomass. *G. australe* was the fastest growing species in the Oct, May and Jul deployments.

For the browns, ANOVA showed a significant interaction of species and deployment on SGR (Table 4). *Ecklonia radiata* showed lower growth in Jan and Mar, and *Sa. linearifolium* showed lower growth in Nov and Mar (Figure 2) compared with other deployment periods. SGR of *C. subfarcinata* and *Sc. dorycarpa* did not vary significantly with deployment. Note that *Sc. dorycarpa* was used only in Oct and Nov, and *Sa. linearifolium* was not used in May, due to insufficient biomass of these species being available. *Scytothalia dorycarpa* had also demonstrated poor survival in holding tanks when collected for the first two deployments, and so was not used in subsequent deployments or considered further. SGR was not significantly different between the brown species except in the Jul deployment, where SGR of *E. radiata > Sa. linearifolium > C. subfarcinata.*

Table 4. Number of seaweed specimens retrieved for each deployment of the initial 2012-2013 field trial (total deployed shown in brackets) by species and attachment method (only one attachment method was used for brown seaweeds). Specimens were regarded as functionally lost if specific growth rate (SGR) < -3 for reds or < -1 for browns and excluded from counts of retrieved specimens and SGR analysis. NA indicates where a species was not used in that deployment.

		Deployment					
Species	**Method**	**Oct**	**Nov**	**Jan**	**Mar**	**May**	**Jul**
G. australe	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)
Pt. lucida	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)
So. robusta	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)
Pl. angustum	Bag	4 (4)	4 (4)	4 (4)	NA	NA	NA
	Tie	4 (4)	4 (4)	1 (4)	NA	NA	NA
C. subfarcinata	Threaded	4 (4)	3 (4)	5 (5)	4 (5)	5 (5)	6 (6)
E. radiata	Threaded	4 (4)	4 (4)	5 (5)	3 (5)	6 (10)	6 (6)
Sa. linearifolium	Threaded	4 (4)	2 (4)	1 (5)	5 (5)	NA	6 (6)
Sc. dorycarpa	Threaded	3 (4)	1 (4)	NA	NA	NA	NA

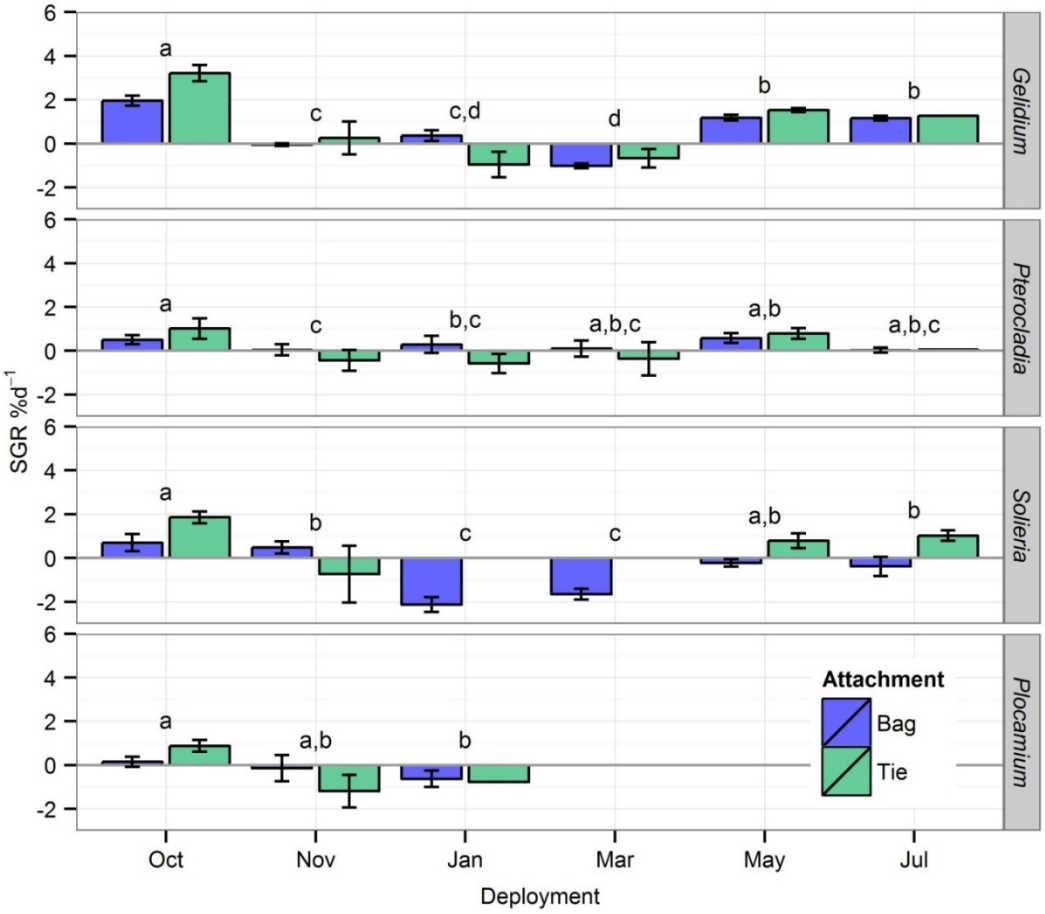

Figure 2. Mean SGR of red seaweeds (*Gelidium = G. australe, Pterocladia = Pt. lucida, Solieria = So. robusta, Plocamium = Pl. angustum*) over the six deployments of the initial trial from October 2012 – October 2013. Error bars show standard error (n = number of retrieved specimens, range 0 – 6, see Table 3). Shared letters indicate no significant differences between deployments (across attachment method) within each species. Note, no tied specimens were retrieved for *So. robusta* in Jan or Mar; *Pl. angustum* was used only in Oct, Nov, Jan.

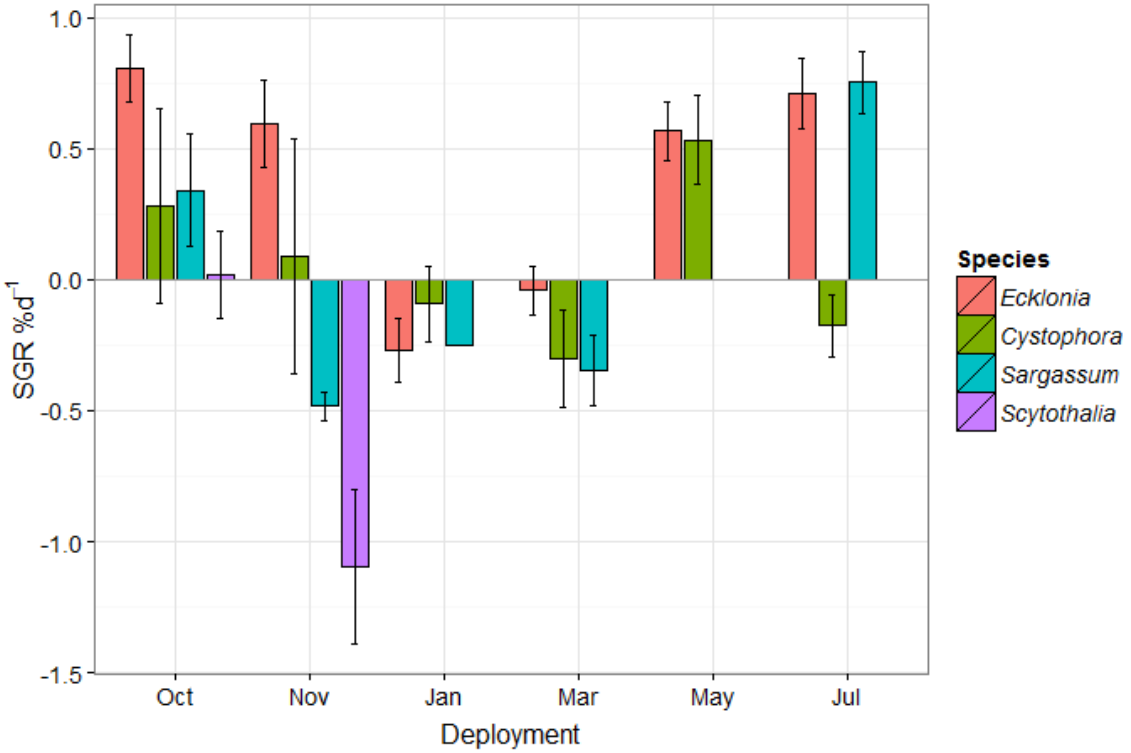

Figure 3. Mean SGR of brown seaweeds (*Ecklonia = E. radiata, Cystophora = C. subfarcinata, Sargassum = Sa. linearifolium, Scytothalia = Sc. dorycarpa*) over the six deployments of the initial trial from October 2012 – October 2013. Error bars show standard error (n = number of retrieved specimens, range 1 – 6, see Table 3). Note, *Sc. dorycarpa* was used only in Oct, Nov, *Sa. linearifolium* was not used in May.

The pattern of losses (actual + functional) for the red seaweeds during the initial trial were similar to the patterns in SGR and 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 angustum* was not included in this analysis due to 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. 30 tied specimens (plus 3 tied *Pl. angustum*) were lost compared to 4 bagged specimens, with losses of tied specimens occurring mainly in Jan (8, + 3 *Pl. angustum*), Mar (9) and Jul (7), while most losses of bagged

specimens (3) occurred in May. 17 of 55 specimens of *So. robusta* were lost, mainly in Jan (5) and Mar (6), compared to 10 of 55 specimens of *G. australe*, mainly in Mar (3) and Jul (4), and 6 of 48 *Pt. lucida* (3 in Jan).

For the browns, the likelihood of loss over the initial trial 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 dorycarpa* was not included in this analysis because it was used in only two deployments; 4 of the 8 *Sc. dorycarpa* specimens were lost (1 in Oct, 3 in Nov). For the other three brown seaweeds, most losses occurred in May (5), followed by Jan (4), with 3 specimens lost in each of the Mar and Oct deployments. 6 of 24 *Sa. linearifolium* specimens were lost, 3 of 30 *C. subfarcinata* and 6 of 30 *E. radiata. Sargassum linearifolium* specimen losses occurred mainly 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 *Sargassum* species (Womersley, 1987).

N% of *G. australe* and *S. robusta* (Table 5) over the initial trial was highly variable, with a significant deployment x species x attachment method interaction (Table 4). Due to poor growth of *P. angustum* and *P. lucida*, chemical analyses were not performed for these species. Bagged specimens of *G. australe* and *S. robusta*, plus tied *G. australe*, had highest N% in Jan and lowest in Oct and July, while there was no significant difference in N% of tied *S. robusta* between deployments. N% of *G. australe* was greater than *S. robusta* for bagged specimens in Oct, Nov, Jan and Mar, and for tied specimens in Jan. Bagged specimens had higher N% than tied for both species in Jan and for *G. australe* also in Oct and Nov. Not all pair-wise tests could be performed because in some deployments only one or no tied specimens were retrieved (Table 3).

There was a significant deployment x species interaction for N% of *E. radiata* and *C. subfarcinata* (Table 5)*. C. subfarcinata* had highest N% in Mar, while *E. radiata* had highest N% in May (Table 5). N% of *C. subfarcinata* was greater than *E. radiata* in Jan and Mar. N% was not analysed for *Sc. dorycarpa* due to poor growth performance or for *Sa. linearifolium* due to lack of specimens caused by unavailability of material for deployments or by losses during deployments.

Table 5. Three-way ANOVA results for specific growth rate (SGR) and Nitrogen content (N%) data for brown and red seaweeds from the initial (2012-2013) field trial. Note that N% data for brown seaweeds was log transformed to achieve homoscedasticity. A single attachment method was used for brown seaweeds.

Factor	**SS**		**df**		**F**		**p-value**	
Brown species	SGR	logN%	SGR	N%	SGR	logN%	SGR	logN%
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
Red species	SGR	N%	SGR	N%	SGR	N%	SGR	N%
Deployment (Dep)	1.19	10.073	5	5	16.31	35.76	<0.001	<0.001
Species (Sp)	41.38	0.521	3	1	4.91	9.31	0.002	0.003
Attachment (Attach)	7.47	0.232	1	1	0.03	4.15	0.520	0.046
Dep x Sp	0.01	0.695	12	5	3.63	2.48	0.003	0.042
Dep x Attach	22.12	0.460	5	5	3.47	1.64	0.005	0.164
Sp x Attach	8.81	0.003	3	1	0.25	0.061	0.714	0.807
Dep x Sp x Attach	0.38	0.660	10	4	0.93	2.95	0.521	0.028

Table 6. Mean nitrogen content (N%) ± standard error of seaweeds *G. australe* and *So. robusta* (reds) and *C. subfarcinata* and *E. radiata* (browns) from the initial (2012-2013) field trial. n = number of specimens.

Deployment	**Species**	**n**		**N (% d.w.)**	
	Red	Bag	Tie	Bag	Tie
Oct	*G. australe*	4	4	1.42 (±0.06)	1.05 (±0.1)
	So. robusta	4	4	0.91 (±0.14)	0.96 (±0.18)
Nov	*G. australe*	4	4	1.98 (±0.03)	1.57 (±0.07)
	So. robusta	4	1	1.1 (±0.04)	1.09
Jan	*G. australe*	4	3	2.78 (±0.04)	2.51 (±0.08)
	So. robusta	4	1	2.44 (±0.1)	1.31
Mar	*G. australe*	5	5	1.96 (±0.07)	1.68 (±0.22)
	So. robusta	6	2	1.42 (±0.09)	1.95 (±0.02)
May	*G. australe*	3	3	1.56 (±0.21)	1.76 (±0.1)
	So. robusta	2	4	1.45 (±0.09)	1.34 (±0.16)
Jul	*G. australe*	1	4	1.01	0.94 (±0.08)
	So. robusta	3	4	0.81 (±0.03)	1.42 (±0.06)
	Brown	Threaded			
Oct	*C. subfarcinata*	4		0.82 (±0.31)	
	E. radiata	4		0.54 (±0.09)	
Nov	*C. subfarcinata*	4		0.7 (±0.04)	
	E. radiata	4		0.5 (±0.15)	
Jan	*C. subfarcinata*	5		0.94 (±0.16)	
	E. radiata	5		0.5 (±0.07)	
Mar	*C. subfarcinata*	5		1.14 (±0.24)	
	E. radiata	3		0.6 (±0.09)	
May	*C. subfarcinata*	5		0.87 (±0.14)	
	E. radiata	6		0.83 (±0.07)	
Jul	*C. subfarcinata*	6		0.72 (±0.13)	
	E. radiata	6		0.67 (±0.05)	

3.1. Fish farm field trial results

Growth of the red seaweeds during the 2014 Port Lincoln fish farm field trial was highly variable (Figure 3). 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 (pseudo-$F_{2,60}$ = 5.06, p_{perm} = 0.041). For specimens of both species that were deployed in March, there was no difference in SGR between 5- and 8-month cultivation periods (Mar-Aug or Mar-Nov), but SGR was greater for specimens deployed in August (Aug-Nov deployment) than for either March deployment cultivation period. There was no significant difference between species except in the 8-month Mar-Nov cultivation period, where *So. robusta* specimens lost more biomass than *G. australe.*

PERMDISP analysis showed that multivariate dispersion (equivalent to variance in the univariate case as applied here) was different between species x cultivation period groups (pseudo-$F_{5,103}$ = 9.23, p_{perm} = 0.001), due to much greater dispersion for *So. robusta* in Aug-Nov than all other species x cultivation period groups. This was due to some *So. robusta* specimens demonstrating SGR up to 3.5 % d^{-1} in the Aug-Nov cultivation period, while maximum SGR of *G. australe* specimens was 1.5 % d^{-1}, and biomass losses of other *So. robusta* specimens were greater than those of *G. australe.* Although differences in mean growth between species were not significant, *So. robusta* showed potential for greater maximum growth rates, but was also more severely affected by biomass losses.

PERMANOVA of SGR for the brown seaweeds showed no significant differences. The analysis lacked power due to a large number of specimens being lost. Overall growth of the browns was low, but with a trend for greater growth of *E. radiata* than *C. subfarcinata,* with most specimens of the latter losing biomass (Figure 4).

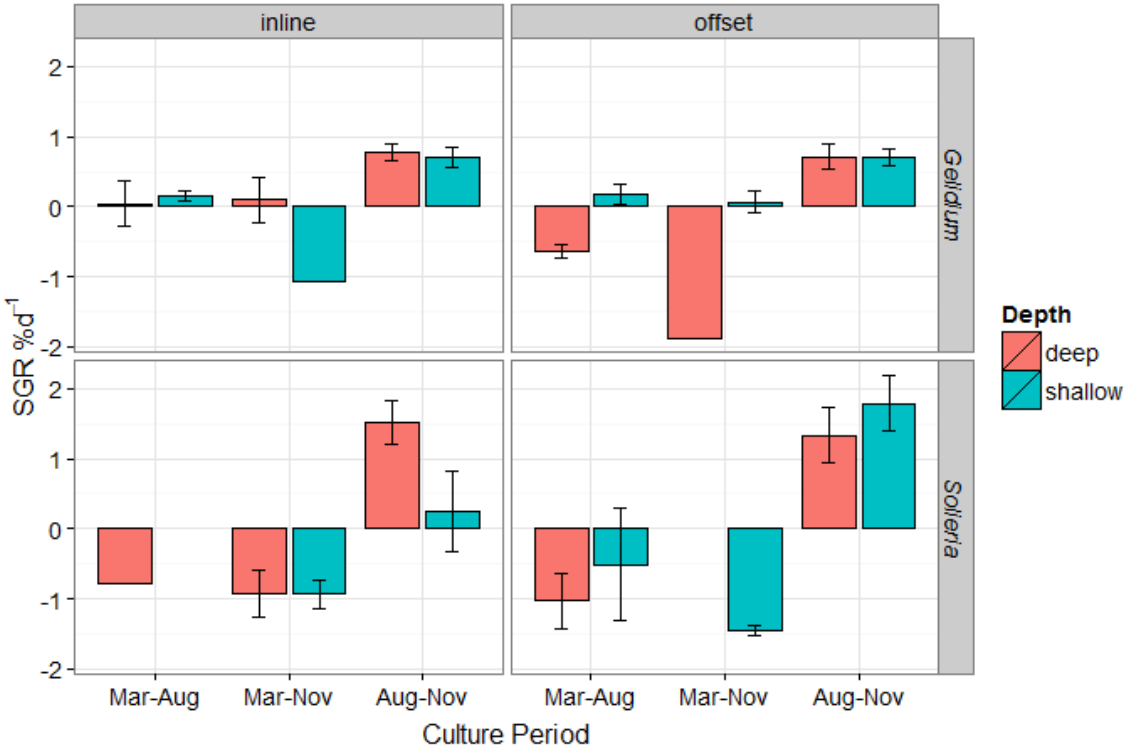

Figure 4. Mean SGR of red seaweeds (*Gelidium* = *G. australe, Solieria* = *So. robusta*) over three cultivation periods of the fish farm trial from March – November 2014. Specimens were grown on longlines either inline with or offset from prevailing current. Deep specimens were at 5 m and shallow specimens at 2 m depth. Error bars show standard error (n = number of retrieved specimens, range 0 – 9, see Table 6). Note, there were no retrieved specimens for some treatment combinations.

For both red and brown seaweeds, there was a significant effect of cultivation period x species on the pattern of losses (reds: $\chi^2_2 = 186.6$, $p = 0.048$, browns: $\chi^2_2 = 165.4$, $p < 0.001$). For the reds deployed in March, more *So. robusta* specimens were lost over the 5-month trial (Mar-Aug), while more *G. australe* were lost in the 8-months (Mar-Nov). For the browns, more *E. radiata* were lost than *C. subfarcinata* over both the 5- and 8-month trials. There were few losses of the Aug-Nov specimens for any species (Table 6).

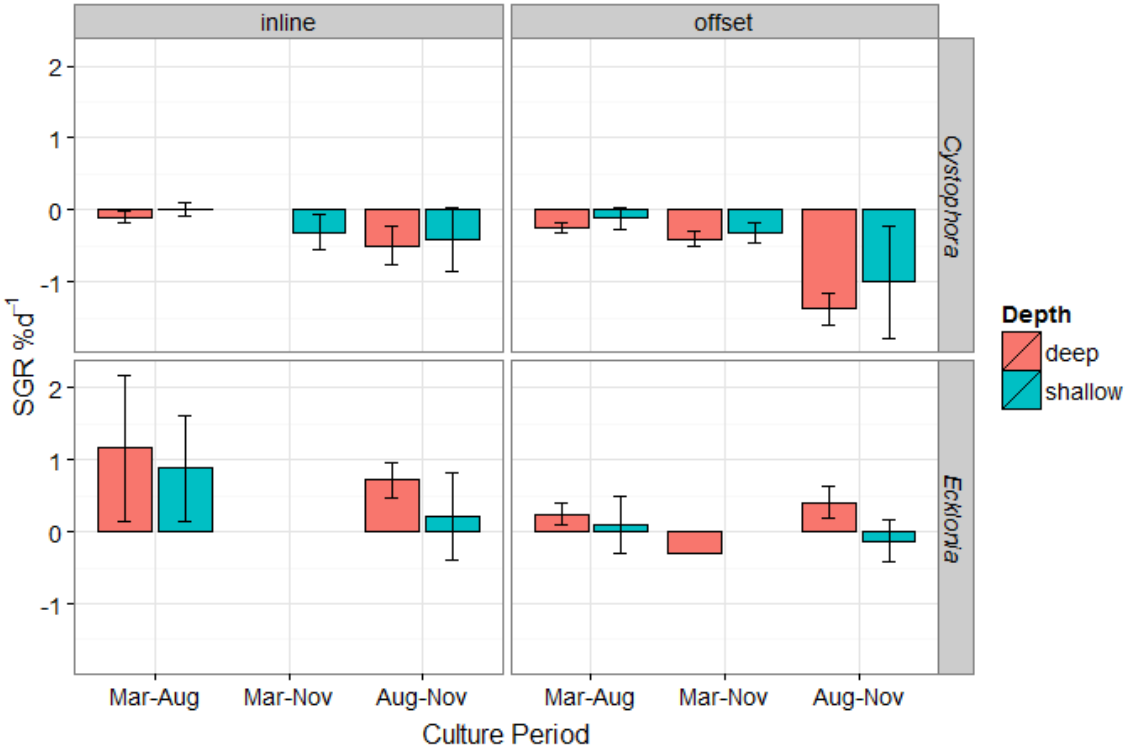

Figure 5. Mean SGR of brown seaweeds (*Cystophora* = *C. subfarcinata, Ecklonia* = *E. radiata*) over three cultivation periods of the fish farm trial from March – November 2014. Specimens were grown on longlines either inline with or offset from prevailing current. Deep specimens were at 5 m and shallow specimens at 2 m depth. Error bars show standard error (n = number of retrieved specimens, range 0 – 10, see Table 6). Note, there were no retrieved specimens for some treatment combinations.

N% was only analysed for *So. robusta* specimens from the Aug-Nov deployment because there were insufficient specimens retrieved of other species and from other cultivation periods for meaningful analysis. There was no significant difference in N% with depth or site, with specimens having average N% ± s.e. (n = 33) of 1.60 ± 0.05 %. The low N content of *So. robusta* suggests that specimens were N limited, despite being cultivated near to fish cages.

Heavy fouling growth, consisting of bivalves (primarily *Mytilus* sp.) and nuisance algae (Ectocarpaceae and *Ulva* sp.) occurred on specimens from all cultivation periods of the fish farm trial, with fouling being particularly prolific on samples from the first 5-month cultivation period (Mar-Aug). Fouling made counting of fluke eggs difficult, so only presence/absence

was recorded for the Mar-Aug samples. Counts of fluke eggs were made for specimens from the 3-month Aug-Nov cultivation period but should be considered approximate, as fouling may have obscured some eggs. The number of samples found to have fluke eggs and the total examined from each cultivation period are shown in Table 7.

Table 7. Number of retrieved seaweed specimens for each species and cultivation period in the 2014 fish farm trial. The total number of bags/ropes retrieved is shown in brackets.

Deployment	**Species**	**Offset**		**Inline**	
	Red	Shallow	Deep	Shallow	Deep
Mar-Aug	*G. australe*	5 (9)	2 (7)	3 (12)	4 (12)
	So. robusta	2 (8)	2 (8)	0 (12)	1 (9)
Mar-Nov	*G. australe*	2 (9)	0 (9)	1 (4)	5 (6)
	So. robusta	5 (10)	1 (9)	2 (5)	5 (6)
Aug-Nov	*G. australe*	9 (9)	9 (9)	9 (9)	8 (9)
	So. robusta	9 (9)	9 (9)	9 (9)	7 (9)
	Brown				
Mar-Aug	*C. subfarcinata*	7 (7)	10 (10)	10 (11)	11 (12)
	E. radiata	3 (10)	3 (7)	2 (9)	2 (9)
Mar-Nov	*C. subfarcinata*	6 (1)	2 (4)	2 (2)	0 (1)
	E. radiata	0 (2)	1 (3)	0 (3)	0 (1)
Aug-Nov	*C. subfarcinata*	5 (7)	7 (8)	5 (5)	4 (8)
	E. radiata	7 (7)	5 (7)	4 (6)	7 (9)

Fluke eggs were found on a greater proportion of cultivation items (bags or ropes) from the Mar-Aug than Aug-Nov (χ^2_1 = 15.62, p < 0.001) cultivation period, and predominantly on samples from the cultivation system that was in line with prevailing tidal flow (χ^2_1 = 9.79, p = 0.002).

Table 8. Number of samples (n) of cultivation equipment items (bag for red seaweeds, rope for brown seaweeds) examined and number having eggs of skin or gill flukes present (and total having either type) for the two cultivation periods assessed in the 2014 fish farm trial. The number of eggs present is shown in brackets where counted. Note that the total number of samples with fluke eggs present is typically less than the sum of samples having skin or gill fluke eggs because some samples had both egg types present.

		Mar-Aug				**Aug-Nov**			
			Fluke eggs present				Fluke eggs present		
Item	Site	n	Skin	Gill	Either	n	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

4 Discussion

Of the red seaweeds, *G. australe* was the best performing species in the initial (2012 – 2013) field trial, exhibiting SGR of up to 3.2 % d^{-1}. It grew at average SGR of < 2 % d^{-1} in the 2014 fish farm trial, however, while some specimens of *So. robusta* exhibited promising growth rates of up to 3.5 % d^{-1} during the 3-month spring (August-November) deployment. Spring is generally the best season for seaweed growth in temperate regions (Titlyanov and Titlyanova, 2010), and this was found in both the initial and fish farm trials.

Our initial field trial tested tied and bagged cultivation methods for red seaweeds. There was little difference in growth performance between these systems, but bag cultivation resulted in lower specimen loss and is less labour intensive (Góes and Reis, 2011), prompting selection of this method for the fish farm trial. Environmental data suggests that losses during the initial trial were not related to rough weather because the Oct deployment experienced a high frequency of strong westerly winds, but no specimen loss. Seasonal growth patterns and temperature tolerances of the red species we used have not been studied, but summer

temperatures may have exceeded their physiological tolerances, especially given a marine heatwave during this trial. The sea surface temperatures in southern Australia were the highest on record in January-March 2013, exceeding the historic average by ~ 5 °C, with temperatures more than 0.5 °C above average persisting from November 2012 until May 2013 (Bureau of Meteorology, 2014; Roberts, et al., 2019).

There was little difference in the performance of the brown seaweeds *E. radiata, C. subfarcinata,* and *Sa. linearifolium* over the 12 months of the initial field trial, although *E. radiata* grew best of the brown seaweeds in the Jul deployment. Results for seasonal growth of *E. radiata, C. subfarcinata* and *Sa. linearifolium* were consistent with previous observations of poor growth in summer in these species due to erosion or to shedding of spent reproductive branches (Kendrick and Walker, 1994; Klemm, 1988; Miller, et al., 2000; Novaczek, 1984; Wernberg and Goldberg, 2008). The seasonal development and shedding of reproductive branches in *Sa. linearifolium,* which is common to southern Australian members of this genus (Womersley, 1987), means that this species would only be suitable for cultivation for part of the year, and failure to harvest at an appropriate time could lead to large losses of material. Additionally, we were unable to successfully obtain gametes from *Sa. linearifolium* despite the presence of apparent fertile structures, while gametes were obtained from *C. subfarcinata*, and spores from *E. radiata,* indicating reproduction is more easily achieved for these latter two species. Initial investigations therefore suggested *E. radiata* and *C. subfarcinata* were the best candidate brown seaweeds for further investigation.

The brown species may also have been adversely impacted by the marine heatwave during the initial field trial. Growth of *E. radiata* in natural populations is negatively correlated with

water temperature at temperatures above 21 °C (Bearham, et al., 2013) and the peak water temperature reached 27 °C in early March (Roberts, et al., 2019). Temperature responses of the other species have not been established, but distribution modelling has shown a strong temperature dependence for both *C. subfarcinata* and *Sa. linearifolium* occurrence, with maximum summer temperatures defining the latitudinal range of these species (Martínez, et al., 2018).

The site for the initial field trial 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. Many southern Australian brown seaweeds contain terpenoid compounds that make them unpalatable to herbivores (Steinberg and van Altena, 1992), but herbivory is another possible cause for biomass losses of the red seaweeds in this trial, particularly in *So. robusta* which has relatively soft branches filled with filaments and mucilage (Womersley, 1994).

In the fish farm trial, growth of all species was likely negatively impacted by overgrowth due to fouling by mussels and nuisance algae, and possibly also by N limitation, given N contents of < 2 % in *So. robusta*. Such low N levels are somewhat surprising given the close proximity of seaweed to fish cages (~ 150 m) in this trial. Over the fish farm trial period the cages on the lease had unusually low kingfish stocking density (CleanSeas operations, personal communication), so fish feed and the expected waste nutrient input would have been low, potentially contributing to the low N levels overserved in seaweed samples. We did not measure water N concentration at our field sites, because the transient nature of water N concentrations means that tissue N of seaweeds more accurately reflects nutritional history than water N (Fong, et al., 1994).

Seedling performance of brown seaweeds in offshore cultivation is affected by preceding nursery cultivation conditions, with fertilisation improving success (Rößner, et al., 2014). We used wild-collected material for seeding, therefore the nutritional status of the specimens was unknown, and may have been suboptimal. Better performance could be expected from optimally fertilised hatchery-produced seedlings. For both red and brown seaweeds, performance would be improved by selection of fast-growing specimens from which to obtain seedstock.

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 egg numbers observed were very low. Fluke eggs occurred more frequently on the more heavily fouled specimens from the Mar-Aug cultivation period than in Aug-Nov. The longer duration (5 months Mar-Aug. c.f. 3 months Aug-Nov) of this cultivation period or greater amount of fouling present may have caused more eggs to be captured. Seasonal differences may also have been influential, such as possible slower biodegradation of eggs at lower water temperatures over winter. The number of fluke eggs present is also likely to depend strongly on the fluke abundance on nearby farmed fish and in-water cage net cleaning, but data on fluke occurrence on the farmed fish and cage management were not available.

Over 60 % of gill fluke eggs produced in aquaculture entangle on the net (SARDI unpublished data). The sparsity of eggs found in this study suggests that while fluke eggs become entangled on seaweed cultivation infrastructure, the overall effect on fluke egg environmental loads, and hence parasite transmission and management, is likely to be negligible. Dispersal of fluke eggs is strongly influenced by tidal currents with the majority of eggs and greater infection rates observed in line with, rather than across, prevailing currents

(Chambers and Ernst, 2005), as also shown by our results. Capture of fluke eggs is therefore likely to be further reduced where seaweed infrastructure is not in line with prevailing currents and may also be less where seaweed is cultivated further from fish cages than in our experiments, although fluke eggs may be transported > 8 km from farms (Chambers and Ernst, 2005).

Aside from limited experimental cultivation of *E. radiata* in New Zealand (Neill, et al., 2009), the initial field trial reflects the first attempt at offshore cultivation of any of the eight candidate species, and the first trial of at-sea seaweed cultivation in southern Australia. The initial and fish farm field trials reported here highlight several issues in the establishment of novel species and systems for offshore seaweed aquaculture. Herbivory and fouling, which impacted our results, are recognised problems in seaweed aquaculture (Titlyanov and Titlyanova, 2010; Troell, et al., 2009). Performance of large-scale cultivation is difficult to predict from small-scale trials such as the ones described here, because performance varies with stocking density and biomass (Troell, et al., 2009). A larger trial with higher stocking density than we applied, for example, may prove more successful by providing sufficient initial seaweed biomass to outcompete fouling organisms or withstand herbivory (Ask and Azanza 2002; Titlyanov and Titlyanova 2010).

Seaweed farms should, however, be located away from areas with high natural herbivore abundance where possible (Ask and Azanza, 2002; Kim, et al., 2017). Prescribed aquaculture zones in SA are located away from reef and seagrass habitats (PIRSA, 2013) and are therefore likely to support lower abundance of herbivorous fish than the site of our initial field trial, which was located adjacent to seagrass. We were also unable to tend to the fish farm trial for a prolonged period, whereas seaweed farms are typically supervised regularly to check for

fouling and perform preventative maintenance (Ask and Azanza 2002; Kim, et al., 2017; Troell et al., 2009). More frequent monitoring would also allow identification of the most suitable length of cultivation period. Our deployment durations may have been longer than is optimal, especially for the red seaweeds. The cultivation period of farmed red seaweeds is typically in the range of 2 – 3 months (Ask and Azanza, 2002; Titlyanov and Titlyanova, 2010), while out-planting periods of brown seaweeds range from 3 to > 6 months (Titlyanov and Titlyanova, 2010). The fish farm trial was impacted by breakage of some of the frames used to hold the seaweed specimens. More frequent maintenance may also have alleviated this issue, but cultivation systems using seaweeds attached directly to long lines are likely more suitable for off-shore cultivation than the raft-type system we used (Kim, et al., 2017). Due to limited biomass availability and the use of wild-collected rather than hatchery produced seedstock, we were unable to implement this type of cultivation system.

Careful timing of out-planting is used to minimise fouling of seaweeds grown around salmon farms in Canada (Troell, et al., 2009), and fouling also varies with location (Abreu, et al., 2009; Neill, et al., 2009). Abreu et al. (2009) found that seaweeds grew better 800 m from cages than at 100 m, with the lines at 100 m suffering fouling growth. In a trial of *E. radiata* cultivation in New Zealand, sites with high water movement displayed much less fouling than calm-water sites (Neill, et al., 2009). We were only able to test two on-farm locations and could not test further distances from cages due to the arrangement of fish cages within the farm lease that was available for our trial. Seaweed farming is permitted within many existing aquaculture zones under current SA legislation (PIRSA, 2013), therefore, future research could assess additional locations and arrangements of seaweed in relation to fish cages within these zones.

Growing demand for seafood is likely to drive expansion of fish aquaculture in Australia. For this expansion to be sustainable, methods for nutrient mitigation will be needed. The benefits of IMTA using seaweeds have been demonstrated globally (Barrington, et al., 2009; Neori, 2008; Troell, et al., 2009), and demand for seaweed products is also increasing, creating further interest in the development of seaweed industries in Australia (Kirkendale, et al., 2010; Lee, 2010; Lorbeer, et al., 2013).

We have demonstrated potential feasibility for cultivation of three native Australian seaweeds: the reds *So. robusta* and *G. australe*, and the brown *E. radiata*, but to develop cultivation of these or other species, further research is needed. To facilitate larger-scale trials, hatchery techniques for the production of seeding biomass need to be developed and refined, and the best conditions for seedling cultivation prior to out-planting determined. Seedstock should be produced from specimens selected for desirable traits, e.g. suitable growth rates (Kim, et al., 2017). Field trials should use a range of planting densities and different locations and arrangements relative to fish cages. Suitable sites for cultivation may also be informed by further investigation of the biology of seaweed species to determine optimal conditions for their growth. Further study of the biology of southern Australian native seaweed species, many with strong economic potential, will also assist in identifying additional candidate species for aquaculture, for development of Australian seaweed industries and of integrated multi-trophic systems to achieve profitable and sustainable aquaculture.

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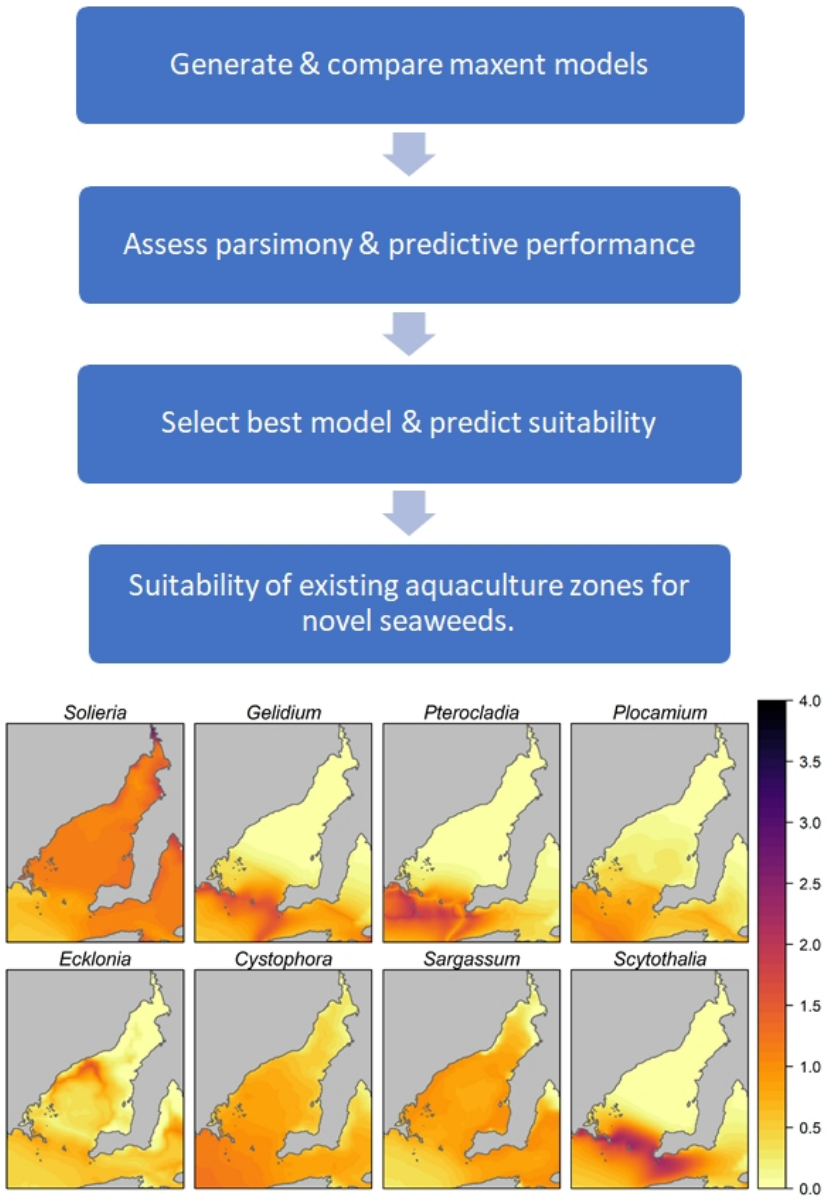
Chapter 3. Comparing maximum entropy modelling methods to inform aquaculture site selection for novel seaweed species



Schematic of the model development and selection process to predict suitability for candidate seaweed species within aquaculture zones.

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Kathryn H. Wiltshire		
Contribution to the Paper	Conceived and designed the study, developed and ran statistical models, interpreted, analysed and mapped model results, wrote original draft manuscript and acted as corresponding author		
Overall percentage (%)	95		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	11/4/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Jason E. Tanner		
Contribution to the Paper	Assisted with modelling method development and interpretation of results, and provided suggestions, comments and editing assistance on manuscript drafts		
Signature		Date	11/4/2020

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Comparing maximum entropy modelling methods to inform aquaculture site selection for novel seaweed species

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ABSTRACT

Maximum entropy (maxent) modelling is a widely used method for developing species distribution models (SDMs), but default maxent modelling methods can result in overly complex models with poor transferability. Methods suggested to reduce overfitting include increasing regularisation, using only linear and quadratic features, or applying forward selection of predictors using maximum likelihood (ML) methods. We built models using these options to determine environmental suitability within existing aquaculture zones for eight seaweed species, four red (Rhodophyta: Florideophyceae) and four brown (Ochrophyta: Phaeophyceae), that are being investigated for aquaculture in southern Australia. Forward selection models were the most parsimonious, but we encountered failure of ML methods for *Pterocladia lucida* (Rhodophyta) due to separation. Separation is a known issue for logistic regression and has recently been recognised in maxent models. Separation occurs where a variable, or combination of variables, is a perfect predictor for a binary response, here, species occurrence, and results in ML parameter estimates tending to infinity. One method for obtaining finite parameter estimates under separation is to apply a Cauchy prior distribution for coefficients. We therefore also built models for each species using a Cauchy-prior version of the forward selection method, and found that these models performed similarly to those built with ML methods. Default models achieved marginally higher predictive performance than other options based on training data metrics, but simpler models performed equivalently to, or better than, default models at predicting independent presence-absence test data. Predictive performance using test data varied considerably between species, but the difference in performance between models within each species was generally small. Our results confirm the concern that default maxent models may suffer from over-fitting and poor transferability. Model transferability and interpretability were important for our purpose, hence, based on the principle of parsimony, forward selection models were preferred. We also found that forward selection models retained similar predictive performance to the best model as assessed by each metric, further supporting use of these models. Where ML methods failed due to separation, the use of the Cauchy-prior method was a viable alternative. Predictions for the region of interest (Spencer Gulf, South Australia) were generated using the most parsimonious models, and *Solieria robusta* (Rhodophyta) showed the highest predicted suitability of the eight candidate species within existing aquaculture zones, especially in northern Spencer Gulf. Predicted suitability was low for the other Rhodophyta considered, while each of the Phaeophyceae showed moderate to high suitability in at least some southern Spencer Gulf aquaculture zones. These model results help to inform selection of the best candidate species and suitable farming areas for future research.

1. Introduction

Species Distribution Modelling (SDM) is commonly used to predict distributions of terrestrial species, with increasing use for aquatic species (Elith and Leathwick, 2009; Robinson et al., 2011). Where the biological knowledge required to build mechanistic models is lacking, correlative SDM, which relates known species occurrences to environmental, or sometimes spatial, predictors, can be applied (Elith and Leathwick, 2009). The resulting models can be used to obtain ecological insight or predict distributions, with applications in spatial management, biosecurity, climate change and theoretical ecology (Elith and Leathwick, 2009; Robinson et al., 2011). In recent years, correlative SDM has been applied for aquaculture site selection, especially for species where there is insufficient knowledge to develop habitat suitability indices (e.g.Castelar et al., 2015; Falconer et al., 2016; Linhoss et al., 2016; Oyinlola et al., 2018; Vincenzi et al., 2007;

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Vincenzi et al., 2011).

In South Australia (SA), several finfish species are farmed in declared aquaculture zones developed and regulated by Primary Industries and Regions SA (PIRSA) (PIRSA, 2013, 2017). There is also interest in developing seaweed aquaculture in SA, and Australia more generally, due to growing demand for seaweed products, of which Australia is a net importer (Lee, 2010), and also the potential for seaweeds to offset nutrient inputs from finfish aquaculture (Chopin et al., 2001; Chung et al., 2002; Neori, 2008; PIRSA, 2013; Troell et al., 2009; Wiltshire et al., 2015). Seaweed farming is not an established industry in Australia, however, and few species with established farming technology are native to Australia, so it is likely that local seaweed species that have not been previously cultivated will need to be utilised. Eight native seaweed species have been identified as candidates for farming in temperate Australia, and their potential for aquaculture in SA is being investigated (Wiltshire et al., 2015). The candidate species comprise four red (Rhodophyta: Florideophyceae) species: *Pterocladia lucida* (R.Brown ex Turner) J. Agardh (Pterocladiaceae), *Gelidium australe* J. Agardh (Gelidiaceae); *Solieria robusta* (Greville) Kylin (Solieriaceae), and *Plocamium angustum* (J.Agardh) J.D. Hooker & Harvey (Plocamiaceae); and four brown (Ochrophyta: Phaeophyceae) species: *Ecklonia radiata* (C.Agardh.) J.Agardh (Lessoniaceae), *Scytothalia dorycarpa* (Turner) Greville (Seirococcaceae), *Cystophora subfarcinata* (Mertens) J. Agardh, and *Sargassum linearifolium* (Turner) C.Agardh (Sargassaceae). These eight candidate species will be referred to by their genus names through the rest of the manuscript.

Several aquaculture zone policies (PIRSA, 2017) list seaweed farming as a permitted class of aquaculture despite the industry not yet being developed in SA. PIRSA's aquaculture zone policies consider community, stakeholder, industry and environmental concerns in selecting suitable areas for aquaculture, however the factors determining environmental suitability for cultivation of seaweeds in SA are not well understood (PIRSA, 2013). Experimental investigation of the biology of the candidate seaweed species will assist in determining their responses to factors such as light, temperature and nutrients, and hence in identifying locations that may be suitable for their farming (Wiltshire et al., 2015), but this biological knowledge is lacking. We therefore applied correlative SDM to predict habitat suitability for each of the candidate seaweed species in existing aquaculture zones in SA.

Maximum entropy (maxent) modelling is widely used for developing correlative SDMs, particularly where available occurrence data are presence-only (Elith et al., 2011; Merow et al., 2013). It is relatively easy to apply using the freely-available Java software MaxEnt.jar (Phillips et al., 2006, 2017). The default methods employed by MaxEnt.jar have, however, been criticised for producing overly complex models, leading to poor transferability of the model over space or time, and difficulty in interpretation of ecological responses (Halvorsen et al., 2015; Radosavljevic and Anderson, 2014; Syfert et al., 2013; Verbruggen et al., 2013; Warren and Seifert, 2011). More parsimonious models may be produced by species-specific tuning of the regularisation multiplier applied by Maxent.jar (Anderson and Gonzalez Jr, 2011; Muscarella et al., 2014; Radosavljevic and Anderson, 2014), but high regularisation can result in biased parameter estimates (Dormann et al., 2013; Halvorsen et al., 2015; Royle et al., 2012; Vollering et al., 2019) and over-generalised predictions (Ashford et al., 2014; Radosavljevic and Anderson, 2014). Another approach is to limit the complexity of response curves by using only linear and quadratic feature types (Elith et al., 2010; Merow et al., 2013; Syfert et al., 2013). Forward step-wise variable selection using likelihood ratio or *F*-tests under a maximum likelihood (ML) interpretation of maxent has also been proposed as a method for limiting model complexity and preventing over-fitting (Bendiksby et al., 2014; Halvorsen, 2013; Halvorsen et al., 2015, 2016; Mazzoni, 2016; Mazzoni et al., 2015; Vollering et al., 2019).

Maxent is now recognised as being equivalent to the inhomogeneous Poisson process (IPP) model (Aarts et al., 2012; Fithian and Hastie, 2013; Renner and Warton, 2013), and options for fitting maxent models using weighted logistic regression (Fithian and Hastie, 2013) have been implemented using existing algorithms for fitting generalised linear models (GLM). These include the *R* (R Core Team, 2019) packages *maxnet* (Phillips, 2017), which replicates the analysis of Maxent.jar v3.4.0 including construction of the same feature types and application of equivalent regularisation (Phillips, 2017; Phillips et al., 2017), and *MIAmaxent* (Vollering et al., 2018, 2019), which is described as a "Modular, Integrated Approach" to maxent (Mazzoni, 2016; Mazzoni et al., 2015), and implements the alternative approach described by Halvorsen et al. (2015, 2016). In addition to applying forward selection with ML tests, this alternative approach seeks to provide more control over constructed feature types than the default Maxent.jar methods, and to generate simpler response curves that are ecologically relevant (Bendiksby et al., 2014; Halvorsen et al., 2015, 2016; Mazzoni et al., 2015; Støa et al., 2018; Vollering et al., 2019).

For our purpose of predicting suitability of existing aquaculture zones for novel aquaculture species, specifically seaweeds, model transferability and ease of ecological interpretation are important considerations. We therefore compared the performance of default maxent models, as implemented in *maxnet*, to that of models applying each of the proposed strategies to avoid over-fitting: increased regularisation, restricted feature types, and the alternative approach as applied by *MIAmaxent*, using a range of performance metrics. We also compared predictions of suitability of existing aquaculture zones across the modelling methods for each species. Results of these models will help to inform which species may be most suitable within Spencer Gulf, SA and which current aquaculture zones may be most suitable for seaweed farming.

2. Methods

2.1. Aquaculture zones

Aquaculture zone policies (PIRSA, 2017) for each of the declared zones in Spencer Gulf, SA were examined to determine those in which seaweed farming could be permitted. A number of zones consist of sectors that allow different classes of aquaculture. Zones, or sectors as relevant, were selected for consideration where they included seaweed farming as a permitted class of aquaculture. Some zones where seaweed farming is permitted are located in intertidal areas; these are used currently for aquaculture of intertidal molluscs such as Pacific oyster (*Crassostrea gigas*) but were not considered in this study as being suitable for cultivation of the candidate seaweeds, all of which occur strictly subtidally.

Spatial polygons of all South Australian aquaculture zones (Location SA, 2018) were obtained from PIRSA. The relevant zones within Spencer Gulf, i.e. subtidal zones where farming of seaweed is a permitted activity, were extracted from this data set and are shown in Figure 1.

2.2. Data sources

Occurrence data for the eight seaweed species of interest was obtained for a geographic area covering all coasts of mainland Australia and Tasmania (bounding box: 10 – 45 °S, 110 – 155 °E) from three online databases: Atlas of Living Australia (ALA, 2019), Australasia's Virtual Herbarium (AVH, 2017) and the Macroalgal Herbarium Portal (MHP, 2017). Spatial duplicates were removed from the combined dataset. These data are presence only and primarily from herbarium records, so are likely to have a strong sampling bias, which can influence presence only species distribution modelling results (Phillips et al., 2009). We therefore also obtained records of all other seaweeds of relevant classes (Rhodophyta: Florideophyceae and Ochrophyta: Phaeophyceae) from these databases to use as target-group samples (TGS) for

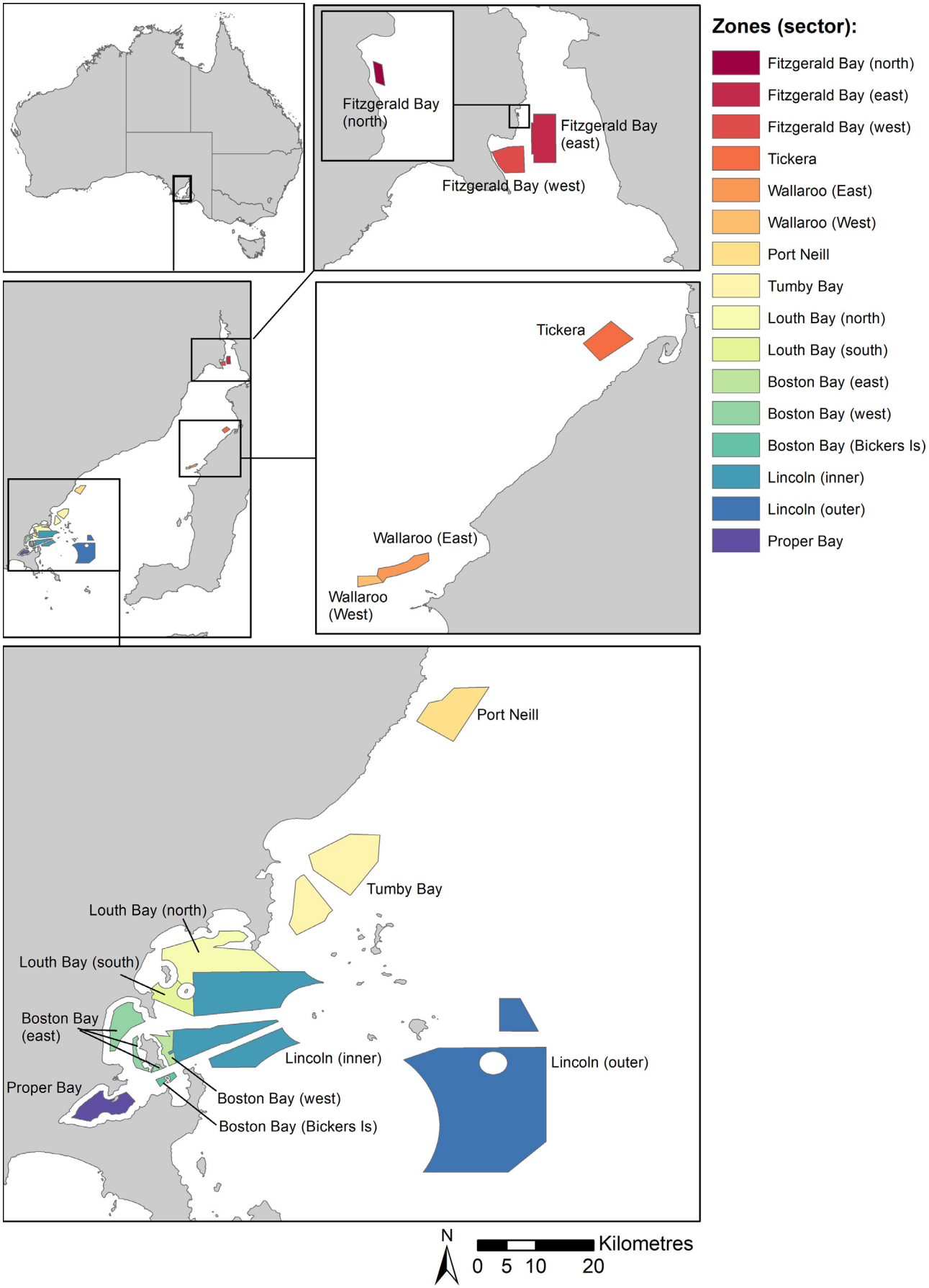

Figure 1. Map of subtidal aquaculture zones in Spencer Gulf, South Australia within which seaweed farming is permitted under current zone policies.

characterising the background environment (Elith et al., 2011; Merow et al., 2013; Phillips and Dudík, 2008; Phillips et al., 2009; Støa et al., 2018;). All occurrence and TGS data used in the models were natural seaweed occurrences given the lack of current seaweed farming in Australia, and a total of 7967 spatially independent TGS points were used in models.

Our aim was to predict environmental suitability for seaweed farming, therefore we selected a suite of relevant predictors from those available based on knowledge of seaweed physiology (Wiencke and Bischof, 2012) and with consideration of variables shown to be important in SDMs for other seaweeds (Castelar et al., 2015; Jueterbock et al., 2013; Lutchminarayan, 2017; Yesson et al., 2015). We excluded predictors that are not relevant in an aquaculture scenario, where seaweeds are typically grown suspended and at optimal depth. Bathymetry and sediment properties were therefore not used. Depth and sediment type may be important factors in determining suitability for culture systems (e.g. longlines) and environmental carrying capacity (Radiarta et al., 2011; Ross et al., 2013), but in the case of Spencer Gulf aquaculture, these factors have already been considered in defining aquaculture zone locations and the classes of aquaculture permitted in each zone (PIRSA, 2013). The predictors selected for consideration were:

1 Sea surface temperature (SST) - annual mean, maximum, minimum and range;
2 Light availability - Photosynthetically available radiation (PAR) annual mean and maximum, Diffuse attenuation (K_d) annual mean, maximum and minimum
3 Water quality – Salinity and pH
4 Nutrients – nitrogen and phosphorus
5 Water movement – average and maximum current velocity (CV), 90th percentile of wave energy flux (wave energy)

Environmental data layers were obtained from Bio-ORACLE (Assis et al., 2017; Tyberghein et al., 2012) using the *R* (R Core Team, 2019) package *sdmpredictors* (Bosch, 2017) with the exception of wave energy, which was obtained from the Australian Wave Energy Atlas (Durrant et al., 2013). Wave energy was resampled to the same resolution as the Bio-ORACLE layers using the *R* package *raster* (Hijmans, 2016). Some predictors were highly correlated ($|r| > 0.7$), e.g. mean SST was highly correlated with both minimum and maximum SST and with mean PAR. We selected variables from these correlated sets based on their average performance in single variable models (Ashford et al., 2014; Braunisch et al., 2013; Dormann et al., 2013; Merow et al., 2013) run using each modelling method (see section 2.3 for details). The candidate set of environmental variables for each species was selected in order of fractional deviance explained, also known as the deviance ratio (D^2), which is equivalent to the R^2 of ordinary least squares regression (Guisan and Zimmermann, 2000; Vollering et al., 2019), excluding variables that were correlated at $|r| > 0.7$ with any of greater deviance explained. The candidate variables used in modelling for each species are shown in Table 1, along with the number of available occurrence records for each species.

2.3. Species distribution modelling approaches

To compare the recommended modelling approaches for avoiding overfitting, we constructed models using the following approaches to generate SDMs for each species: (1) default regularisation and feature types, which in the *maxnet* implementation and v3.4.0 of the MaxEnt.jar software include linear, quadratic, hinge and product features (Phillips, 2017; Phillips et al., 2017), (2) regularisation multipliers from 2 to 8 with all feature types (Anderson and Gonzalez Jr, 2011; Muscarella et al., 2014; Radosavljevic and Anderson, 2014), (3) default regularisation with linear and quadratic features only (following Elith et al., 2010); all implemented using the *maxnet* package (Phillips, 2017) in *R* (R Core Team, 2019); and (4) forward selection using maximum likelihood (ML) methods (Halvorsen, 2013; Halvorsen et al., 2015; Mazzoni et al., 2015; Vollering et al., 2019) built with the *R* package *MIAmaxent* (Vollering et al., 2018). The forward selection models were built in three steps (following Halvorsen, 2013; Halvorsen et al., 2015; Mazzoni et al., 2015; Vollering et al., 2019): (1) five derived variables were obtained for each environmental predictor (one linear, one monotonous zero-skew, and three deviation type), (2) the most parsimonious set of derived variables was selected for each environmental predictor, and (3) models were built using combinations of environmental predictors each represented by the selected derived variable set. For both steps 2 and 3, nested models were compared using likelihood ratio tests (LRTs), and model selection based on $\alpha = 0.01$. The ML model for *Pterocladia* was not identifiable due to complete separation, a potential issue for IPP models where the average value of a covariate at presence locations is at the upper or lower limit of the range for that covariate (Hefley and Hooten, 2015). Where this problem occurs, ML estimates become infinite, but models may be estimated using penalised likelihoods (e.g. regularisation, as applied in *maxnet*), or by using a weakly informative prior, such as proposed by Gelman et al. (2008) for logistic regression, to restrict parameter estimates to finite, plausible values (Hefley and Hooten, 2015). We therefore applied a modification of the forward selection method by replacing calls to the standard *R glm* function in *MIAmaxent* with the *bayesglm* function of the *arm* package (Gelman and Su, 2018), which implements the Cauchy prior recommended by Gelman et al. (2008) for logistic regression, and shown to be effective for the IPP case (Hefley and Hooten, 2015). The Cauchy prior method was otherwise identical to the ML method, including the use of the derived variables generated by *MIAmaxent* and forward variable selection based on LRTs, and was used to generate models for all species, not just *Pterocladia*, in order to compare its performance with the other modelling approaches used. The *MIAmaxent* package automatically adds presence points to the background data (Vollering et al., 2019); this is an option in the MaxEnt.jar software but not in the *maxnet* package. We therefore manually added presence points to the TGS background samples for use in all *maxnet* models.

2.4. Model performance assessment

A number of metrics are commonly used to assess SDM performance; of these, Aikikes Information Criterion with small sample size correction (AICc) best selects models that have good transferability (Warren and Seifert, 2011). We calculated AICc as per Burnham et al. (2011) using code adapted from the *R* package *enmSdm* (Smith, 2019), which calculates AICc based on the method proposed by Warren and Seifert (2011) and adapted by Wright et al. (2015) for the case where non-random background points (e.g. TGS) are used. For comparison, we also calculated other common performance metrics: Area under the receiver operating characteristic curve (AUC) based on training data (AUCtrain) and on independent test data (AUCtest), deviance ratio (D^2), and model calibration (Fieberg et al., 2018). The presence-absence data set for determining test data AUC was compiled from unpublished data held by SARDI Aquatic Sciences that was collected during temperate reef surveys (Collings et al., 2008; Turner et al., 2007) and field visits to identify potential collection sites for seaweed seed stock (Wiltshire et al., 2015). These records were not included in the training data set, but, as with the training data, represent natural seaweed occurrence, not farmed seaweed locations. The purpose of our models was to predict environmental suitability in an aquaculture setting, and we therefore excluded some environmental variables that are likely to be important in determining natural seaweed distributions, e.g. depth and substrate characteristics. We note that our models are likely to be suboptimal for predicting natural species occurrences in comparison to models including all biologically relevant predictors, and may over-predict in areas where depth or substrate would be unsuitable

Table 1
Number of occurrence records and variables used in modelling from the candidate set for each species. Shared superscript letters indicate variables correlated at $|r| > 0.7$. Only one variable per correlated set was used per species. X indicates variable used for modelling. Variables shown in grey were not used for any species.

Species:	Red seaweeds				Brown seaweeds			
Category Variable	*Solieria*	*Gelidium*	*Pterocladia*	*Plocamium*	*Ecklonia*	*Cystophora*	*Sargassum*	*Scytothalia*
Temperature								
SST range	X	X	X	X	X	X	X	X
SST mean[a]		X	X	X		X	X	
SST min[a]					X			X
SST max[a]	X							
Light								
PAR mean[a]								
PAR max	X	X	X	X	X	X	X	X
K_d mean[b]		X			X			
K_d min[b]								
K_d max[b]	X		X	X		X	X	X
Water quality								
Salinity	X	X	X	X	X	X	X	X
pH	X	X	X	X	X	X	X	X
Nutrients								
Nitrate[c]			X	X	X	X		
Phosphate[c]	X	X					X	X
Water movement								
CV mean	X	X	X	X	X	X	X	X
CV max	X	X	X	X	X	X	X	X
Wave energy	X	X	X	X	X	X	X	X
# occurrence records	274	195	214	634	625	351	182	161

for natural species occurrence. The ability of each model to predict to this independent test data is, however, a valid measure of relative model performance, as all models were based on the same suite of environmental variables. The interpretation of training AUC for presence-only models is not at straight-forward as interpretation of training AUC based on presence-absence data. The maximum training AUC possible for models built using presence-only data is < 1 and is dependent on apparent prevalence, i.e. the ratio of occurrence to background points, and additionally the AUC of the null or random model may be different to 0.5 (Raes and ter Steege, 2007; Yackulic et al., 2013). We therefore used a null-model approach for assessment of training AUC, which involved building 99 null models for each of the eight modelled species using randomly selected TGS points as presences (following Merckx et al., 2011; Raes and ter Steege, 2007). The number of TGS points used in null models was equal to the number of species occurrence records in each case. The rank of training AUC in comparison to the 99 null model AUC values can be used as a statistical test of predictive performance (Raes and ter Steege, 2007), i.e. where model AUC is greater than at least 95 of the 99 null AUCs, the model is considered better than random at $p \leq 0.05$ (one-sided), and a model AUC higher than all 99 null model AUCs indicates prediction better than random at $p = 0.01$. D^2 and adjusted D^2 of each model were calculated based on Guisan and Zimmermann (2000). Adjusted D^2 and AICc are both measures that account for the number of parameters and number of observations, and penalise more complex models or those based on fewer data (Guisan and Zimmermann, 2000; Warren and Seifert, 2011). Calibration of each individual species model was assessed using the continuous Boyce index (Boyce et al., 2002; Fieberg et al., 2018; Hirzel et al., 2006), a quantitative version of the presence-only calibration plot recommended by Phillips and Elith (2010). Boyce indices were calculated using the *R* package *ecospat* (Broennimann et al., 2018; Di Cola et al., 2017). The Boyce index ranges from -1 to 1, with positive values indicating predictions in agreement with expectation, 0 indicating predictions no better than random, and negative values indicating predictions contrary to expected.

2.5. Predicted environmental suitability

Prediction rasters were generated for Spencer Gulf using raw output of all models for each species. The raw output of MaxEnt.jar and *maxnet* is a relative occurrence rate but its value is dependent on the number of background + presence points used (Elith et al., 2011). The default output of *MIAMaxent* is the probability ratio output (PRO) proposed by Halvorsen (2013). PRO output is a rescaling of raw scores to remove the dependence on the number of points used, such that the mean suitability of a random cell in the model domain = 1 (Bendiksby et al., 2014; Halvorsen, 2013; Halvorsen et al., 2015; Mazzoni, 2016; Vollering et al., 2019). PRO scores through the model domain for a species therefore show suitability for that species relative to an average site and allow comparison between models built using different numbers of points for the same species. Without strong assumptions or data about prevalence (i.e. the proportion of available sites that a species occupies), however, raw output, and hence PRO scores, can only be interpreted as relative indices of suitability, and not probabilities of occurrence or absolute abundance (Elith et al., 2011; Merow et al., 2013; Yackulic et al., 2013), and these scores are not directly comparable between species (Elith et al., 2011; Merow et al., 2013). MaxEnt.jar v3.4.0 uses a complimentary log-log transform as the default output; this transform has a stronger theoretical basis than the logistic transform that was the default output for earlier versions, however, it is still not directly comparable between species where prevalence is unknown (Phillips et al., 2017). The *maxnet* package includes both transforms as prediction options, along with raw output (Phillips, 2017). For each species and modelling method, we determined the mean raw score at the occurrence points used for modelling (equivalent to the 'meanPred' method of Liu et al., 2013), and used this to scale the raw suitability predictions. For our purposes, this mean occurrence point score is not taken to be a threshold for defining presence/absence (or suitable/unsuitable areas), but rather as a reference value to permit comparison between species. The derived relative suitability results illustrate suitability relative to that at an average presence site for each species. In comparing between species, a higher relative suitability score should not be interpreted as higher absolute suitability for one species over others for the area of interest, but does indicate higher suitability relative to known occurrences for that species in comparison to a lower scoring species. To assess suitability for each species within existing aquaculture zones, mean relative suitability using the modelling method with lowest AICc for that species was extracted for all relevant aquaculture zones. To examine how

predictions varied between modelling methods, relative suitability for each species was extracted for 200 random points across all aquaculture zones for each model. Gamma generalised linear modelling (GLM) was applied to determine how predicted suitability varied between models for each species. The Gamma family was used as it is suitable for strictly positive continuous data (Zuur et al., 2013). Initial data exploration showed that the effects of modelling methods were not consistent across species, and as we were primarily interested in exploring the effects of modelling method, we ran a separate GLM analysis for each species. Each GLM was run in a Bayesian framework using JAGS v. 4.3.0 (Plummer, 2017) with three chains for 10 000 iterations, thinned at a rate of 10, following 2000 iterations for adaptation and 10 000 iterations for burn-in. Diffuse normal priors were used for all coefficients, and factor levels were considered different to the reference default model where the 95% credible interval of the posterior coefficient estimate did not include zero. JAGS was run using the *R2jags* package (Su and Yajima, 2015). Convergence was assessed using the Gelman-Rubin convergence statistic, and confirmed by visual inspection of trace, density and autocorrelation plots generated using the *mcmcplots* package (McKay Curtis, 2015). To visualise spatial variation in model predictions using different options we generated maps for each species for the default model, regularisation multipliers of 2,4,6, and 8, linear-quadratic model and each forward selection model, and also of the mean prediction across all models and standard deviation x 5 across models for that species. For mapping, standard deviation was multiplied by 5 in order to better visualise areas where predictions varied. No model weighting was applied to the average predictions in these maps as our aim was to illustrate overall model agreement or difference, not to derive average models for the purpose of spatial prediction.

Raster extraction and calculation was performed using the *R* package *raster* (Hijmans, 2016), and maps generated using *rasterVis* (Perpiñán and Hijmans, 2019).

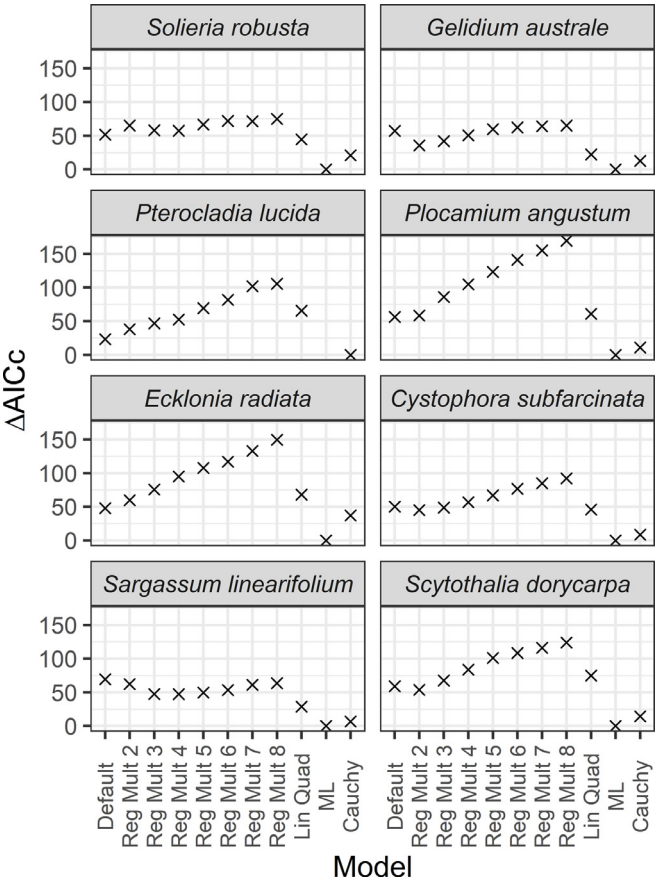

Figure 2. Comparison of AICc across modelling methods for each species. In each case, the difference in AICc relative to the model with lowest AICc is shown.

3. Results

3.1. Model performance

Forward selection models were the most parsimonious for all species as assessed by AICc (Table 2, Figure 2), with the ML models having the lowest AICc for all species except *Pterocladia*, where this model was not identifiable; data exploration revealed that salinity was a near-perfect predictor for this species leading to separation in the data. The Cauchy-prior model had the lowest AICc for *Pterocladia*, and the second lowest for all other species, being marginally higher than that of the ML model in each case, though less than 0.5 greater in the case of *Cystophora* and *Sargassum* (Figure 2, Table 3). Within regularised models for *Solieria, Gelidium, Pterocladia* and *Sargassum*, AICc was lower at regularisation mutlipliers of 2 – 4 than for default models before increasing with further regularisation, while AICc increased consistently with increasing regularisation for the remaining species. Linear-quadratic models had lower AICc than default models for *Solieria, Gelidium* and *Sargassum,* but higher for other species, generally similar to that of high regularisation models. Default models always had the greatest number of parameters (37 – 55) (Table 3). Increasing the regularisation multiplier decreased the number of parameters for each species, to a minimum of 7 – 16 across species at a regularisation multiplier of 8. Linear-quadratic models had a similar number of parameters (13 – 16) to models with moderate to high regularisation, while forward selection models typically had the fewest or equal fewest parameters for each species: 3 – 14 for the ML method and 3 – 9 for the Cauchy-prior method. Default models explained the highest proportion of deviance for each species as measured by D^2 (Figure 3); D^2 declined slightly with increasing regularisation, with linear-quadratic and forward selection models typically having intermediate D^2, but overall differences in D^2 between models were minor ($\leq$ 0.04; Figure 3). Adjusted D^2 was consistently lowest for default models and highest for forward selection

Table 2
Model performance measures for the most parsimonious model (lowest AICc), and null AUC (AUC of null or random model) for each species. For each performance metric, the difference between the selected and best model for each species as assessed by that metric is shown in brackets. AICc = AIC with small sample size correction, AUC test = AUC based on independent test data, AUC train = training data AUC, D^2 = deviance ratio, Adj D^2 = adjusted deviance ratio.

Species	Lowest AICc	null AUC	AUC train	AUC test	D^2	Adj D^2	Boyce Index
Solieria robusta	FwdSel - ML	0.51	0.70 (-0.06)	0.92 (0.00)	0.03 (-0.02)	0.01 (0.00)	0.77 (-0.11)
Gelidium australe	FwdSel - ML	0.51	0.83 (-0.02)	0.74 (0.00)	0.08 (-0.02)	0.05 (0.00)	0.59 (-0.37)
Pterocladia lucida	FwdSel - Cauchy	0.51	0.82 (-0.04)	0.84 (-0.02)	0.08 (-0.03)	0.04 (0.00)	0.88 (-0.10)
Plocamium angustum	FwdSel - ML	0.51	0.86 (0.00)	0.89 (0.00)	0.10 (-0.01)	0.09 (0.00)	0.87 (-0.13)
Ecklonia radiata	FwdSel - ML	0.51	0.80 (0.00)	0.70 (-0.01)	0.07 (-0.01)	0.05 (0.00)	0.91 (-0.08)
Cystophora subfarcinata	FwdSel - ML	0.51	0.84 (-0.01)	0.76 (-0.08)	0.09 (-0.02)	0.09 (0.00)	0.73 (-0.26)
Sargassum linearifolium	FwdSel - ML	0.52	0.78 (-0.04)	0.56 (-0.02)	0.06 (-0.02)	0.04 (0.00)	0.81 (0.00)
Scytothalia dorycarpa	FwdSel - ML	0.52	0.90 (-0.02)	0.83 (-0.04)	0.13 (-0.02)	0.10 (0.00)	0.72 (-0.12)

Table 3
Mean difference in model performance across eight species ± standard deviation, plus number of parameters. Difference calculated in comparison to best model by each metric for each species. AICc = AIC with small sample size correction, AUC test = AUC based on independent test data, AUC train = training data AUC, D^2 = deviance ratio, Adj D^2 = adjusted deviance ratio, K = mean number of parameters with minimum and maximum across species shown in brackets. Best performing models overall by each metric are highlighted in bold.

Model	ΔAICc	ΔAUC train	ΔAUC test	ΔD^2	ΔAdj D^2	ΔBoyce Index	K
Default	86.0 (± 85.6)	**0.00 (± 0.00)**	-0.08 (± 0.12)	**0.00 (± 0.00)**	-0.14 (± 0.06)	-0.09 (± 0.05)	45.0 (37 – 55)
RegMult2	78.7 (± 82.6)	-0.01 (± 0.01)	-0.06 (± 0.06)	-0.01 (± 0.00)	-0.07 (± 0.04)	**-0.02 (± 0.03)**	28.3 (19 – 35)
RegMult3	78.9 (± 86.5)	-0.02 (± 0.01)	-0.04 (± 0.03)	-0.01 (± 0.00)	-0.04 (± 0.02)	**-0.02 (± 0.02)**	20.8 (16 – 26)
RegMult4	84.1 (± 87.0)	-0.02 (± 0.01)	-0.04 (± 0.03)	-0.02 (± 0.00)	-0.03 (± 0.02)	-0.09 (± 0.09)	16.9 (14 – 23)
RegMult5	90.9 (± 85.9)	-0.03 (± 0.01)	-0.04 (± 0.03)	-0.02 (± 0.01)	-0.03 (± 0.02)	-0.12 (± 0.12)	15.4 (12 – 19)
RegMult6	95.9 (± 86.5)	-0.03 (± 0.01)	-0.04 (± 0.03)	-0.02 (± 0.01)	-0.03 (± 0.01)	-0.11 (± 0.11)	13.4 (11 – 17)
RegMult7	101.4 (± 85.9)	-0.03 (± 0.01)	-0.04 (± 0.03)	-0.02 (± 0.01)	-0.03 (± 0.01)	-0.14 (± 0.08)	12.5 (9 – 16)
RegMult8	105.5 (± 87.9)	-0.04 (± 0.01)	-0.03 (± 0.03)	-0.02 (± 0.01)	-0.02 (± 0.01)	-0.13 (± 0.09)	11.3 (7 – 16)
LinQuad	103.8 (± 88.7)	-0.04 (± 0.01)	-0.05 (± 0.04)	-0.02 (± 0.01)	-0.03 (± 0.02)	-0.17 (± 0.10)	14.3 (13 – 16)
FwdSel - ML	**0.0 (± 0.0)**	-0.02 (± 0.02)	**-0.02 (± 0.03)**	-0.02 (± 0.00)	**0.00 (± 0.00)**	-0.15 (± 0.12)	7.1 (3 – 14)
FwdSel - Cauchy	7.0 (± 10.5)	-0.03 (± 0.02)	-0.03 (± 0.03)	-0.02 (± 0.00)	**0.00 (± 0.00)**	-0.16 (± 0.10)	6.5 (3 – 9)

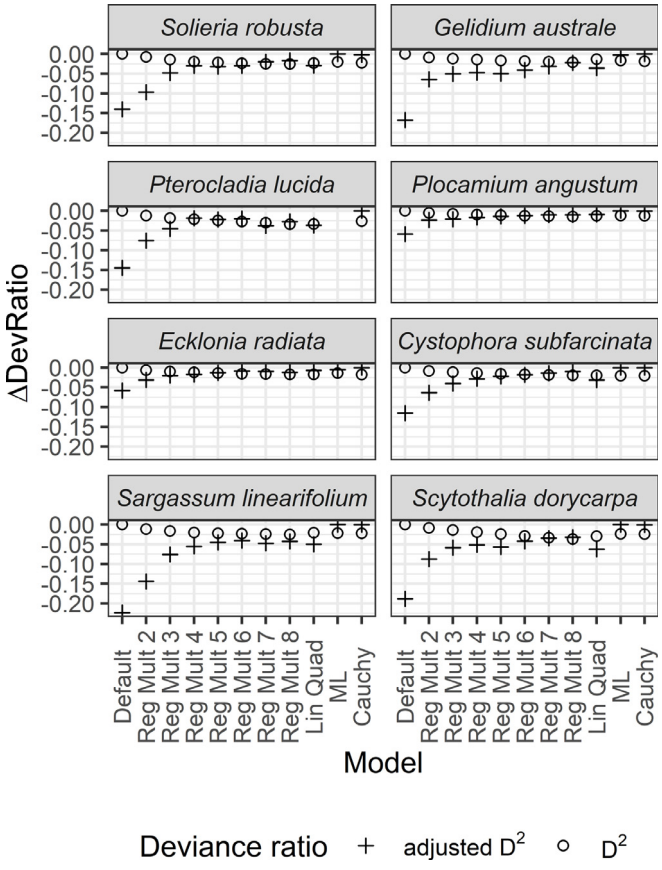

Figure 3. Comparison of deviance ratio and adjusted deviance ratio across modelling methods for each species. In each case, the difference in deviance ratio relative to the model with highest deviance ratio is shown.

models, specifically, for the ML option for *Solieria, Plocamium, Cystophora, Sargassum* and *Scytothalia*, and the Cauchy prior option for the remaining species, but with <0.005 difference in adjusted D^2 between the forward selection models for each species (Figure 3).

Predictive performance based on training data was similar within each species across modelling methods (Table 3, supplementary material) and all models had good discriminatory power, with a minimum difference of 0.19 between training and null AUC, and AUCtrain of each model > all 99 null AUCs (therefore AUC better than random, $p=0.01$). Average null AUC for each species and modelling method was close to 0.5 (Table 2). The model selected by AICc had the highest or equal highest AUCtrain for *Plocamium* and *Ecklonia*; the difference in AUCtrain between the selected model and highest AUCtrain for each species was minor (≤ 0.06) (Table 2). On average, AUCtrain was highest for the default method across species, but differences to other models were minor (average difference ≤ 0.04; Table 3). Regularisation and use of restricted feature types (linear and quadratic only) led to marginally reduced AUCtrain, with a regularisation multiplier of 8 and linear-quadratic models having lowest AUCtrain overall, while AUCtrain for forward selection models was intermediate (Table 3). Performance based on AUCtest showed greater variation across modelling methods, although average differences were still minor (≤ 0.08; Table 3). Forward selection ML models had the highest AUCtest on average, while default models had the lowest average AUCtest across species (Table 3). Default models, however, had the highest or equal highest AUCtest for *Pterocladia, Plocamium* and *Scytothalia*. The models selected by AICc had the highest or equal highest AUCtest for *Solieria, Gelidium* and *Plocamium* (Table 2). A regularisation multiplier of 4 resulted in the highest AUCtest for *Sargassum,* with high regularisation multipliers giving the highest AUCtest for *Scytothalia* and *Cystophora*, and the linear-quadratic model had the highest AUCtest for *Ecklonia* (see supplementary material). AUCtest scores were notably different between species, with the highest AUCtest scores being between 0.84 and 0.92 for most species, but lower for some: 0.71 and 0.74 for *Ecklonia* and *Gelidium* respectively, and 0.58 for *Sargassum.* The difference in AUCtest between the selected model and the highest AUCtest for each species was ≤ 0.08 (Table 2).

Calibration, as measured by the continuous Boyce index, varied across models without displaying consistent patterns. Calibration generally increased with increasing regularisation to a multiplier of between 2 and 6, but not always monotonically (supplementary material), with average calibration being highest at regularisation multipliers of 2 – 3 and lowest for models with restricted feature types (Table 3). The model selected by AICc had the highest calibration for *Sargassum,* while the AICc selected models for *Gelidium* and *Cystophora* showed reduced calibration in comparison to the best models for those species, although the selected models still showed good (> 0.5) calibration (Table 2). Forward selection models had lower calibration, on average, than default or high regularisation models, but the reduction was minor (Table 3), and average calibration across all modelling methods was good (≥ 0.76 in all cases; supplementary material). The Boyce index was positive for all models, ranging from 0.48 – 1, showing predictions consistent with expectation.

3.2. Environmental suitability of Spencer Gulf and aquaculture zones

The red seaweed *Solieria* was predicted to have high relative suitability (> 1) throughout Spencer Gulf by the selected model (Figure 4) with relative suitability being greatest in northern Spencer Gulf (> 2 in parts). All other species showed generally higher relative suitability in southern than northern Spencer Gulf (Figure 4). Relative suitability for

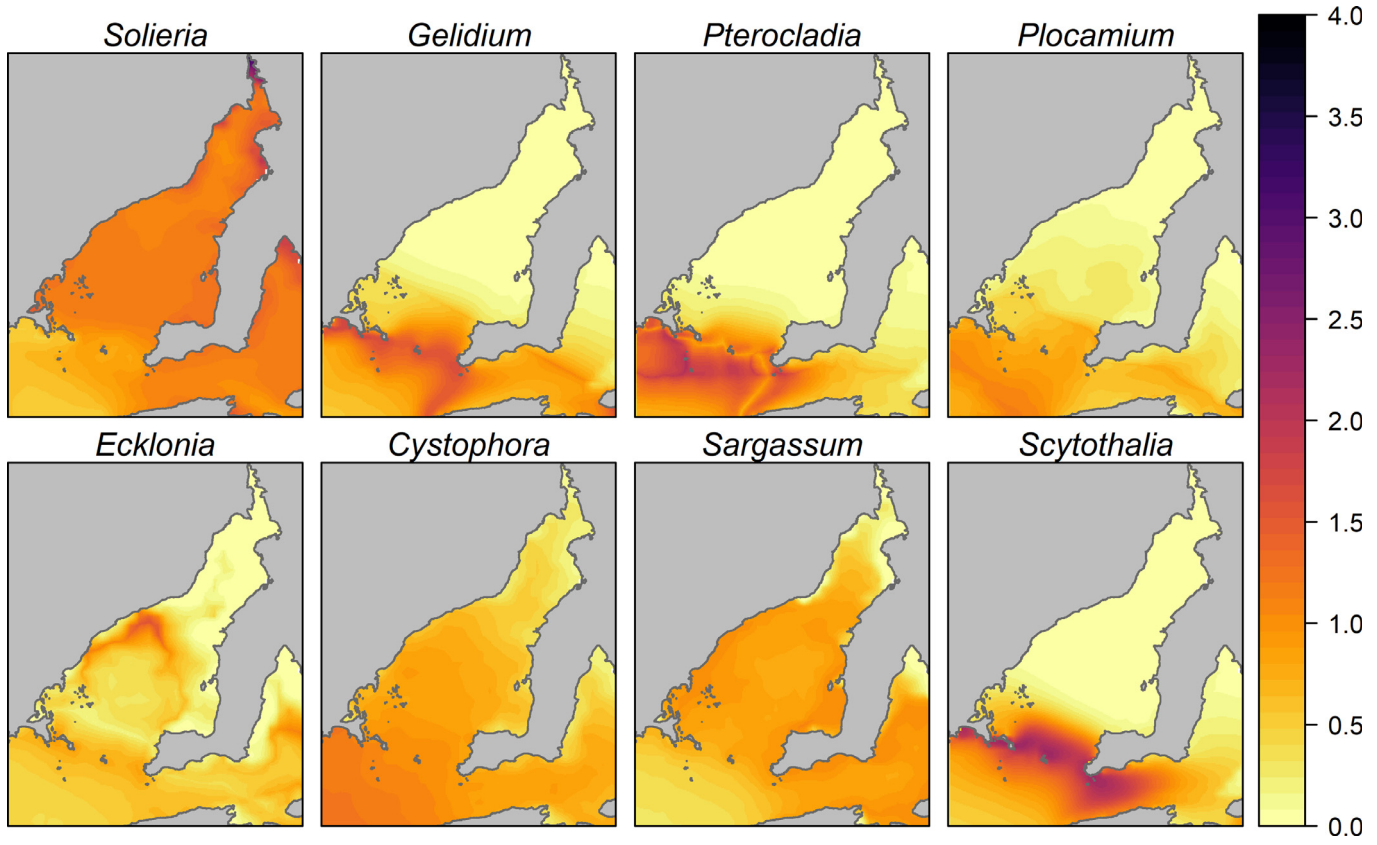

Figure 4. Map of relative suitability predictions for the Spencer Gulf region for the most parsimonious model for each species (Forward selection – maximum likelihood, except for *Pterocladia*, Forward selection – Cauchy prior). Refer to Figure 1 for a map of zone locations.

the brown seaweeds *Cystophora* and *Sargassum* was predicted to be > 1 for much of central and southern Spencer Gulf, and the brown *Ecklonia* also showed areas of relative suitability > 1 in central Spencer Gulf (Figure 4). Predictions were generally similar across modelling methods for most species, being most variable for *Ecklonia*; the default model for this species predicted somewhat lower relative suitability in central Spencer Gulf than the selected model, although still with areas of relative suitability > 0.5 (supplementary material). For *Cystophora* the selected model also predicted slightly higher suitability through much of Spencer Gulf and to the south of the gulf than the default model (supplementary material) and for *Solieria* the selected model predicted higher relative suitability in northern Spencer Gulf than the default model. Relative suitability from the selected model for *Gelidium, Pterocladia* and *Scytothalia*, however, was slightly lower in southern Spencer Gulf than for the default model in each case, while predictions were generally similar between the selected and default models for *Plocamium*. Maps of standard deviation across models show that the greatest discrepancies between predictions across all modelling methods were generally in southern Spencer Gulf and outside the gulf, except for *Solieria* where the greatest discrepancies were in northern Spencer Gulf (supplementary material). For all species, the greatest discrepancies were in areas where the highest relative suitability was predicted, with predictions for some modelling methods in these regions being considerably greater than others, while differences between models were relatively minor in areas of moderate to low suitability for each species.

Based on the selected model, *Solieria* had relative suitability > 1 for all existing aquaculture zones (Figure 5). Of the brown seaweeds, the highest relative suitability across aquaculture zones was predicted for *Sargassum*, which had predicted relative suitability > 0.5 except for Fitzgerald Bay, Tickera and Proper Bay, and suitability close to 1 for the Port Neill and Lincoln (inner) zones. Predicted relative suitability for *Cystophora* was > 0.5 for the Port Neill, Tumby Bay, Louth Bay, Boston Bay and Lincoln zones, with highest suitability in the Lincoln (outer) zone. *Ecklonia* had relative suitability ~ 1 in the Port Neil zone and ~ 0.5 in Tumby Bay and Louth Bay (south). Predicted relative suitability for *Scytothalia* was ~ 0.5 for the Boston Bay and Lincoln (inner) zones of southern Spencer Gulf, and approached 1 for the Lincoln (outer) zone. For the remaining red seaweeds, *Gelidium* and *Plocamium* had predicted relative suitability ~ 0.5 for the Lincoln (outer) zone, but predicted suitability was < 0.5 for other zones for these species, and in all zones for *Pterocladia* (Figure 5).

3.3. Differences between default and alternative model predictions

Differences between relative suitability predictions within aquaculture zones from default and alternative modelling methods did not display consistent patterns across species as assessed by the gamma GLMs (Table 4). The most parsimonious forward selection models predicted higher relative suitability on average across the aquaculture zones than the default models for *Solieria* and *Plocamium*, but lower for *Gelidium, Ecklonia* and *Sargassum*, while predictions were similar between the selected and default models for *Pterocladia, Cystophora* and *Scytothalia.* In all cases, however, the scale of the difference was small, the greatest discrepancy being ~20% higher relative suitability predictions (on average) by the forward selection model than the default model for *Solieria* (Table 4). Predictions from the Cauchy-prior forward selection model were lower than the ML model for *Solieria,* higher for *Gelidium* and *Ecklonia*, and similar for the other species, but differences, where present, were minor (Table 4). Increasing regularisation also had variable effects across species, leading to increasingly higher suitability predictions with greater regularisation for each of the red seaweeds and *Ecklonia,* lower predictions at a regularisation multiplier of 8 for *Cystophora,* and, for *Sargassum,* higher predictions than the default model at regularisation multipliers of 2 – 4, but with decreasing predictions at

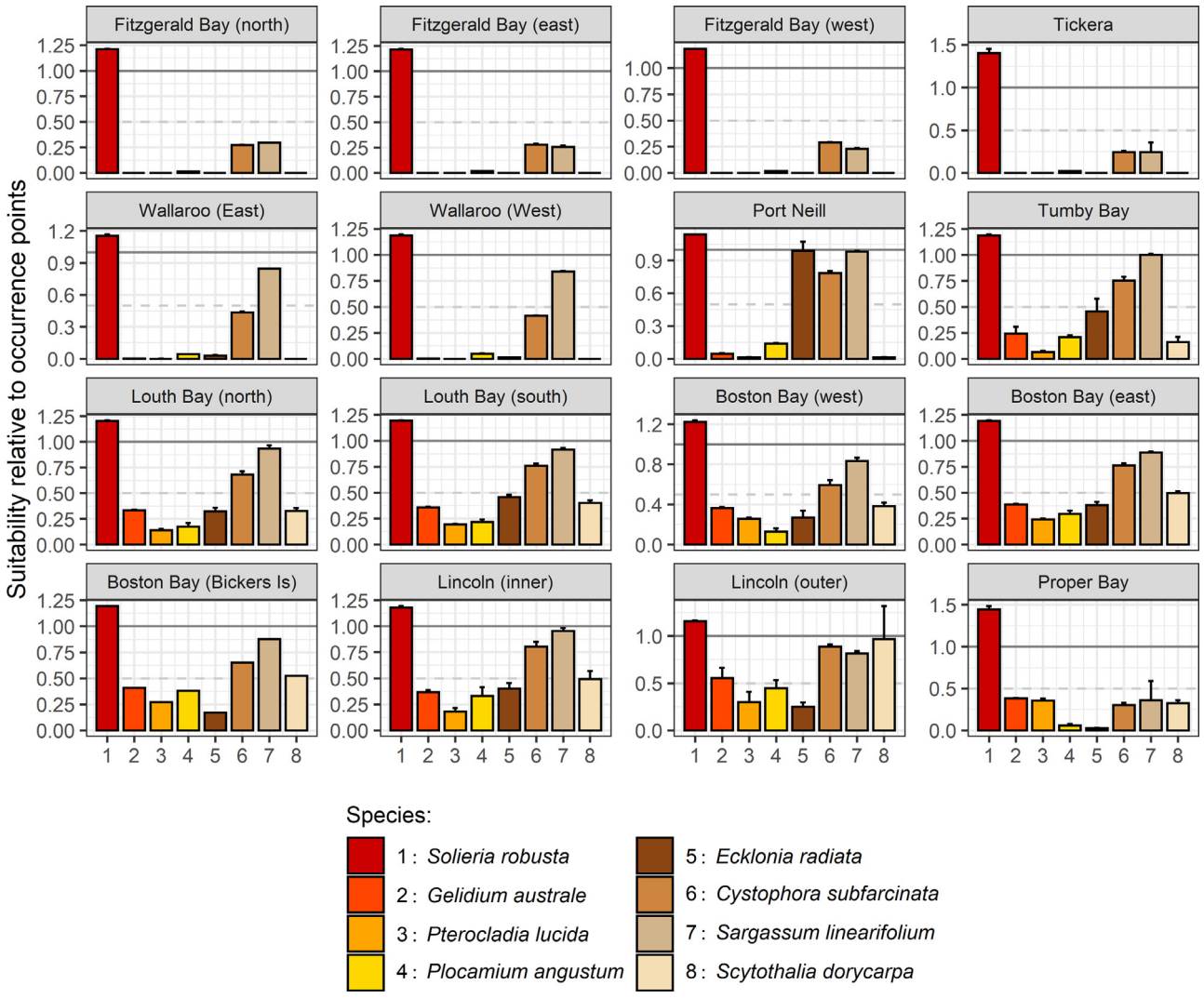

Figure 5. Average predicted relative suitability from the most parsimonious model for each species (Forward selection – maximum likelihood, except for *Pterocladia lucida*, Forward selection – Cauchy prior) for existing aquaculture zones in Spencer Gulf, South Australia. Solid line shows relative suitability = 1 and dashed line shows relative suitability = 0.5 for reference. Refer to Figure 1 for a map of zone locations.

higher regularisation, while predictions were similar for *Scytothalia* between default and higher regularisation models. In most cases the change to the scale of the predictions with regularisation was minor, but, for *Pterocladia*, predicted suitability under high regularisation was almost three times higher than under the default model. Using restricted feature types (linear-quadratic only) led to higher average predictions than the default model for *Solieria, Pterocladia, Plocamium, Ecklonia* and *Sargassum,* with predictions being similar between these modelling methods for the remaining species. The scale of differences between the linear-quadratic and default models was generally small, again with the exception of *Pterocladia*, for which the linear-quadratic model predictions were approximately twice as high as those of the default model (Table 4).

4. Discussion

Default methods for maximum entropy SDM produce overly complex models (Halvorsen et al., 2015; Radosavljevic and Anderson, 2014; Syfert et al., 2013; Vollering et al., 2019; Warren and Seifert, 2011); our results also demonstrate that default models are over-parameterised, leading to increased AICc and decreases in adjusted D^2, and generally lower predictive performance (AUC) using independent test data. We found that forward selection methods, following the *MIAmaxent* approach (Halvorsen, 2013; Halvorsen et al., 2015; Vollering et al., 2019) produced the most parsimonious models, but, as recognised by Hefley and Hooten (2015), the ML method may fail in some circumstances where ML estimates are not finite due to separation. We encountered this problem in the model for the red seaweed *Pterocladia.* Applying a weakly informative Cauchy prior (as per Gelman et al., 2008) to the weighted logistic regression allowed a forward selection model to be produced for *Pterocladia*, with this model being most parsimonious for this species. For each of the remaining species, the Cauchy prior model had marginally higher AICc than the ML forward selection model, but lower than other modelling methods. The other proposed approaches to limit model complexity: increasing the regularisation multiplier (Anderson and Gonzalez Jr, 2011; Muscarella et al., 2014; Radosavljevic and Anderson, 2014), or using only linear and quadratic feature types (Elith et al., 2010; Merow et al., 2013; Syfert et al., 2013), each resulted in models having fewer parameters than the default method. These methods, however, also resulted in poorer model fit, i.e. reduced D^2 and log likelihood, and hence AICc similar to or higher than default models.

Default models had the best predictive performance as assessed using training data AUC, but differences to other model options were minor, and all models performed well as assessed by this metric. AUC is often used for SDM assessment, but the usefulness of AUC for this

Table 4
Difference in average predicted relative suitability and 95% credible intervals across modelling methods for each species from Bayesian gamma GLM. Arrows indicate predictions that are higher (↑) or lower (↓) than the default model based on non-overlapping 95% credible intervals.

Model	Average relative suitability prediction (95% credible interval) by species							
	Solieria	*Gelidium*	*Pterocladia*	*Plocamium*	*Ecklonia*	*Cystophora*	*Sargassum*	*Scytothalia*
Default	1.05 (1.02 – 1.08)	0.41 (0.37 – 0.46)	0.24 (0.21 – 0.27)	0.23 (0.21 – 0.26)	0.48 (0.44 – 0.53)	0.75 (0.71 – 0.80)	0.88 (0.85 – 0.92)	0.45 (0.39 – 0.53)
RegMult2	1.04 (1.01 – 1.07)	0.53 (0.48 – 0.60) ↑	0.42 (0.37 – 0.47) ↑	0.25 (0.23 – 0.28)	0.55 (0.50 – 0.60) ↑	0.79 (0.74 – 0.84)	1.05 (1.01 – 1.09) ↑	0.45 (0.39 – 0.53)
RegMult3	1.10 (1.07 – 1.13)↑	0.57 (0.51 – 0.63) ↑	0.43 (0.39 – 0.49) ↑	0.27 (0.25 – 0.30) ↑	0.60 (0.55 – 0.65) ↑	0.78 (0.73 – 0.83)	1.05 (1.01 – 1.10) ↑	0.45 (0.39 – 0.52)
RegMult4	1.15 (1.11 – 1.18) ↑	0.59 (0.53 – 0.66) ↑	0.49 (0.43 – 0.55) ↑	0.28 (0.26 – 0.31) ↑	0.61 (0.56 – 0.67) ↑	0.76 (0.71 – 0.81)	1.00 (0.96 – 1.04) ↑	0.45 (0.39 – 0.53)
RegMult5	1.18 (1.15 – 1.22) ↑	0.60 (0.54 – 0.67) ↑	0.54 (0.48 – 0.61) ↑	0.29 (0.26 – 0.32) ↑	0.62 (0.57 – 0.67) ↑	0.74 (0.69 – 0.79)	0.95 (0.91 – 0.99)	0.45 (0.38 – 0.52)
RegMult6	1.22 (1.19 – 1.26) ↑	0.61 (0.55 – 0.68) ↑	0.60 (0.53 – 0.67) ↑	0.29 (0.26 – 0.32) ↑	0.62 (0.57 – 0.68) ↑	0.71 (0.67 – 0.76)	0.92 (0.88 – 0.96)	0.42 (0.36 – 0.49)
RegMult7	1.26 (1.23 – 1.30) ↑	0.61 (0.55 – 0.68) ↑	0.65 (0.58 – 0.73) ↑	0.29 (0.26 – 0.32) ↑	0.62 (0.57 – 0.68) ↑	0.69 (0.65 – 0.73)	0.89 (0.85 – 0.92)	0.42 (0.36 – 0.48)
RegMult8	1.28 (1.24 – 1.32) ↑	0.61 (0.55 – 0.68) ↑	0.71 (0.63 – 0.80) ↑	0.29 (0.26 – 0.31) ↑	0.62 (0.57 – 0.68) ↑	0.67 (0.63 – 0.72) ↓	0.86 (0.83 – 0.90)	0.42 (0.35 – 0.48)
LinQuad	1.10 (1.07 – 1.14) ↑	0.45 (0.41 – 0.50)	0.51 (0.45 – 0.58) ↑	0.28 (0.26 – 0.31) ↑	0.59 (0.54 – 0.64) ↑	0.77 (0.73 – 0.82)	0.99 (0.95 – 1.04) ↑	0.53 (0.46 – 0.62)
FwdSel - ML	1.19 (1.15 – 1.22) ↑	0.35 (0.32 – 0.39)↓		0.28 (0.26 – 0.31) ↑	0.32 (0.29 – 0.34) ↓	0.74 (0.69 – 0.78)	0.82 (0.79 – 0.86) ↓	0.50 (0.43 – 0.58)
FwdSel - Cauchy	1.07 (1.04 – 1.11)	0.45 (0.40 – 0.50)	0.18 (0.16 – 0.20) ↓	0.29 (0.26 – 0.31) ↑	0.53 (0.48 – 0.58)	0.73 (0.69 – 0.78)	0.82 (0.79 – 0.86) ↓	0.47 (0.41 – 0.55)

purpose has been questioned (Jiménez-Valverde, 2012; Lobo et al., 2008; Peterson et al., 2008). Training AUC in particular has been criticised as a metric for model selection, because it typically selects overfitted models (Warren and Seifert, 2011). Interpretation of AUC for presence-only models can be problematic, but use of null model AUC for comparison can assist with this issue (Merckx et al., 2011; Raes and ter Steege, 2007). In contrast to other studies (Hijmans, 2012; Raes and ter Steege, 2007) we found null AUC for each species to be close to 0.5, the value expected for presence-absence models or in the absence of sampling bias, but this will not always be the case where presence only data are used, hence the null model AUC value serves as a useful comparison for assessment of model AUC values. The use of independent, and ideally presence-absence, test data for model selection and validation based on test data AUC has been recommended, and is recognised as a more appropriate measure of model performance than training AUC (Halvorsen et al., 2016; Liu et al., 2013; Phillips and Dudík, 2008; Radosavljevic and Anderson, 2014; Warren and Seifert, 2011). Forward selection models had the highest AUCtest scores on average, however we did not find that test data AUC consistently selected the most parsimonious models. Differences in predictive performance to test data between models were generally minor, and although default models had decreased AUCtest in some cases, this pattern was not consistent across species. We also found that AUCtest varied more widely between models for different species than between models within any individual species, and was consistently lower for some species than others. The differences in AUC between species likely occurred because AUC is typically higher for species with a well-defined geographic or ecological niche than for more widespread, generalist species (Lobo et al., 2008; van Proosdij et al., 2016). While some limitations of using AUC for model comparison can be overcome (Raes and ter Steege, 2007; van Proosdij et al., 2016), or may not apply in all cases (Halvorsen et al., 2016), it is clear that AUC for assessment of presence-only models is only valid for comparison within, and not across, species models. For our purpose we wanted to predict potential suitability, but in addition to its other drawbacks, AUC is typically a poor metric for selecting models of the potential, as opposed to realised, distribution of a species (Jiménez-Valverde, 2012).

Calibration measured by the continuous Boyce index did not show consistent patterns with modelling method across species, although overall calibration was marginally higher for models with increased regularisation than for default models. The forward selection models selected by AICc showed reduced calibration on average, but were still well calibrated, having a minimum Boyce index of 0.59. Calibration is an often overlooked aspect of model validation (Fieberg et al., 2018; Hirzel et al., 2006) but its suitability as a metric for model selection has not been thoroughly assessed.

We selected models using AICc based on the principal of parsimony, because simpler models are likely to have greater transferability and ecological interpretability than more complex models (Halvorsen et al., 2016; Radosavljevic and Anderson, 2014; Verbruggen et al., 2013; Vollering et al., 2019; Warren and Seifert, 2011), considerations that are more important to our purpose than accuracy of prediction. We found that AICc was lowest and adjusted D^2 was highest for forward selection models, with AICc selecting the ML model (except where not identified) and adjusted D^2 being very similar between ML and Cauchy prior models for each species. To our knowledge, adjusted D^2 has not been used for selection of maxent models, although it can be applied for comparison in logistic regression (Gelman and Su, 2018; Hirzel et al., 2006); the recognition of maxent as an IPP opens up the possibility of using adjusted D^2 to assess and compare model fit for these models. AICc does not necessarily select models with the greatest predictive accuracy, as demonstrated by Velasco and González-Salazar (2019) and also by our results, and the same is likely true of adjusted D^2. We note however, that differences in AUC and calibration between the most parsimonious models and best performing models as assessed by these metrics were minor. In our case, therefore, there was little cost to

predictive accuracy in selecting models based on AICc. Further validation of our model results is, however, difficult due to the lack of existing seaweed farms or larger sets of systematic survey data for the modelled species. The relative suitability predictions for our area of interest varied between modelling methods, but the scale of the differences was generally not large, and in most cases would not change the interpretation of areas as being broadly suitable or unsuitable for each species. We found, however, that increased regularisation or the use of restricted feature types, resulted in considerably (2 – 3 x) higher predictions than the default for *Pterocladia*. It appears therefore, that predictions based on these particular alternative modelling methods could change interpretations of results in some cases. The *R* packages now implemented to run the various maxent modelling methods via weighted logistic regression are faster than the MaxEnt.jar software, hence it is more feasible to run and compare multiple modelling methods across several species than previously. We therefore encourage further exploration of the effects of using alternative modelling strategies on model performance and predictions. Choice of modelling method, and of the metric applied to assess models, should depend on the modelling purpose at hand, and the relative importance of transferability, interpretability and predictive accuracy for that purpose.

Aquaculture site selection is a relatively new application of SDM, and the use of SDM for this purpose is not well established, though the potential of this method has been recognised (Falconer et al., 2016; Linhoss et al., 2016; Oyinlola et al., 2018). SDM applications for aquaculture site selection to date have primarily used the location (Falconer et al., 2016; Oyinlola et al., 2018) or commercial yield (Vincenzi et al., 2007; Vincenzi et al., 2011) of existing farms for model-building, and have assessed model results relative to the outputs of the more established methods (e.g. multi-criteria evaluation) that rely on pre-existing knowledge of suitable conditions for farming (Falconer et al., 2016; Vincenzi et al., 2007). Falconer et al. (2016) found that Mahalanobis Typicality model outputs were more consistent with multi-criteria evaluation results than those of the default MaxEnt.jar method for predicting site suitability for fish farms. Our aims and method varied from theirs for several reasons: we were considering novel aquaculture species, for which there are no existing farms, and the lack of detailed biological knowledge of these species prevented us from applying a multi-criteria evaluation for comparison. Our application of SDM for aquaculture also differed as we considered suitability for candidate species within existing aquaculture zones, with these zones having already been spatially defined following assessment for social, logistical and general environmental suitability for aquaculture (PIRSA, 2013). Our interest was therefore solely habitat suitability for the species being considered, and we elected to use maximum entropy modelling as this is a well-suited method for the presence-only occurrence data that were available. Our results demonstrate, however, that the default maxent method may not be the most appropriate where model transferability to different areas is likely to be important, such as for predicting aquaculture site suitability.

The models developed here show relative suitability for the native seaweeds that are being investigated for cultivation across the existing aquaculture zones in SA. It is unclear how well predicted suitability from the models, which is based on relative occurrence rate, will relate to seaweed performance in aquaculture. We interpret the results with some caution in light of this limited validation, but given the paucity of data on the species considered, the results help to inform future research directions. Specifically, the results show which zones may be most suitable for seaweed aquaculture, and will assist in identification of the species most suitable for cultivation in each area. All existing aquaculture zones in Spencer Gulf SA where seaweed farming is permitted are likely to be suitable for at least one of the candidate seaweeds. *Solieria* appears the best suited for cultivation in Spencer Gulf, especially in the northern zones, with the brown seaweeds *Sargassum, Ecklonia, Cystophora* and *Scytothalia* each being potentially suited to at least some areas of southern Spencer Gulf. The other candidate red seaweeds, *Gelidium, Pterocladia* and *Plocamium,* show generally low suitability throughout the Spencer Gulf aquaculture zones, and appear less suited to cultivation in this area. Spencer Gulf is an inverse estuary, with evaporation exceeding precipitation, leading to increasingly hypersaline conditions towards the head (north) of the gulf; northern Spencer Gulf also experiences warmer temperatures and a greater annual temperature range than the southern gulf (Nunes and Lennon, 1986). These environmental gradients are likely to be important drivers for seaweed habitat suitability. We note that the spatial resolution of the environmental data used is too low to distinguish fine scale distributional preferences, such as occurrence on exposed or sheltered sides of a reef, thus only broad scale responses to variables such as wave energy are captured in the models. The environmental data used are also annual averages or seasonal extremes, and so model results reflect suitability for each species to occur long-term. For those species with low predicted suitability, it is possible that conditions in some areas may be suitable for growth at certain times of year and permit seasonal cultivation. Species with greater relative suitability in an area are, however, likely to perform better over a greater proportion of the year, and it is unlikely that a species will grow well in an area of very low relative suitability for its occurrence. The models also provide insight into ecological responses which can be used to guide further investigation of candidate species. Models built using the alternative modelling approach of *MIAmaxent* provide relatively simple response curves to ecological parameters (Halvorsen et al., 2016; Støa et al., 2018; Vollering et al., 2019), which can be explored in order to determine potential suitable environmental conditions, and apparent optima, for each species. Response curves from SDMs do not always reflect physiological or ecological responses due to the influence of correlated variables (Elith and Leathwick, 2009), or because a species may be restricted from occurring at sites with favourable conditions of a given predictor by other factors (Marcelino and Verbruggen, 2015). The curves can still provide insight where biological knowledge is lacking, and help to inform further investigation (Marcelino and Verbruggen, 2015). Such experimental investigation of optimal conditions for growth will help to inform the best seasons for growth as well as assisting in identifying the best potential areas for seaweed aquaculture.

CRediT authorship contribution statement

Kathryn H Wiltshire: Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing - original draft, Visualization. **Jason E Tanner:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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Supplementary material

Supplementary tables

Table S1. Model assessment results for *Solieria.* The best performing model as assessed by each metric is shown in bold

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	4815	**0.051**	-0.132	0.51	**0.76**	0.57
Reg Mult 2	4818	0.043	-0.088	0.51	0.74	0.74
Reg Mult 3	4817	0.036	-0.040	0.51	0.73	0.86
Reg Mult 4	4830	0.031	-0.022	0.51	0.72	0.85
Reg Mult 5	4839	0.029	-0.024	0.51	0.71	0.89
Reg Mult 6	4845	0.027	-0.022	0.51	0.71	0.89
Reg Mult 7	4844	0.026	-0.011	0.51	0.70	0.89
Reg Mult 8	4845	0.025	-0.008	0.51	0.70	0.90
LinQuad	4844	0.028	-0.021	0.51	0.71	0.88
Fwd ML	**4787**	0.030	**0.009**	0.51	0.70	**0.92**
Fwd Cauchy	4798	0.028	0.006	0.51	0.70	0.89

Table S2. Model assessment results for *Gelidium.* The best performing model as assessed by each metric is shown in bold

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	3278	**0.095**	-0.111	0.51	**0.85**	0.67
Reg Mult 2	3259	0.086	-0.008	0.51	0.83	0.66
Reg Mult 3	3261	0.083	0.006	0.52	0.83	0.66
Reg Mult 4	3267	0.081	0.009	0.51	0.82	0.67
Reg Mult 5	3275	0.078	0.007	0.51	0.82	0.67
Reg Mult 6	3276	0.077	0.016	0.51	0.82	0.66
Reg Mult 7	3275	0.075	0.025	0.52	0.82	0.66
Reg Mult 8	3275	0.074	0.034	0.51	0.81	0.67
LinQuad	3260	0.082	0.021	0.51	0.82	0.67
Fwd ML	**3230**	0.079	0.054	0.51	0.83	**0.74**
Fwd Cauchy	3236	0.076	**0.057**	0.52	0.82	0.72

Table S3. Model assessment results for *Pterocladia.* The best performing model as assessed by each metric is shown in bold. Note that the Fwd ML model for *Pterocladia* was not identifiable.

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	3571	**0.105**	-0.103	0.51	**0.86**	**0.86**
Reg Mult 2	3572	0.093	-0.034	0.51	0.85	0.80
Reg Mult 3	3577	0.086	-0.003	0.52	0.83	0.80
Reg Mult 4	3573	0.083	0.023	0.52	0.83	0.80
Reg Mult 5	3584	0.080	0.020	0.52	0.83	0.80
Reg Mult 6	3592	0.077	0.022	0.51	0.82	0.80
Reg Mult 7	3610	0.074	0.004	0.51	0.82	0.80
Reg Mult 8	3618	0.070	0.015	0.51	0.81	0.76
LinQuad	3622	0.071	0.005	0.52	0.81	0.83
Fwd Cauchy	**3555**	0.078	**0.042**	0.52	0.82	0.84

Table S4. Model assessment results for *Plocamium.* The best performing model as assessed by each metric is shown in bold

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	10599	**0.116**	0.033	0.51	0.85	**0.89**
Reg Mult 2	10578	0.110	0.069	0.51	0.85	0.88
Reg Mult 3	10587	0.108	0.071	0.51	0.84	0.88
Reg Mult 4	10590	0.106	0.075	0.51	0.84	0.88
Reg Mult 5	10594	0.104	0.078	0.51	0.84	0.87
Reg Mult 6	10600	0.103	0.080	0.51	0.84	0.87
Reg Mult 7	10603	0.102	0.082	0.51	0.83	0.86
Reg Mult 8	10609	0.101	0.082	0.51	0.83	0.86
LinQuad	10604	0.103	0.083	0.51	0.83	0.88
Fwd ML	**10339**	0.104	**0.092**	0.51	**0.86**	**0.89**
Fwd Cauchy	10344	0.103	**0.092**	0.51	**0.86**	**0.89**

Table S5. Model assessment results for *Ecklonia.* The best performing model as assessed by each metric is shown in bold

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	10714	**0.084**	-0.003	0.51	**0.80**	0.63
Reg Mult 2	10716	0.077	0.024	0.51	0.79	0.65
Reg Mult 3	10722	0.074	0.035	0.51	0.79	0.68
Reg Mult 4	10733	0.072	0.038	0.51	0.79	0.66
Reg Mult 5	10739	0.070	0.043	0.51	0.78	0.67
Reg Mult 6	10745	0.068	0.047	0.51	0.78	0.67
Reg Mult 7	10752	0.068	0.046	0.51	0.78	0.67
Reg Mult 8	10761	0.067	0.044	0.51	0.78	0.69
LinQuad	10761	0.067	0.048	0.51	0.78	**0.71**
Fwd ML	**10541**	0.070	0.051	0.51	**0.80**	0.70
Fwd Cauchy	10572	0.066	**0.055**	0.51	**0.80**	0.68

Table S6. Model assessment results for *Cystophora.* The best performing model as assessed by each metric is shown in bold

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	5820	**0.113**	-0.028	0.51	**0.86**	0.81
Reg Mult 2	5817	0.104	0.023	0.51	0.85	0.82
Reg Mult 3	5811	0.101	0.047	0.51	0.84	0.82
Reg Mult 4	5811	0.099	0.059	0.51	0.84	0.83
Reg Mult 5	5814	0.097	0.065	0.51	0.84	0.83
Reg Mult 6	5819	0.096	0.069	0.51	0.84	0.83
Reg Mult 7	5821	0.094	0.073	0.51	0.84	**0.84**
Reg Mult 8	5823	0.093	0.077	0.51	0.83	**0.84**
LinQuad	5841	0.093	0.056	0.51	0.83	0.78
Fwd ML	**5746**	0.092	**0.087**	0.51	0.84	0.76
Fwd Cauchy	**5746**	0.092	**0.087**	0.51	0.84	0.75

Table S7. Model assessment results for *Sargassum.* The best performing model as assessed by each metric is shown in bold

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	3123	**0.080**	-0.181	0.52	**0.82**	0.51
Reg Mult 2	3123	0.069	-0.102	0.52	0.80	0.55
Reg Mult 3	3110	0.064	-0.034	0.52	0.79	**0.56**
Reg Mult 4	3112	0.060	-0.013	0.51	0.79	0.58
Reg Mult 5	3114	0.058	-0.003	0.51	0.78	0.50
Reg Mult 6	3115	0.057	0.002	0.51	0.78	0.53
Reg Mult 7	3120	0.056	-0.005	0.51	0.78	0.57
Reg Mult 8	3121	0.055	0.000	0.51	0.78	0.56
LinQuad	3112	0.059	-0.008	0.52	0.78	0.47
Fwd ML	**3080**	0.058	**0.042**	0.52	0.78	**0.56**
Fwd Cauchy	**3080**	0.058	**0.042**	0.52	0.79	0.55

Table S8. Model assessment results for *Scytothalia.* The best performing model as assessed by each metric is shown in bold

Model	Parameter AICc	D^2	Adj D^2	null AUC	AUCtrain	AUCtest
Default	2553	**0.154**	-0.092	0.52	**0.91**	**0.87**
Reg Mult 2	2533	0.145	0.009	0.52	**0.91**	**0.87**
Reg Mult 3	2533	0.140	0.038	0.52	**0.91**	0.86
Reg Mult 4	2543	0.135	0.045	0.52	0.90	0.85
Reg Mult 5	2556	0.130	0.040	0.52	0.90	0.85
Reg Mult 6	2562	0.125	0.054	0.52	0.89	0.86
Reg Mult 7	2570	0.121	0.062	0.51	0.89	0.85
Reg Mult 8	2578	0.117	0.065	0.51	0.89	**0.87**
LinQuad	2573	0.125	0.034	0.51	0.89	0.83
Fwd ML	**2508**	0.130	**0.097**	0.52	0.90	0.83
Fwd Cauchy	2510	0.130	0.096	0.52	0.90	0.84

Supplementary figures

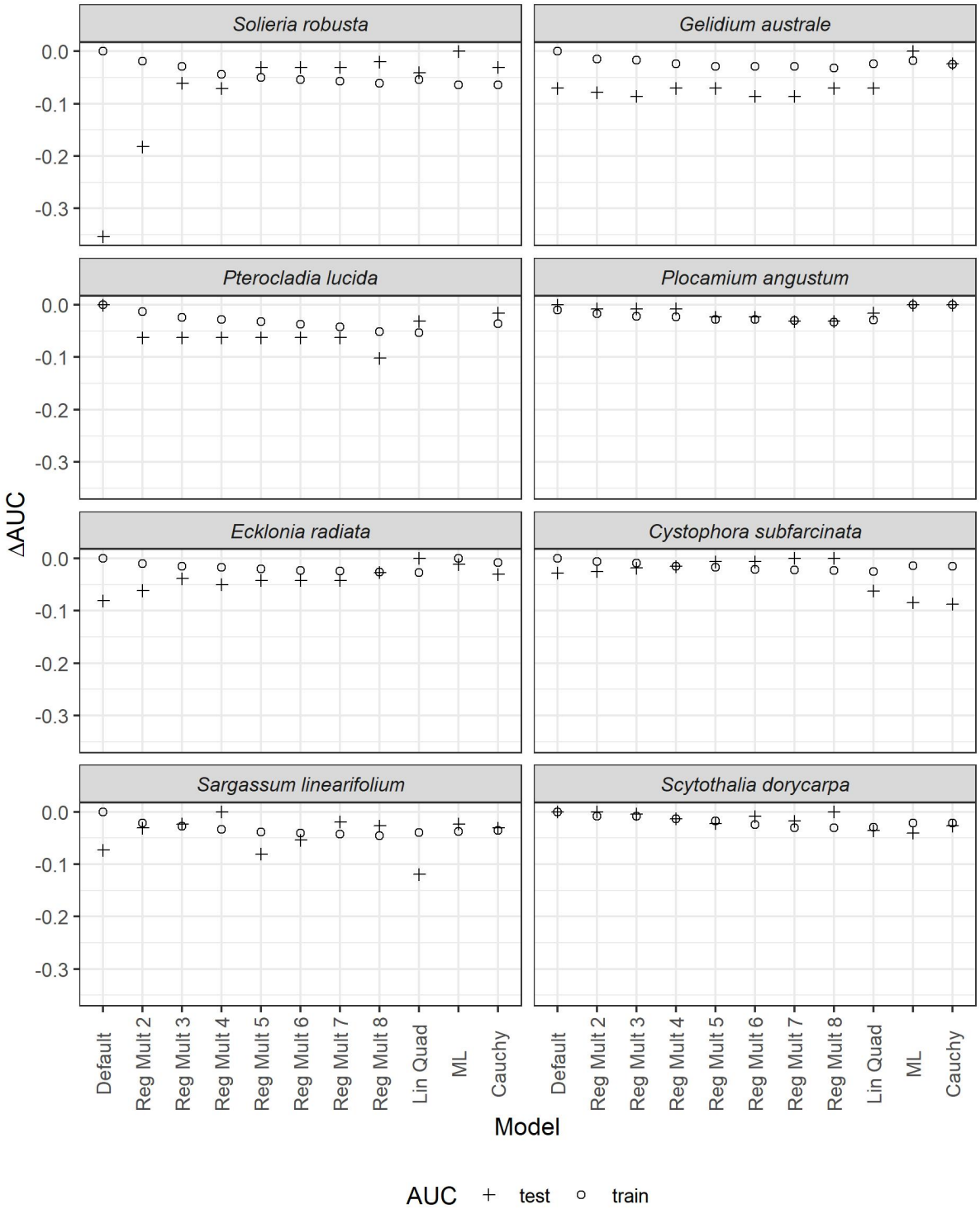


Figure S1. Comparison of AUC based on independent test or training data across modelling methods for each species. In each case the difference in AUC relative to the model with highest AUC is shown.

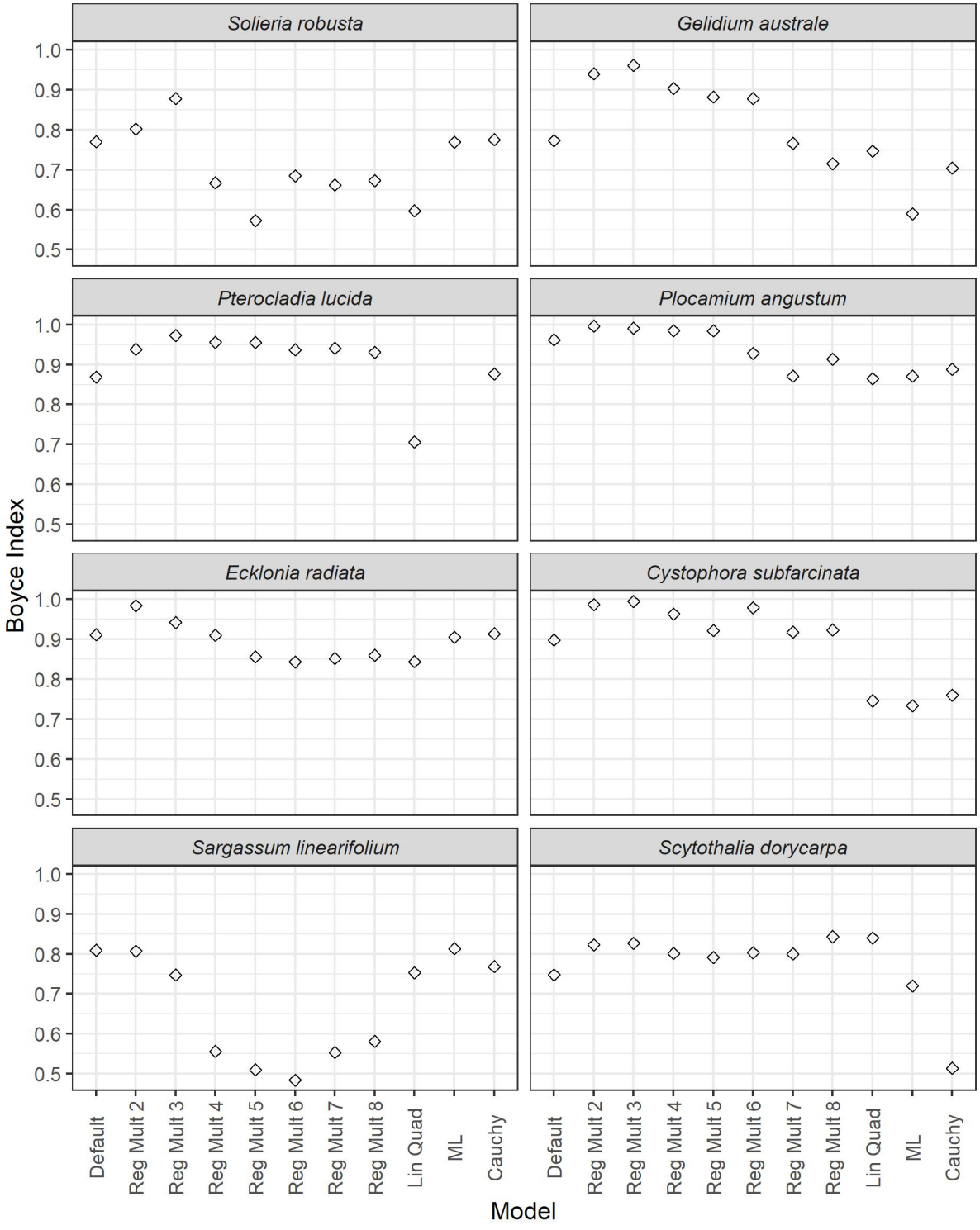


Figure S2. Boyce index across modelling methods for each species. Note that the Boyce index has possible values from -1 to 1, with values > 0 indicating predictions in line with expectation, values ~ 0 indicating performance no better than random, and values < 0 indicating predictions contrary to expectation.

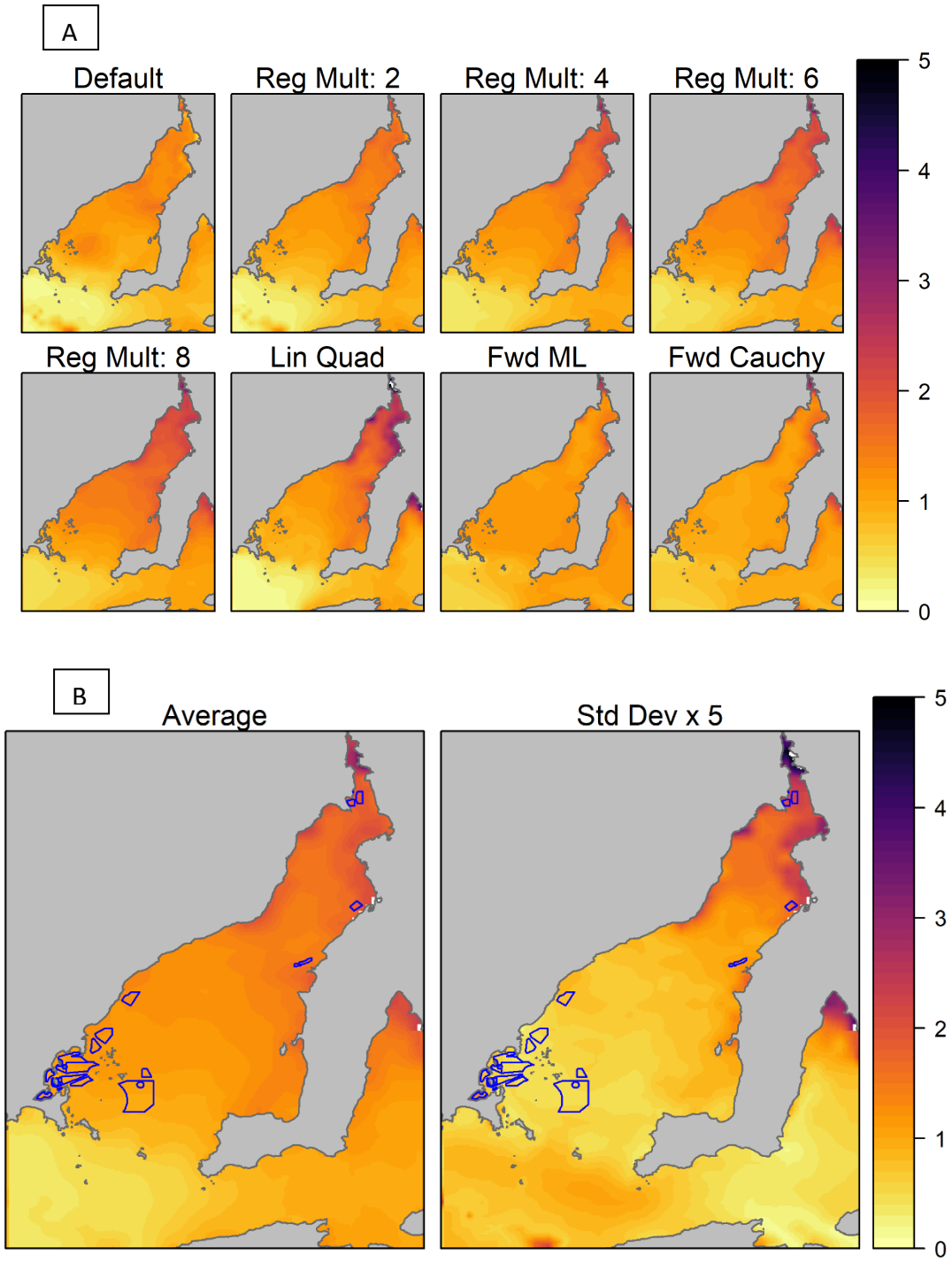


Figure S3. Maps of model predictions for the Spencer Gulf region for *Solieria robusta*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue.

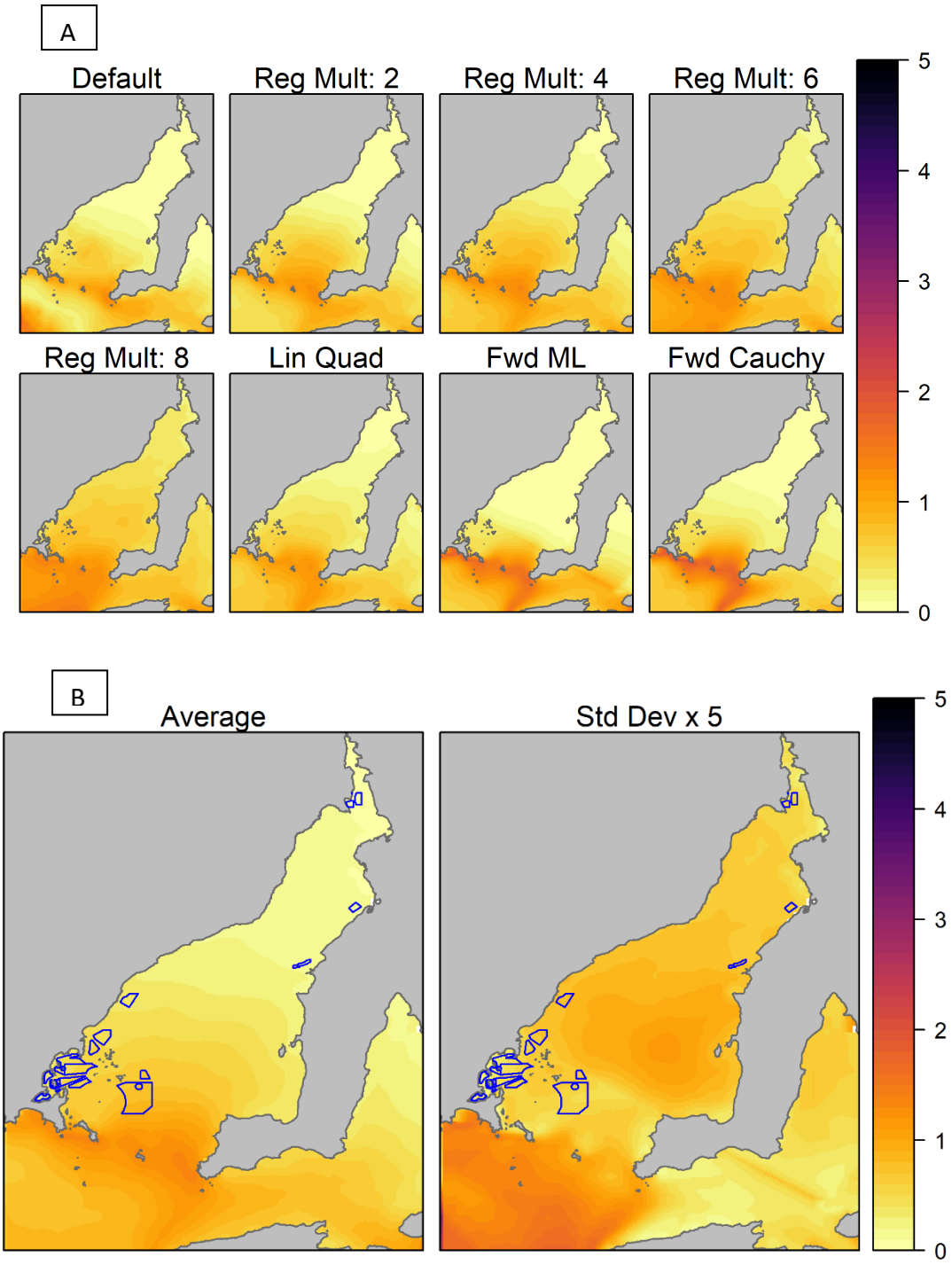


Figure S4. Maps of model predictions for the Spencer Gulf region for *Gelidium australe*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue.

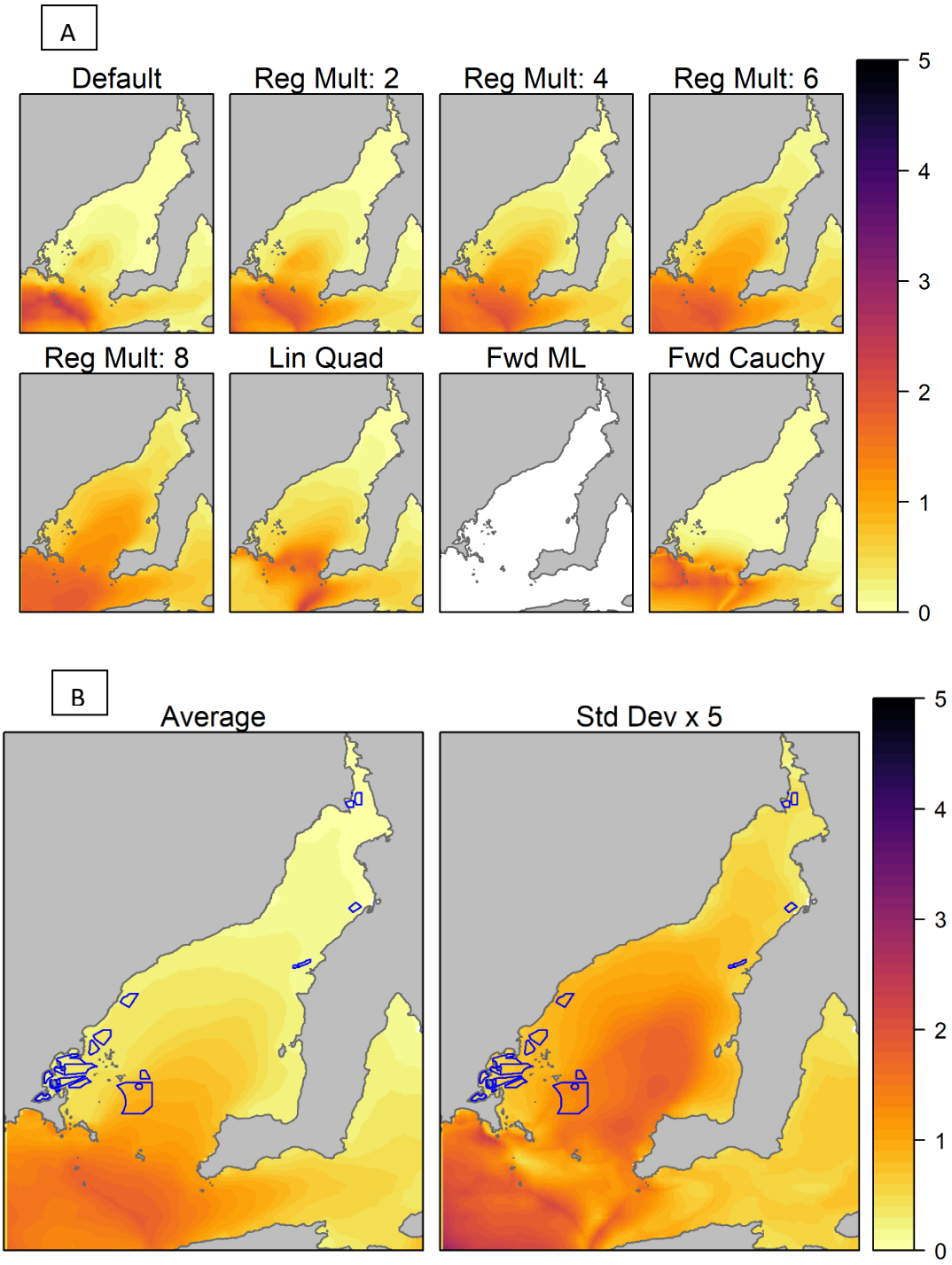


Figure S5. Maps of model predictions for the Spencer Gulf region for *Pterocladia lucida*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood*, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue. *Note: Fwd ML model not identifiable for this species

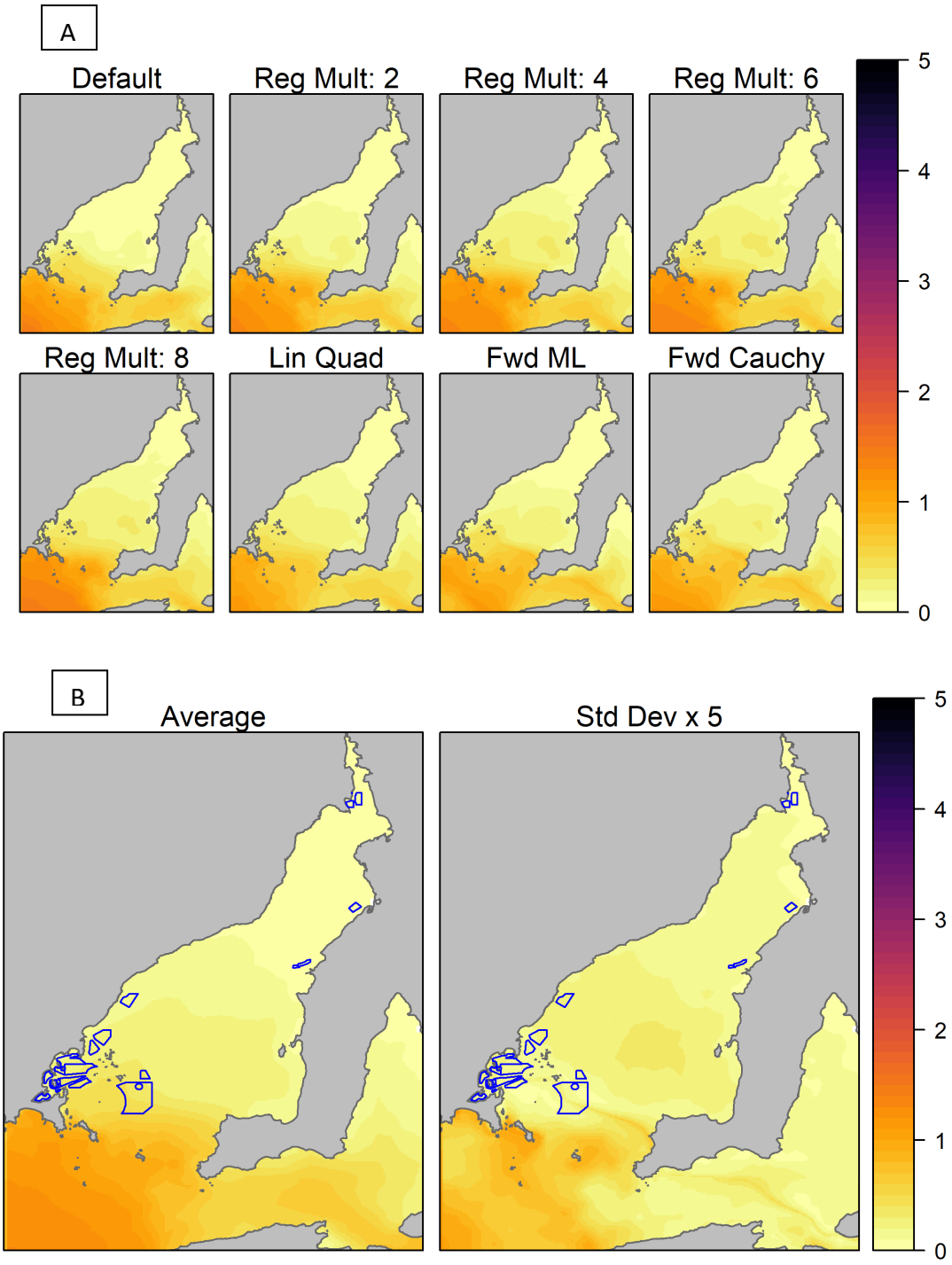


Figure S6. Maps of model predictions for the Spencer Gulf region for *Plocamium angustum*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue.

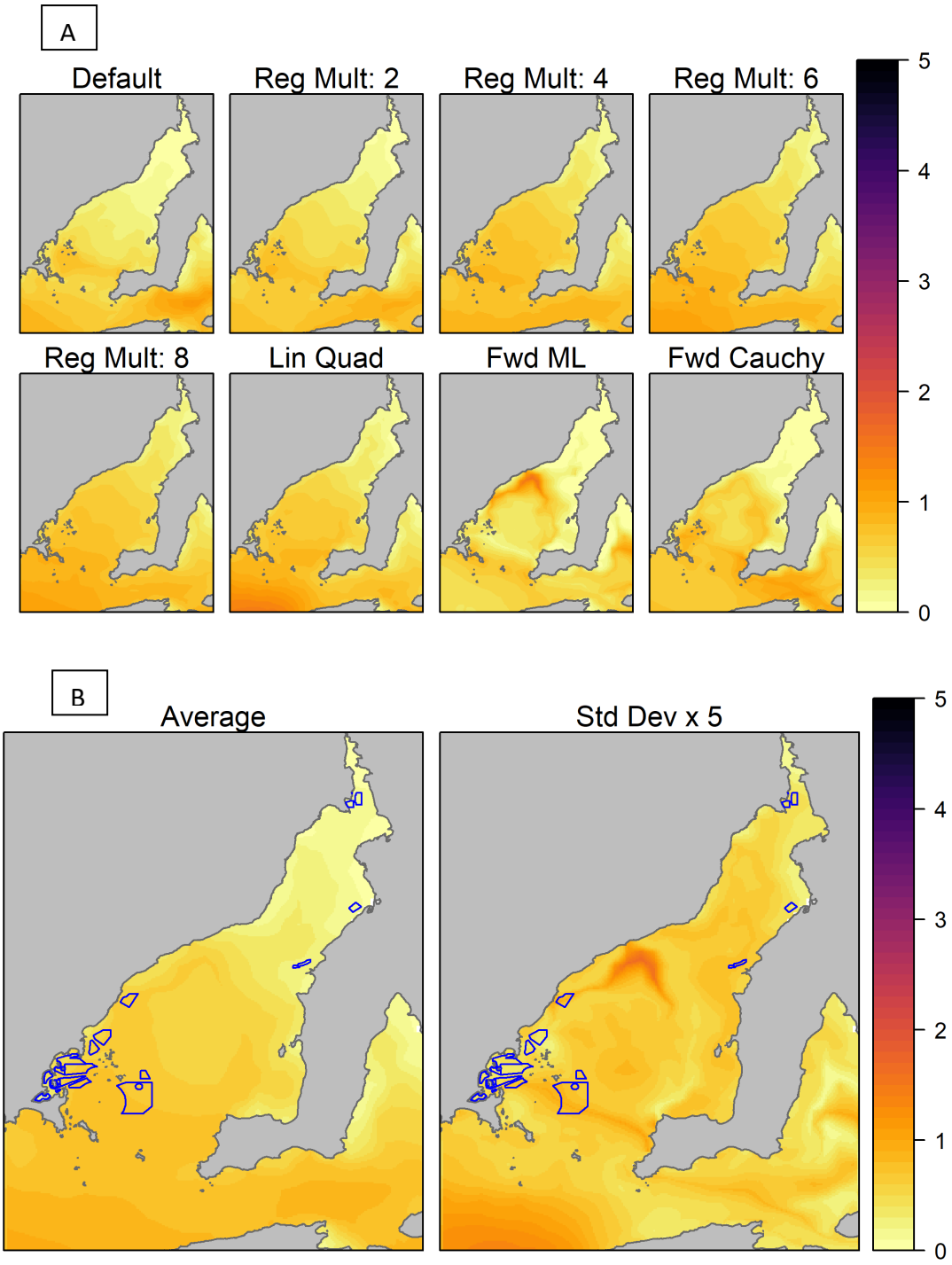


Figure S7. Maps of model predictions for the Spencer Gulf region for *Ecklonia radiata*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue.

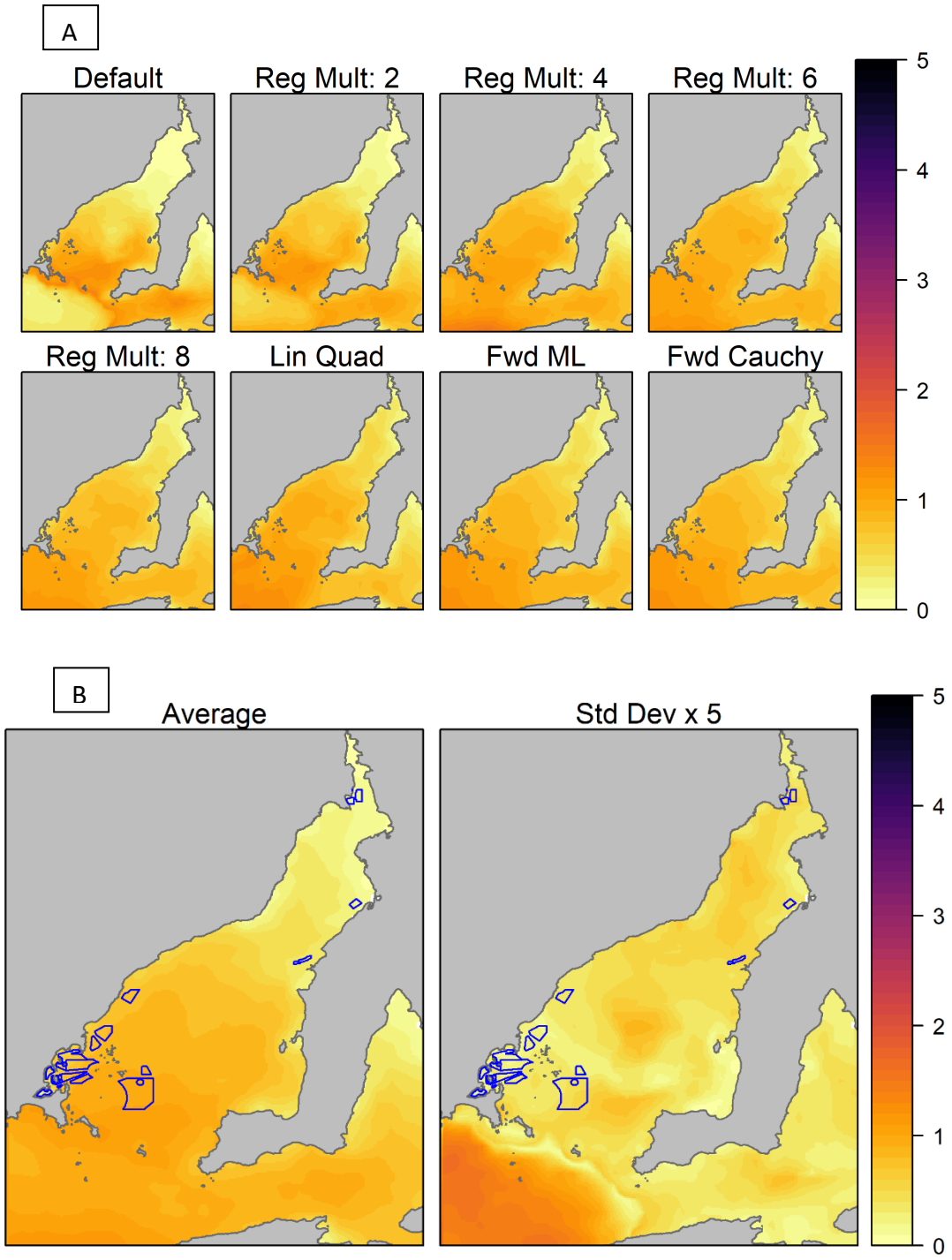


Figure S8. Maps of model predictions for the Spencer Gulf region for *Cystophora subfarcinata*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue.

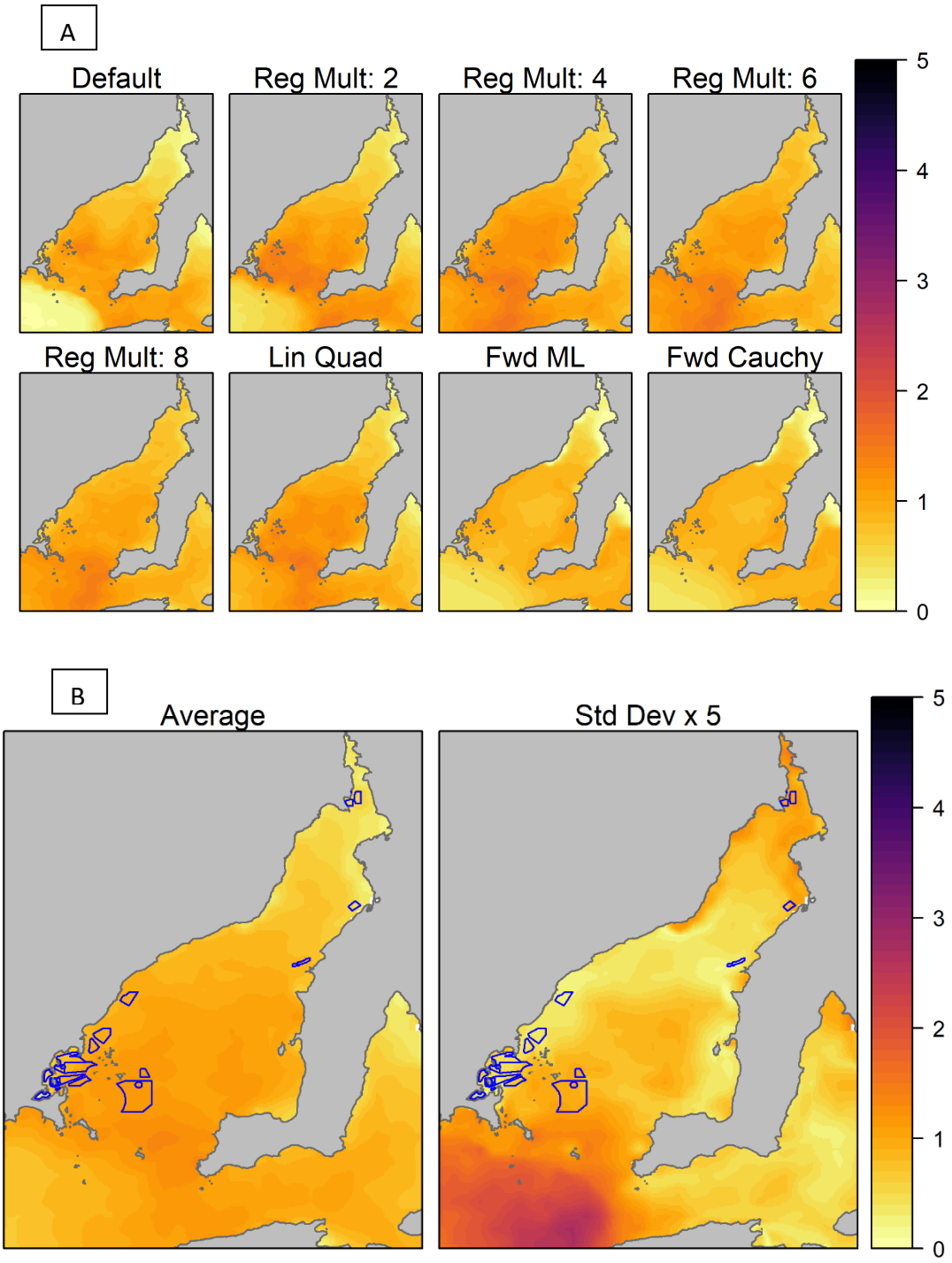


Figure S9. Maps of model predictions for the Spencer Gulf region for *Sargassum linearifolium*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue.

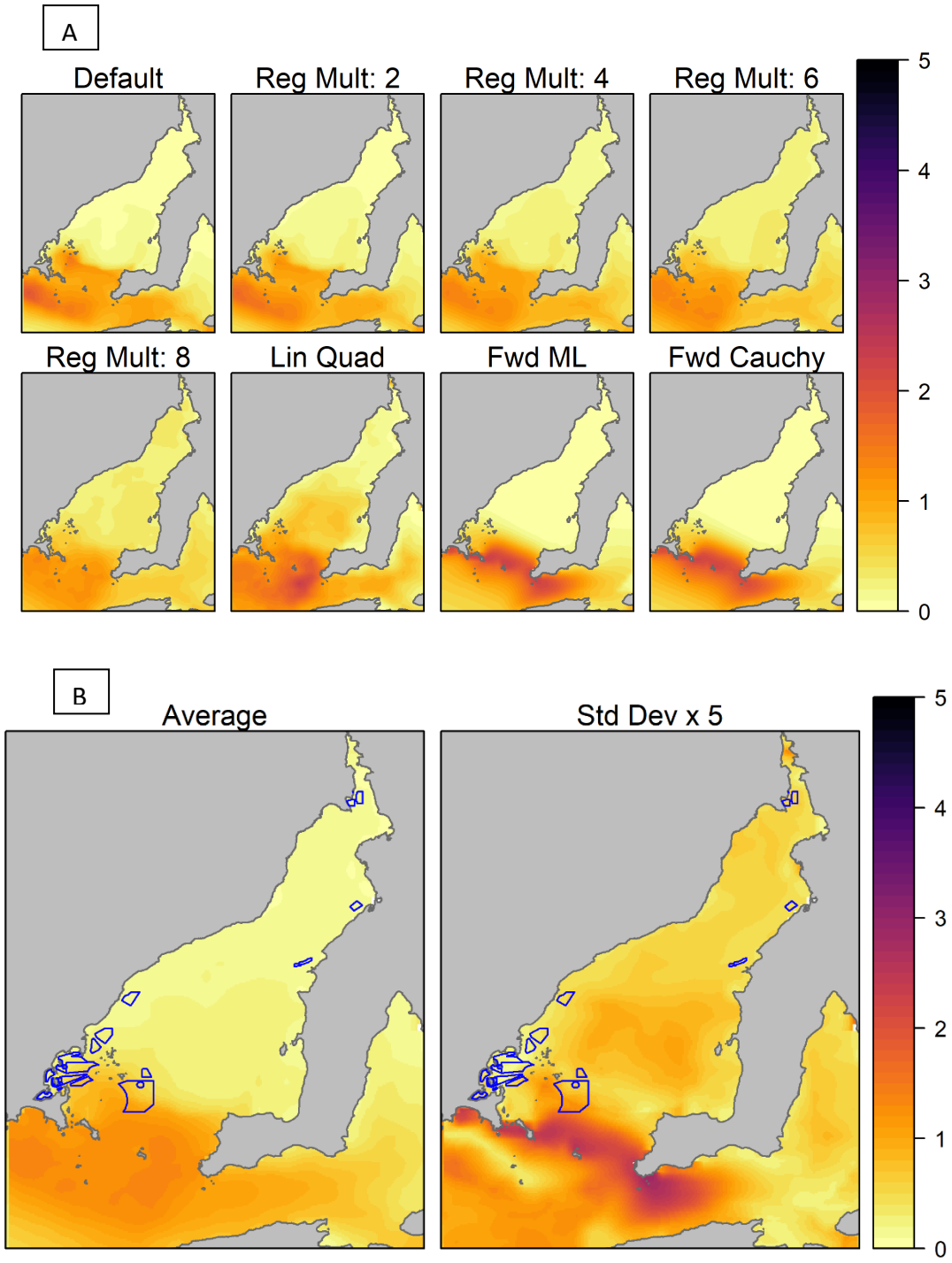
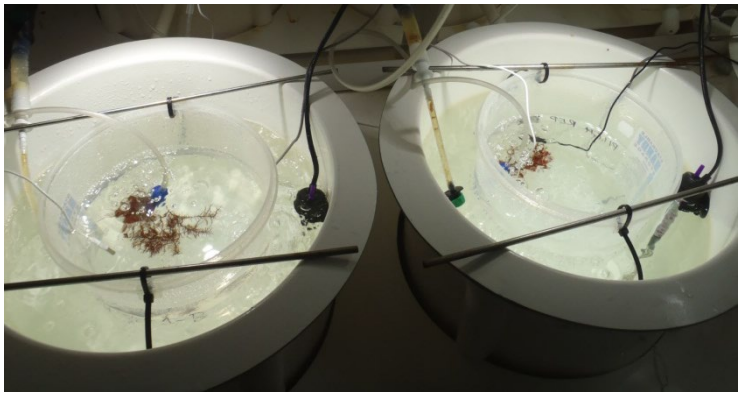


Figure S10. Maps of model predictions for the Spencer Gulf region for *Scytothalia dorycarpa*. (A) Results from modelling methods: default, regularisation multipliers (Reg Mult) 2,4,6,8, restricted feature types (Lin Quad = linear + quadratic only), Forward selection (Fwd ML = Maximum Likelihood, Cauchy = Cauchy prior). (B) Unweighted average of all model predictions and standard deviation (Std Dev) x 5 across all models, with aquaculture zones outlined in blue.

Chapter 4. Exploring novel Rhodophyta species for aquaculture and nutrient remediation



Top: red seaweed (Rhodophyta) specimens in tanks at the start of a laboratory experiment to assess temperature responses. Below: specimen of *Solieria robusta*. This species showed best potential for aquaculture of the Rhodophyta assessed.

Statement of Authorship

Title of Paper	Exploring novel Rhodophyta species for aquaculture and nutrient remediation
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	This is a co-authored manuscript written for submission to the journal *Aquaculture*.

Principal Author

Name of Principal Author (Candidate)	Kathryn. H. Wiltshire		
Contribution to the Paper	Conceived experiments, assisted with funding acquisition, designed and carried out experiments, analysed data and prepared the manuscript		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	17 Jun 2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Marty R. Deveney		
Contribution to the Paper	Provided advice on experimental design, and assisted with review and editing of the manuscript		
Signature		Date	17 June 2020

Name of Co-Author	C. Frederico D. Gurgel		
Contribution to the Paper	Provided advice on seaweed biology to inform experimental designs and assisted with review and editing of the manuscript		
Signature		Date	**17 June 2020**

Name of Co-Author	Jason E. Tanner		
Contribution to the Paper	Obtained funding for the project, provided advice on experimental design and statistical analysis, assisted with review and editing of the manuscript		
Signature		Date	17/6/20

Exploring novel Rhodophyta species for aquaculture and nutrient remediation

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Abstract

Seaweeds comprise almost half of global mariculture production, the majority farmed for human consumption, but demand is growing for a wide range of seaweed products. There is also increasing utilisation of seaweeds for nutrient remediation, including to improve environmental sustainability of fish aquaculture, while also producing a crop of value. Several fish species are farmed in Australia, but expansion of fish aquaculture is limited by the need to keep dissolved nutrients, particularly nitrogen, below levels that cause environmental impacts. Four Rhodophyta species (*Solieria robusta, Gelidium australe, Pterocladia lucida, Plocamium angustum*) that naturally occur in southern Australia but have not been cultivated were investigated to identify candidate species for aquaculture, with specific focus on application to nutrient removal in fish farming regions of South Australia (SA). Specific growth rates (SGR) and nitrogen (N) content of these seaweeds were compared in a 4-week laboratory experiment where nutrient was added to simulate conditions near SA fish farms. Data were used to determine N removal by each species over the experimental period. *Solieria robusta* was the fastest growing species in this initial experiment (average SGR: 5.3 % d^{-1}), while *Gelidium australe* removed the most N due to the combination of its growth rate (2.5 % d^{-1}) and N content (3.2 % DW at end of 4-week experiment). We carried out a second experiment to compare temperature responses of these two species, while *Pterocladia lucida* and *Plocamium angustum*, which both displayed SGR < 2 % d^{-1} in the initial experiment, were not considered further. *Solieria robusta* demonstrated a greater maximum growth rate and wider temperature tolerance than *Gelidium australe* in this second experiment and was selected for further study. We investigated growth responses of *Solieria robusta* to light and ammonium-N conditions relevant to SA fish farming regions; N uptake rates for both ammonium and nitrate; and propagation methods for seedstock

production. Our results help to inform cultivation methods and selection of suitable farming sites and seasons for the development of *Solieria robusta* aquaculture. Data to incorporate seaweed N removal into biogeochemical models is also provided.

1 Introduction

Seaweed aquaculture, carried out predominantly in Asia, comprises almost half of global aquaculture production by biomass (FAO, 2018). Growing demand for seaweed products and diminishing wild harvests have led to the expansion of seaweed cultivation in many other countries (Buchholz, et al., 2012; Buschmann, et al., 2017). Australia, however, has little wild harvest and no commercial seaweed cultivation (Roos, et al., 2018), and is a net importer of seaweed products (Lee, 2010). Establishment of seaweed culture is therefore of interest (Lee, 2010; Roos, et al., 2018). Farming native species is clearly desirable to ensure they are appropriate for the habitat and to avoid the risks involved with introduced species (Barrington, et al., 2009; Williams and Smith, 2007). Few species with established farming technologies are native to Australia, and it is likely that local seaweed species that have never been cultivated will need to be used. Australia, and in particular southern Australia, has a diverse native seaweed flora, with around 2000 species and a high degree of endemism (Phillips, 2001). The value of this unique seaweed diversity has been recognised, including the potential for native species to yield novel bioactive products, further supporting the development of a local seaweed industry using species that are not farmed elsewhere (Lee, 2010; Lorbeer, et al., 2013; Roos, et al., 2018; Winberg, et al., 2011).

Increasing global interest in seaweed cultivation has also been driven by the use of seaweeds for nutrient mitigation or bioremediation, for example, in integrated multi-trophic aquaculture (IMTA) systems. One specific IMTA application of seaweeds is to remove and

recycle dissolved nutrient waste from fish aquaculture into valuable biomass, providing economic as well as environmental benefits (Barrington, et al., 2009; Neori, et al., 2004; Troell, et al., 2003). Several fish species are farmed in Australia, and there is a strong emphasis on management of the aquaculture industry to ensure environmental sustainability (Rimmer and Ponia, 2007). In South Australia (SA), two marine fish: Southern Bluefin Tuna, *Thunnus maccoyii* (Castelnau, 1872), and Yellowtail Kingfish, *Seriola lalandi* Valenciennes, 1833, are farmed, primarily in southern Spencer Gulf. Dissolved nitrogen (N) is the nutrient limiting the environmental carrying capacity of both species (Collings, et al., 2007; Middleton, et al., 2013; Tanner, et al., 2007) and IMTA with seaweeds could be used to allow increased fish production while maintaining nutrients at environmentally sustainable levels (Neori, 2008).

We investigated four common Rhodophyta species that occur naturally in southern Spencer Gulf. These were chosen based on a literature review that identified seaweeds with desirable characteristics for aquaculture including suitable size and likely economic value (Wiltshire, et al., 2015; Chapter 1). Very little is known about the biology or chemical composition of any of these species, making it difficult to determine which are most suitable for aquaculture or nutrient remediation.

The species used were: *Pterocladia lucida* (R. Brown ex Turner) J. Agardh (Pterocladiaceae), *Gelidium australe* J. Agardh (Gelidiaceae); *Solieria robusta* (Greville) Kylin (Solieriaceae), and *Plocamium angustum* (J. Agardh) J.D. Hooker & Harvey (Plocamiaceae). These seaweeds are likely to be of commercial utility for a variety of reasons. *Pterocladia lucida* is an agarophyte wild-harvested in New Zealand (Brasch, et al., 1984), while the southern Australian endemic *Gelidium australe* is also an agar producer (Gordon-Mills, et al., 1990). The Solieriaceae contains many commercial carrageenophytes including the κ-carrageenan producers

Kappaphycus alvarezii (Doty) Doty and *K. striatum* (Doty), known in the industry as “cottoni”, and the ι-carrageenan producer *Eucheuma denticulum* (Burman) Collins et Harvey, known as “spinosum” (Ask and Azanza, 2002; McHugh, 2003). *Solieria robusta* produces yields of up to 40 % ι-carrageenan on a fresh weight basis, with a high pyruvate and sulphate content (Chiovitti, et al., 1999). The market for ι-carrageenan is less than that for κ-carrageenan (McHugh, 2003), but novel uses are being investigated, for example, the pyruvated ι-carrageenan from *S. chordalis* is an immunostimulant (Bondu, et al., 2010), and that of *S. filiformis* has antiprotozoal activity (Caamal-Fuentes, et al., 2017). Extracts from *S. robusta* also show hypolipidaemic (Ara, et al., 2002), anti-cancer (Yen, et al., 2014) and anti-fungal activity (Khanzada, et al., 2007). *Plocamium angustum* is a potential feed for farmed abalone (Kirkendale, et al., 2010), and produces bioactive compounds, including anti-bacterial and anti-fungal agents (Timmers, et al., 2012).

We compared growth rates and N storage of these four Rhodophyta in an initial laboratory experiment where nutrients were added to mimic conditions near fish farms. The two best performing species from the initial experiment were used in a second experiment, which compared their temperature responses over the temperature range experienced by Spencer Gulf aquaculture zones. Further investigation was then carried out on the species identified as the best overall candidate by this second experiment. Specifically, light and nutrient responses, N uptake rates, and seedstock production methods were investigated to elucidate potential for aquaculture and nutrient remediation, and inform suitable conditions for growth.

2 Materials and Methods

2.1 Seaweed material

Specimens of *G. australe, Pt. lucida* and *Pl. angustum* were collected at 3 – 8 m depth from Granite Island, SA (35° 33′ 59″ S, 138° 37′ 41″ E). *Solieria robusta* was collected at ~ 3 m depth from Outer Harbor (34° 48′ 14″ S, 138° 28′ 24″ E). Specimens were collected a maximum of 28 days prior to each experiment and were held in outdoor tanks at the South Australian Aquatic Science Centre, West Beach, Adelaide, SA until use. Tanks were continuously supplied with flow-through sand-filtered seawater sourced via a pipe from Gulf St Vincent, at ambient temperature and with no additional nutrient.

2.2 Initial species comparison experiment

A laboratory experiment was used to compare growth and N storage of the four candidate species under nutrient conditions expected around fish farms in southern Spencer Gulf. Seaweed specimens for this experiment were collected between 10 and 15 October 2012. Between 40 and 50 clean specimens of each species were selected from the stock tanks. An apical cutting 5 – 8 cm long was excised from each specimen with a sterile scalpel blade and cleaned with a soft toothbrush in filtered seawater to remove micro-epiphytes. Cuttings were randomly assigned to 20 x 18 L conical-bottomed aquaria (5 per species), such that each aquarium contained an initial biomass of ~ 7g, comprising cuttings from between six and ten different specimens.

Aquaria were supplied with flow-through filtered (10 µm) 17.5 °C natural seawater, sourced from Gulf St Vincent, at a rate of 8 L. h^{-1}, and with aeration. Lighting of 160 µE m^{-2} s^{-1} photosynthetically active radiation (PAR) at the water surface with 10 : 14 h light : dark regime

was supplied by cool-white LED lamps (Brightgreen D900) filtered through shade cloth with ~ 50 % transmission (Coolaroo medium green).

Nutrients were added from a stock solution of $(NH_4)_2SO_4$, KNO_3 and KH_2PO_4 via IV microburettes (B Braun Exadrop), with drip rates adjusted to provide aquaria with a continuous concentration (mean ± s.e. for n = 80 samples) of 3.3 ± 0.3 µM N as ammonium, 5.6 ± 0.1 µM N as oxidized N and 0.45 ± 0.03 µM P as phosphate, when mixed with incoming water. These nutrient concentrations were based on values predicted in the vicinity of SA fish farms by biogeochemical models (Tanner and Volkman, 2009), but total oxidized N concentrations were higher than target concentration of ~ 1.5 µM N due to background levels in the natural seawater.

Seaweed material was 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 acclimation, subsamples were taken from cuttings for initial N content measurement (see section 2.5), leaving between 4.5 g and 5 g fresh weight in each aquarium for the start of the experiment.

Fresh weights were measured at the start of the experiment and weekly thereafter for four weeks after gently patting specimens dry on paper towel to remove excess water. These weights were 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. Samples for final N content measurements were collected after recording fresh weights at the end of the experiment (week four).

Weekly SGR data were analysed using a linear mixed model with species as a fixed effect and aquarium as a random effect to account for the repeated weekly measures. To investigate difference between pre- and post- experiment N in addition to the N content of each species, N content data were analysed with a linear model including species and subsample (initial or final) plus the interaction as fixed factors and aquarium as a random effect because initial and final samples were taken from the same specimens. Analyses were conducted in a Bayesian framework following Zuur, et al. (2013), with details as described in section 2.6. The total N removed by each species over the four-week experimental period was calculated using modelled SGR and N content of each species, with average water content of each species used to convert N content from % DW to % FW.

2.3 Temperature response experiment

A second experiment was carried out to compare temperature responses of the two best performing species from the initial species comparison experiment: *S. robusta* and *G. australe.* Seaweeds for use in this second experiment were collected between 1 and 4 October 2013.

This experiment used the same experimental system and lighting regime described in section 2.2, but with seaweed specimens housed in 3 L plastic tubs suspended in each aquarium and filled with artificial seawater (Dupla Marin) at a salinity of 36; aeration was supplied continuously. Artificial seawater was used to permit greater control of nutrient concentrations than was achieved with natural seawater in the initial species comparison experiment. Salinity in the tubs was checked daily using a conductivity-salinity meter (TPS 90-C) and distilled water added as necessary to compensate for evaporation. Media were exchanged twice-weekly and nutrient added from a stock solution of $(NH_4)_2SO_4$, KNO_3 and

KH_2PO_4 to provide 5.7 µM N as ammonium, 1.4 µM N as oxidized N and 0.25 µM P as phosphate, based on expected nutrient concentration near farms in the tuna farming zone off Port Lincoln, SA during the ranching period (Tanner and Volkman, 2009).

Cuttings were taken from a total of 40 specimens of each species and were prepared as per section 2.2. with average fresh weight 3.5 g, comprising cuttings from 3 – 5 specimens, of *S. robusta* and *G. australe* added to 10 aquaria each. To permit curve-fitting, the experiment was designed to treat temperature as a continuous covariate rather than a factor, and temperatures were not replicated. The 10 aquaria for each species 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 (Petrusevics 1993). Temperature in each aquarium was controlled by supply of chilled (12 °C) seawater and by submersible aquarium heaters (AquaOne 100 W) attached to digital thermostats (BY-LOX 15 A). A digital submersible thermometer (OneTemp CHY805) was used to check the temperature of each aquarium daily. The average temperature recorded in each aquarium over the course of the experiment was used in curve fitting. Total fresh weight of the cuttings in each aquarium was recorded at the start and end of the experiment, with SGR calculated as per section 2.2.

For both species, responses to temperature were non-linear, therefore generalised additive modelling (GAM) was used to assess temperature responses, with temperature fitted as a smooth effect. The effect of species and temperature were tested by comparing a model of the overall temperature response for both species with one that also included a term for the species difference using Akaike's Information Criterion (AIC) (Arnold, 2010; Burnham, et al., 2011), and examining the approximate significance of smooth terms within the selected model (Wood, 2017). To avoid overfitting, the number of knots used (4) was chosen to be less

than half the number of data points. Analysis was performed using the *mgcv* package (Wood, 2017) in *R* (R Core Team, 2019). Predicted temperature optima and maximum SGR for each species were determined from fitted curves.

2.4 Further investigation of *S. robusta*

Additional investigation was carried out for *S. robusta*, which was selected as the best performing species from the first two experiments (see Results). These additional investigations comprised: an experiment to assess light and ammonium responses; determination of ammonium and nitrate uptake rates; and investigation of explant production methods.

2.4.1 Light and ammonium responses

Light and ammonium-N responses of *S. robusta* were investigated using the experimental system described in section 2.3, but with specimens, from collections made on 16 June 2015, housed in 250 ml conical flasks suspended in tubs in the aquaria. The surrounding water was maintained at 18 °C and flasks were aerated to provide water circulation and gas exchange. Each aquarium contained three flasks, comprising one of each of three nutrient treatments: nil, low or high added ammonium. Low-nutrient artificial seawater (Sigma s9883) at a salinity of 36 was used in all flasks and was replaced three times weekly. No nutrient was added to the nil ammonium treatment, while low and high ammonium treatments received tri-weekly doses, at the same time as seawater was replaced, of 1.87 µM and 28 µM ammonium-N respectively from a stock solution of $(NH_4)_2SO_4$ and KH_2PO_4. These doses provide weekly ammonium fluxes equivalent to those expected around SA fish farms at 2010-11 stocking levels and with stocking at maximum environmental carrying capacity as modelled by Middleton, et al. (2013). Phosphate was added in a 10:1 molar ratio to N to avoid P limitation,

and modified Provasoli Enrichment solution (Berges, et al., 2001), made without N or P, was added to all treatments to supply micronutrients and vitamins.

A 10 : 14 h light : dark regime was supplied and combinations of shade cloth (Coolaroo medium green) and curtain material were used over aquaria, giving PAR treatments of (mean ± SE for n = 5 tanks each): 52 ± 3, 136 ± 5, 261 ± 7 and 365 ± 13 µE m^{-2} s^{-1}. PAR treatments were randomly assigned to five aquaria each.

Solieria robusta specimens used in this experiment were grown for 2 weeks in tubs of artificial seawater under the same temperature conditions used for the experiment, with PAR of 160 µE m^{-2} s^{-1} and with no nutrient addition. At the start of the experiment excised fronds of ~ 0.5 g fresh weight from the acclimated material were added to each flask. Cuttings were taken from a single specimen per flask, with different specimens used in each flask. Fresh weights were recorded at the start of the experiment and then weekly for three weeks, with SGR calculated as per section 2.2.

Ammonium removal efficiency was calculated following Kang, et al. (2013) from water samples taken from each flask two days after nutrient addition in the first week of the experiment and three days after nutrient addition in the third week. Ammonium in these water samples was quantified as described in section 2.5.

After three weeks cultivation, effective quantum yield of PSII photochemistry (Genty, et al., 1989) was calculated for each specimen based on fluorescence values taken three hours after the start of the lighting period 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, as a proxy for pigment content, and then frozen for N content analysis. Colour analysis was used instead of pigment quantification because there was insufficient biomass available to analyse both tissue N and pigment content of the specimens. Red, green and blue values were extracted from images using FIJI/ImageJ (Schindelin, et al., 2012) after correcting white balance with the *chart white balance* plug-in, and converted to CIE Lab values using the *colorspace* package (Ihaka, et al., 2015) for *R*.

SGR data were analysed using a linear mixed model with PAR treatment and ammonium addition level as fixed factors, and tank as a random effect. N content of some specimens was below detection limits, therefore, censored regression was used to analyse N content data, with PAR treatment and ammonium addition level as fixed factors, and tank as a random effect as per the SGR analysis. Censored regression was implemented in JAGS following Kruschke (2014). For both analyses, priors were defined, MCMC simulations performed and parameter effects assessed as described in section 2.6.

Multivariate analysis of the effect of light and nutrient on CIE Lab values from colour analysis was undertaken using permutational multivariate ANOVA (with the PERMANOVA routine) in PRIMER v 6.1.15 (Plymouth Routines in Multivariate Ecological Research) with PERMANOVA+ add-on v1.0.5 (Anderson, et al., 2008). Where significant differences were found, based on α of 0.05, pair-wise tests were performed, and PERMDISP was used to assess if multivariate dispersion differed between treatments. Euclidean distances were used in all analyses, with 9999 permutations; Monte-Carlo p-values were used if less than 1000 unique permutations occurred.

2.4.2 Nitrogen uptake rates

Solieria robusta collected on 23 October 2018 was acclimated prior to use in uptake rate investigations and the explant production experiment (section 2.4.3). Excised fronds, comprising a single cutting from each of 96 specimens, were prepared as per section 2.2 and transferred to 2 L flasks containing filtered natural seawater. Flasks were acclimated at 20 °C in a culture cabinet (Climatron 520-DL) under lighting of 100 µE $m^{-2}s^{-1}$ PAR with a 12 : 12 light : dark cycle and gentle aeration for two weeks, during which time seawater was replaced twice weekly with no nutrient addition.

Uptake rates of ammonium and nitrate were determined using the multiple flask method (Harrison, et al., 1989) with a total of 36 x 200 mL flasks. Flasks contained 150 mL low-nutrient artificial seawater (Sigma s9883) with salinity of 36 and N of 10, 25, 50, 100, 200 or 300 µM as either ammonium (from $(NH_4)_2SO_4$) or nitrate (from $NaNO_3$), with three replicate flasks per treatment. Phosphate (P, as KH_2PO_4) was added in a 10:1 N:P ratio to avoid P limitation.

Water samples of 50 mL for N analysis (described in section 2.5) were taken from each flask after addition of nutrient and mixing, immediately prior to specimen addition, and then after one hour. During the hour uptake period, flasks were maintained under illumination in the same culture cabinet with the conditions used for acclimation.

An average of 0.65 g fresh weight, comprising 1-2 cuttings, of *S. robusta* was added to each flask from the acclimated material. The fresh weight of the cuttings in each flask was determined after gently patting dry on paper towel, and converted to dry weight for uptake rate calculation using the average water content determined from the tissue N samples (see section 2.5).

Uptake rates (V) were then determined as: $V = (M_0 - M_t) / (t \times DW)$, where M_0 and M_t are the moles of N at time 0 and t, calculated from concentration x volume at each time, t is the time interval and DW the seaweed dry weight. The tissue N status of the seaweed used in the uptake experiment was determined from subsamples taken from excised fronds immediately prior to their use. Frond subsamples were pooled into two samples for analysis due to the small size of fronds in comparison to the quantity required to estimate tissue N (~ 2 g fresh weight).

Uptake rates were fitted to the Michaelis-Menten equation for each N source individually, and, for comparison, to the overall response, using non-linear curve fitting in JAGS, following Bolker, et al. (2013). The Michaelis-Menten model is given by $V = V_{max} \times S/(K_s + S)$, where S is the substrate concentration, K_s is the half-saturation constant and V_{max} is the maximum uptake rate. Michaelis-Menten models were compared to linear models, which were also fit in JAGS, using the deviance information criterion (DIC) to determine whether the linear or non-linear fit was more parsimonious, i.e. whether responses showed evidence of saturation. Where the responses did not show evidence of saturation kinetics, linear models with and without an interaction term between initial N concentration and N source were compared using DIC. The slope of the response from the linear model equals affinity for the substrate, with the interaction term being used to assess difference in affinity between N sources. Affinity is given by V_{max}/K_s when a Michaelis-Menten curve is fitted, but the individual saturation kinetics parameters cannot be estimated from the linear model (Harrison and Hurd, 2001; Smit, 2002). Priors were defined, MCMC simulations performed and parameter effects assessed as described in section 2.6.

2.4.3 Explant production

Explant production methods for *S. robusta* were explored based on commercial cultivation methods of *Kappaphycus* and *Eucheuma* spp., which are also Solieriaceae (Yong, et al., 2011; 2014). Clean fronds of *S. robusta* were selected from acclimated material (see section 2.4.2), excised with a sterile scalpel blade and cleaned with a soft toothbrush in filtered seawater to remove micro-epiphytes. To compare performance of explant types, explants of ~ 20 mm length were taken from frond tips and stems. Tip explants included branch tips, which in *S. robusta*, contain apical cells (Womersley, 1994), while stem explants were taken at a minimum distance of 50 mm from tips. Segments containing apical cells are expected to perform better than non-apical segments (Yong, et al., 2014), but this is not always the case (Del Carmen Hernández-González, et al., 2010). Each flask contained one tip and one stem explant from the same specimen, with different specimens used as source material for each flask, and specimens randomly assigned to treatment flasks.

Flasks contained 200 mL filtered natural seawater plus enrichment solution. The enrichment solutions used were modified Provasoli Enrichment solution (PES) (Berges, et al., 2001) and Von Stosch medium (VSM) (Harrison and Berges, 2005), at full, half and quarter strength each, with four replicate flasks used per treatment. These are common enrichment solutions used for the propagation of red seaweeds (Yong, et al., 2014).

Flasks were maintained at 20 °C in the same culture cabinet as for acclimation under lighting of 100 µE $m^{-2}s^{-1}$ PAR with a 12 : 12 light : dark cycle and gentle aeration. Seawater and enrichment media in each flask were replaced three times weekly over a four-week experimental period. The N source in both enrichment solutions is nitrate. Samples for

nitrate-N determination were taken from samples of freshly mixed seawater and enrichment solution of each treatment and stored frozen until analysis.

Explant growth (SGR) was determined form fresh weights recorded at the start and end of the experiment, calculated as per section 2.2. At the end of the experiment, explants were scored for the presence or absence of epiphytic growth.

Some explants did not survive the four-week experiment and were not included in the final analyses. For surviving explants, SGR was analysed using a linear mixed model with explant type, enrichment solution type and strength, expressed as equivalent N concentration, as fixed effects, and flask as a random effect, following the method described in section 2.6. Presence of epiphytes was analysed using a logistic mixed model with the same parameters as for the SGR analysis. Some treatment combinations did not include any specimens with epiphytes, resulting in the issue of separation in the data; hence parameters could not be estimated using diffuse normal priors (Gelman, et al., 2008; Ghosh, et al., 2018). We therefore used weakly informative scaled t-priors to restrict parameter estimates to finite, plausible values following Gelman, et al. (2008), with seven degrees of freedom as recommended by Ghosh, et al. (2018). Priors for variance components were defined and MCMC sampling conducted as per section 2.6.

2.5 Chemical analyses

Samples for seaweed tissue N content were frozen, freeze-dried overnight and then ground to a fine powder using a Fritsch stainless steel ball mill. A 100 mg aliquot was analysed on a LECO Truspec CNS Elemental Analyser (LECO, St Joseph, MI, USA). To enable N as a proportion of fresh weight to be determined, water contents were calculated from the difference in

weight between fresh and freeze-dried samples. Water nutrient samples were kept frozen until analysis on a Thermo Scientific™ Aquakem™ for ammonium levels above 3 µM and nitrate levels above 15 µM, with lower level samples analysed by flow injection analysis (FIA) on a Lachat QuickChem 8000 Automated Ion analyser. Ammonium (NH_3 + NH_4^+) was determined using the indophenol blue method (Lachat, 2003b). Nitrate was determined using the sulphanilamide method using hydrazine reduction for the Aquakem™ or a cadmium reduction column for FIA (Lachat, 2003a).

2.6 Bayesian methods

Bayesian analyses using JAGS v 4.3.0 (Plummer, 2017) were run with the *R* (R Core Team, 2019) package *R2jags* (Su and Yajima, 2015). For each analysis, diffuse normal priors (mean zero, precision 0.0001) were used for coefficients of fixed effects and uniform (0,100) priors for variance components. Three chains were used, each comprising a total of 10 000 MCMC iterations, thinned at a rate of 10, following 10 000 iterations for burn-in. Convergence was assessed using the Gelman-Rubin statistic and confirmed by examination of diagnostic plots generated by the *mcmcplots* package (McKay Curtis, 2015).

Hypothesis testing followed Kruschke (2014). Factor levels were considered different where 95 % highest density intervals (HDIs) of the difference between their posterior parameter estimates did not contain zero, and continuous predictors were considered important where their 95 % HDI did not contain zero. HDIs were calculated using the *HDinterval* package (Meredith and Kruschke, 2018). The importance of the interaction terms in models with more than one covariate was assessed by comparing the deviance information criterion (DIC) between nested models.

3 Results

3.1 Initial species comparison results

Solieria robusta was the fastest growing of the four species investigated (Figure 1, Table 1) with SGR (posterior mean with 95 % highest density interval (HDI) in brackets) of 5.2 (4.4 – 6.1) % d^{-1}, followed by *G. australe* with SGR of 2.4 (1.6 – 3.3) % d^{-1}, although 95 % HDIs of differences between estimates for this and both slower growing species included zero (Table 1). SGRs for the remaining species were: *Pt. lucida* 1.9 (1 – 2.7) % d^{-1} and *Pl. angustum* 1.3 (0.4 – 21) % d^{-1}.

Table 9. Mean differences between parameter estimates for species: GA = *Gelidium australe,* PA = *Plocamium angustum,* PL = *Pterocladia lucida*, SR = *Solieria robusta* from Bayesian models of SGR, length and weight. Estimates are mean difference between coefficients and 95 % HDIs of differences. * Different from zero based on 95 % HDIs.

Difference between species:	Coefficient for SGR
PA – PL	−0.58 (−1.79 – 0.64)
PA – SR	−3.96 (−5.11 – −2.71)*
PA – GA	−1.14 (−2.31 – 0.06)
PL – GA	−0.56 (−1.83 – 0.61)
PL – SR	−3.39 (−4.64 – −2.19)*
SR – GA	2.83 (1.66 – 4.06)*

All species increased their N content over 4 weeks (Figure 1), with final N being similar for *Pl. angustum* (mean: 3.4, 95 % HDI: 3.2 – 3.6 % DW), *Pt. lucida* (mean: 3.2, 95 % HDI: 3.0 – 3.3% DW) and *G. australe* (mean: 3.2, 95 % HDI: 3.1 – 3.4 % DW), but lower for *S. robusta* (mean: 2.1, 95 % HDI: 2.0 – 2.3 % DW). Initial N contents were highest for *Pl. angustum* and *Pt. lucida* (mean: 2.7, 95 % HDI: 2.5 – 2.9 % DW for both), followed by *G. australe* (mean: 2.3, 95 % HDI: 2.2 – 2.5 % DW) and *S. robusta* (mean: 1.5, 95 % HDII: 1.4 – 1.7 % DW). Differences in N content between pre- and post- nutrient addition samples, and between species, were

confirmed by 95 % HDIs of differences between coefficients (supplementary material). The model without a species interaction term was most parsimonious based on DIC (ΔDIC -7.2), indicating that the increase in N content was similar for each species, as also demonstrated by the posterior means of initial and final N contents from the model including the interaction term. Examination of HDIs of parameter estimates showed, however, that the increase in tissue N for *Pt. lucida* was less than that for *G. australe*, with the other species showing intermediate increases (supplementary material). Calculation of N removal based on modelled growth and tissue N showed that *G. australe* removed the most N (mmol N g^{-1} FW) over the 28 day experiment (mean 0.83, 95 % HDI 0.53 – 1.18), although 95 % HDIs overlapped, with N removal of other species being: *Pt. lucida* mean 0.59 (95 % HDI 0.33 – 0.92), *S. robusta* mean 0.54 (95 % HDI 0.39 – 0.71), and *Pl. angustum* mean 0.37 (95 % HDI 0.16 – 0.56).

3.2 Temperature responses of *S. robusta* and *G. australe*

Solieria robusta and *G. australe* were selected for use in the temperature response experiment based on their performance in the initial four species comparison (section 3.1). Growth rates (SGR) for both of these species were significantly affected by temperature (approximate significance of smooth temperature term $p<0.001$), and the temperature response was significantly different between the species (approximate significance of species difference $p < 0.001$). AIC also supported the difference in species responses, with AIC of the model including the species difference term being 24 compared to 60 without this term, and 66 for the null (intercept only) model. *Gelidium australe* grew faster than *S. robusta* 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 several became bleached and brittle

after ~2 weeks. By the fourth week of the experiment, the majority of specimens held at > 21 °C appeared to have died and were starting to disintegrate. The growth of *S. robusta* increased with temperature from 12 to 16 °C, and was similar between 16 and 22 °C, declining slightly at temperatures > 22 °C, although specimens still appeared healthy. The selected GAM (Figure 2) predicted a maximum SGR of 4.1% d^{-1} at 20.1 °C for *S. robusta*, and of 2.8% d^{-1} at 17.9 °C for *G. australe.*

3.3 Further investigations of *Solieria robusta*

Solieria robusta was selected for further investigation based on its temperature performance, specifically, faster growth over temperatures relevant to Spencer Gulf aquaculture zones, and greater tolerance of high temperatures than *G. australe.*

3.3.1 Light and ammonium responses

Solieria robusta grew faster in the high ammonium treatment than with low or nil added ammonium (Table 2, Figure 3); within each nutrient treatment, there was little effect of PAR although with a trend to higher growth at 136 µE m^{-2} s^{-1} than other PAR levels, especially in the high ammonium treatment (Figure 3). DIC, however, selected the model without the ammonium x PAR interaction term (ΔDIC −7.5), indicating that effects of added ammonium were similar at each PAR level. SGR of the specimens grown with nil or low ammonium addition was minimal (< 1 % d^{-1} in each case); highest SGR was achieved by specimens grown under 136 µE m^{-2} s^{-1} with high ammonium level (posterior mean: 1.9, 95 % HDI: 1.5 – 2.4 % d^{-1} from the DIC-selected model), although 95 % HDIs of the differences between PAR levels contained zero (Table 2). Effective quantum yield of PSII (Φ_{PSII}) was also higher in the high ammonium treatment than the nil or low treatments (Table 2, Figure 3), with no interaction between PAR and ammonium level as assessed by DIC (ΔDIC −14.4). Specimens grown under

PAR of 136 µE m^{-2} s^{-1} had higher Φ_{PSII} than those grown at the highest PAR level (365 µE m^{-2} s^{-1}) (Table 2).

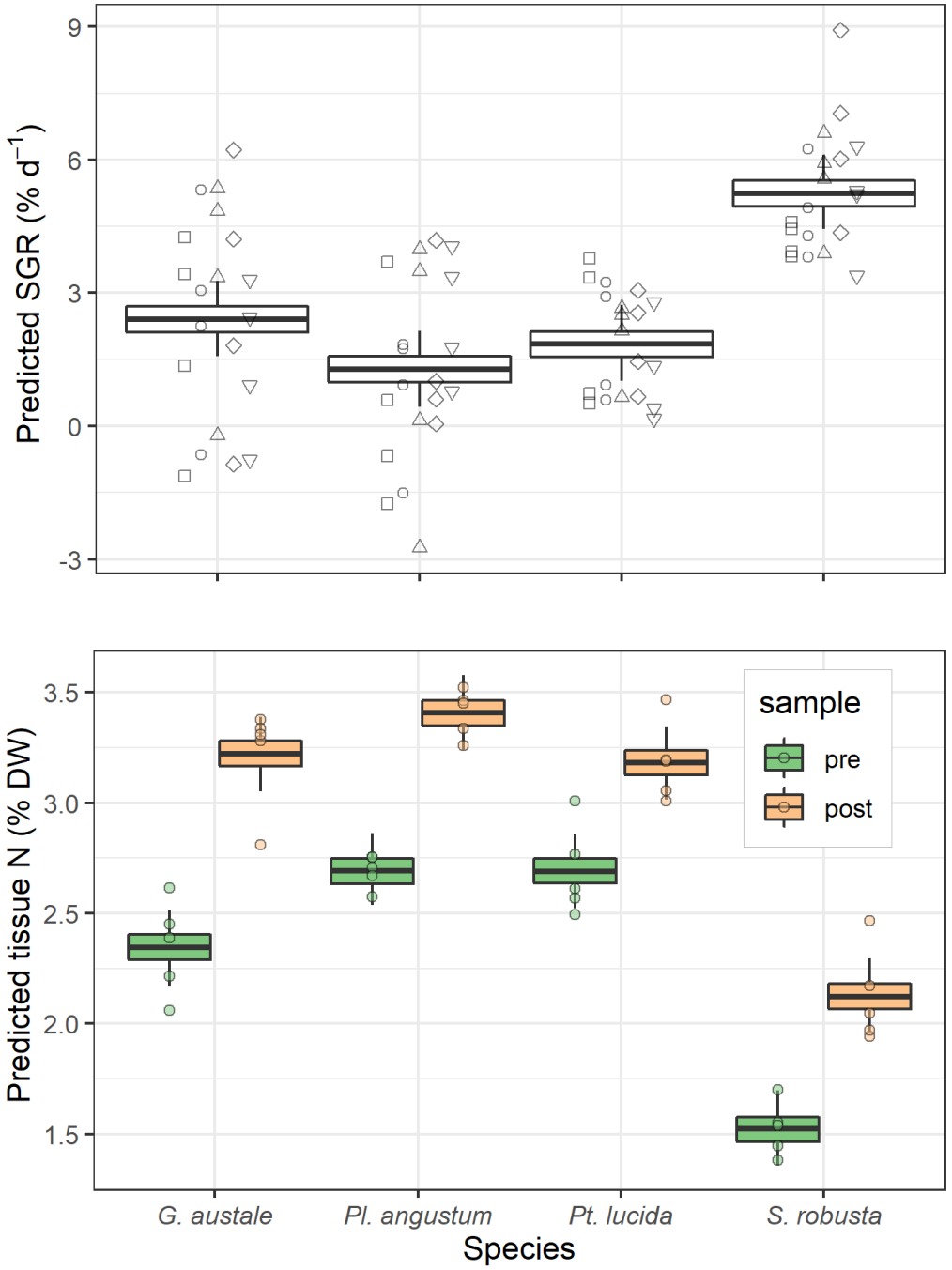

Figure 6. Top: SGR (% d^{-1}) for each species, and Bottom: Tissue N (% DW) by species and sample (pre- or post- experiment). Boxes show mean and interquartile range of posterior predictions, with whiskers showing 95 % HDIs of the posterior predictions. Points show experimental data from 5 replicate tanks per species. For SGR data, the four weekly SGR measurements per replicate tank are shown, with a different symbol used per replicate within each species.

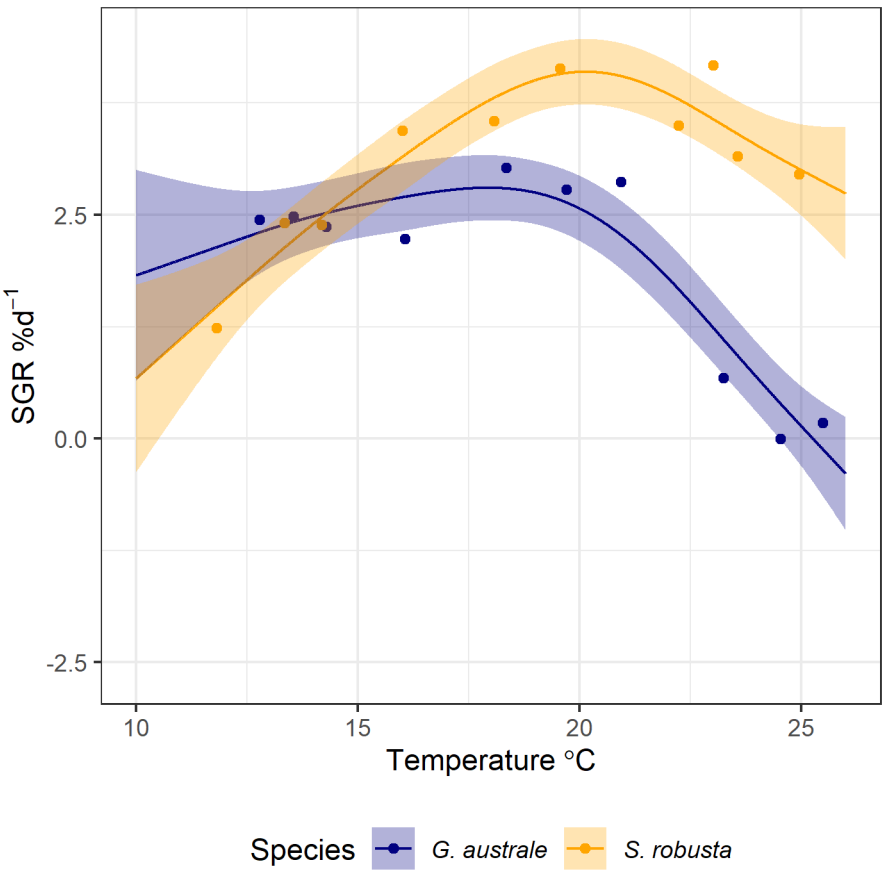

Figure 7. SGR (% d^{-1}) of *S. robusta* and *G. australe* with temperature. Line shows fitted GAM model with shading indicating 95 % confidence interval of the fit. Points show SGR for each tank (10 per species).

N content was also greater in the high ammonium treatment than the nil or low treatments (Table 2, Figure 3). The model with an interaction term could not be estimated for N content because there were no specimens in the nil ammonium-261 PAR treatment combination with N content above the detection limit. Within each ammonium treatment, N content was greater for specimens grown at the lowest PAR (52 µE m^{-2} s^{-1}) than at other PAR levels (Table 2), with the greatest N content overall being 1.84 (95 % HDI: 1.70 – 2.00) % DW. PERMANOVA showed significant effects of ammonium (Pseudo-$F_{2,59}$ = 267 p_{perm} < 0.001) and PAR (Pseudo-$F_{3,59}$ = 12.5, p_{perm} < 0.001) on CIE Lab colour, with no interaction (Pseudo-$F_{6,59}$ = 0.916,

p_{perm} = 0.537). Post hoc tests showed that the high ammonium treatment was different to the low and nil nutrient treatments (p_{perm} < 0.001 in each case), while the two lowest PAR levels were significantly different to the two highest PAR levels (p_{perm} < 0.005 in each case). The two low PAR treatments were similar to each other (p_{perm} = 0.414), as were the two high PAR treatments (p_{perm} = 0.170). PERMDISP showed that there was no difference in multivariate dispersion between treatments (p_{perm} = 0.992 for ammonium, p_{perm} = 0.956 for PAR). Specimens grown under high ammonium or lower PAR (within each ammonium treatment) 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 (Figure 4).

Table 10. Mean differences between parameter estimates for nutrient treatments (level of ammonium addition) and PAR from Bayesian models of SGR, effective quantum yield of PSII (ΦPSII) and tissue N. Estimates are mean difference between coefficients and 95 % HDIs of differences. *Different from zero based on 95 % HDIs

Difference between treatments:	**Regression coefficient for term:**		
	SGR	**Φ_{PSII}**	**Tissue N**
Ammonium:			
high − nil	1.50 (1.04 – 1.91)*	0.14 (0.11 – 0.17)*	0.98 (0.80 – 1.13)*
low − nil	−0.03 (−0.47 – 0.40)	0.03 (0.00 – 0.06)	0.17 (−0.04 – 0.36)
low − high	−1.53 (−1.94 – −1.11)*	−0.11 (−0.14 – −0.08)*	−0.81 (−0.99 – −0.64)*
PAR:			
136 − 52	0.56 (−0.04 – 1.09)	0.03 (−0.02 – 0.07)	−0.21 (−0.44 – −0.02)*
136 − 261	0.44 (−0.15 – 0.99)	0.07 (0.03 – 0.12)	0.12 (−0.12 – 0.34)
136 − 365	0.20 (−0.33 – 0.81)	0.05 (0.01 – 0.10)*	0.08 (−0.13 – 0.30)
261 − 52	0.12 (−0.50 – 0.65)	−0.05 (−0.10 – 0.00)	−0.33 (−0.55 – −0.11)*
261 − 365	−0.24 (−0.80 – 0.36)	−0.02 (−0.07 – 0.02)	−0.04 (−0.25 – 0.19)
365 − 52	0.36 (−0.23 – 0.94)	−0.03 (−0.08 – 0.01)	−0.28 (−0.48 – −0.09)*

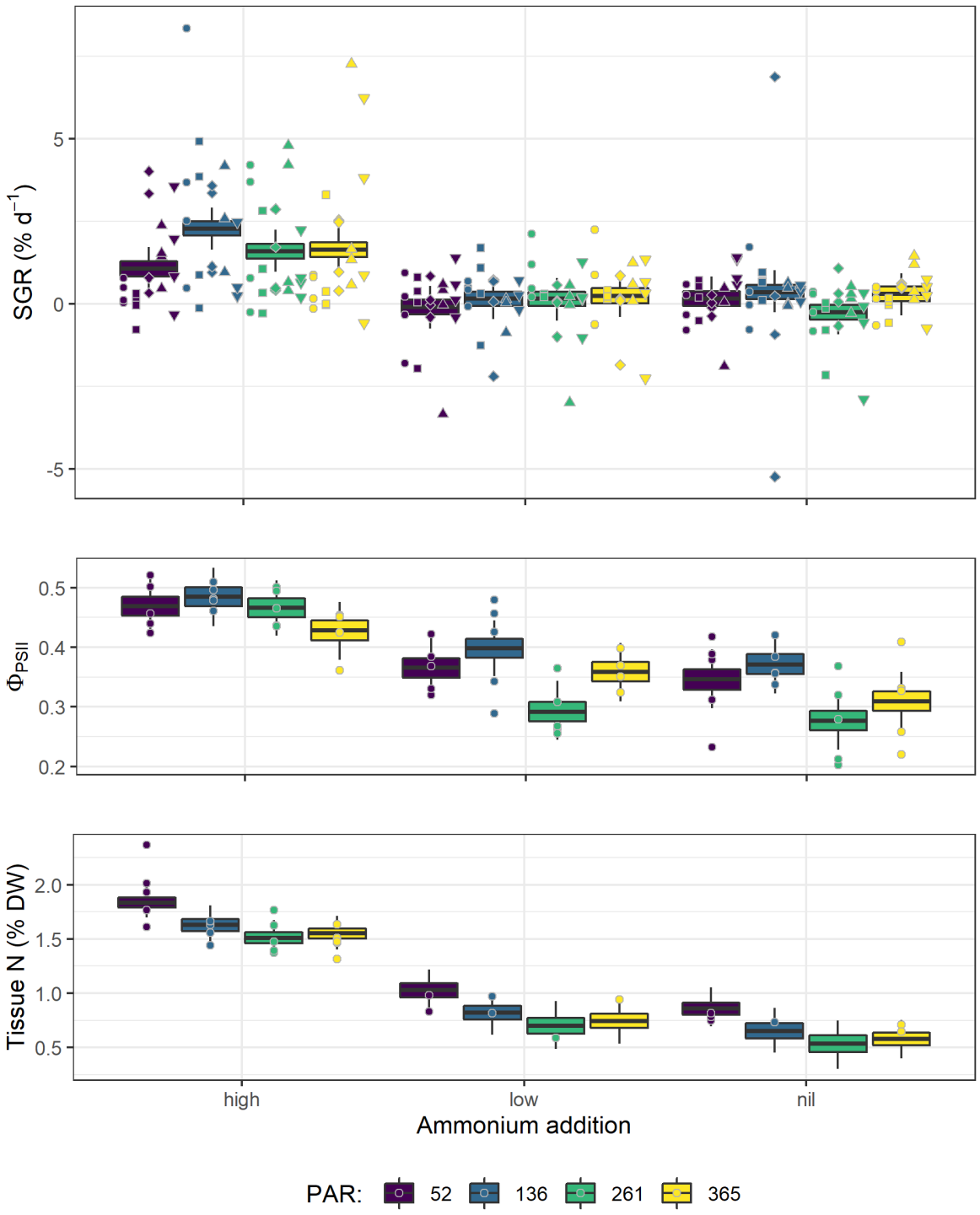

Figure 8. Top: SGR (% d^{-1}), middle: Φ_{PSII}, and bottom: tissue N (% DW) by nutrient treatment (level of ammonium addition) and PAR (µE m^{-2} s^{-1}). Boxes show mean and interquartile range of posterior predictions, with whiskers showing 95 % HDIs. Points show data (n = 5 replicates per treatment). Note that Tissue N content is not shown for specimens having N content below the analysis detection limit. For SGR data, the four weekly SGR measurements per replicate are shown, with a different symbol used per replicate within each treatment combination.

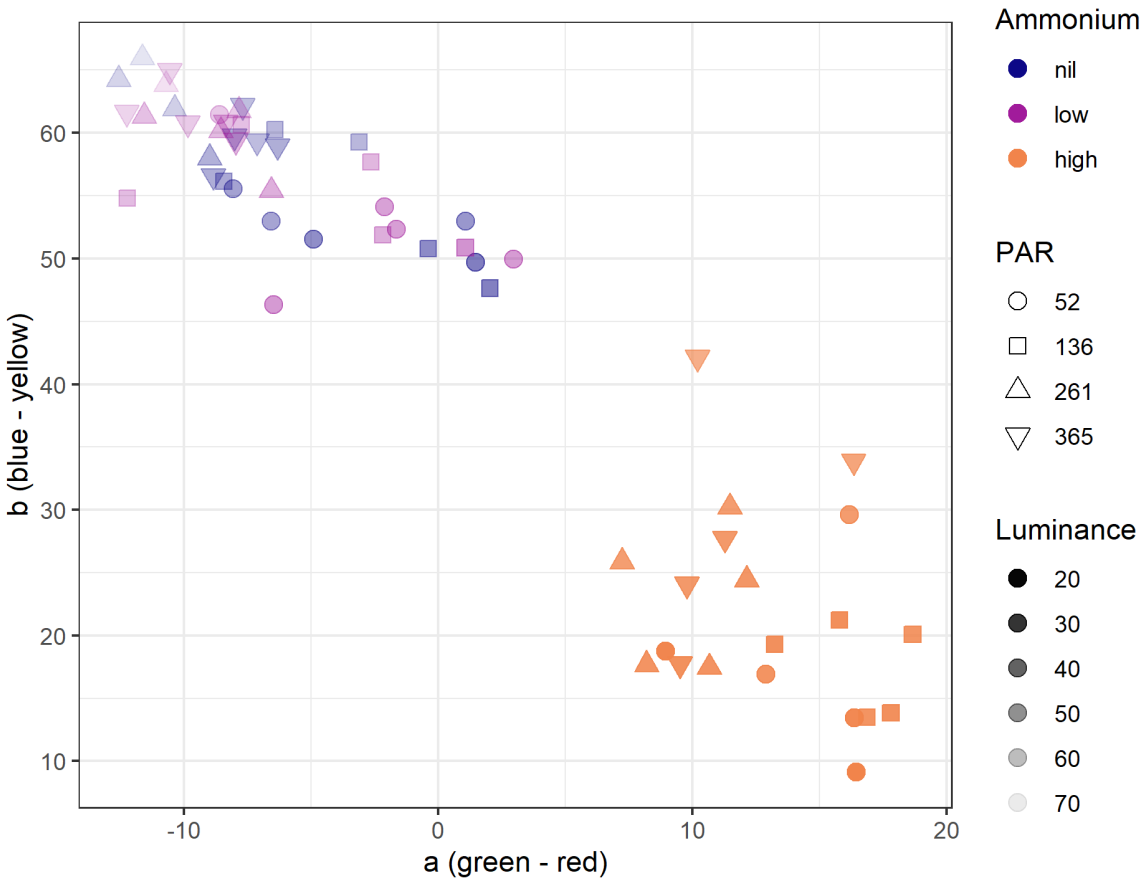

Figure 9. CIE Lab colour results for *S. robusta* specimens grown with no, low or high ammonium addition and four PAR (µE m^{-2} s^{-1}) levels with 5 replicates per treatment combination. Lab colour components are: L – Luminance or lightness, lower values indicate darker colour (0 = black, 100 = white), a – green (negative) to red (positive) value, b – blue (negative) to yellow (positive) value.

Ammonium in the flasks used for the experiment was depleted between media exchanges. Water nutrient samples taken during week 1 and week 3 showed that, after 2 and 3 days of *S. robusta* cultivation respectively, the remaining ammonium concentration in flasks was between 0.2 and 0.5 µM N. For week one, this represented removal efficiency of 98.9 % of the initial 54 µM dose in the high ammonium treatment, 71.0 % of the 5 µM dose in the low ammonium treatment, and 46.0 % in the nil treatment, which contained trace ammonium (< 0.5 µM N) prior to use either from contaminants in the salt or absorbed from the atmosphere. In week 3, where final samples were taken 3 days after media were renewed,

S. robusta in the high, low and nil treatments removed 99.8 %, 98.4 % and 96.8 % of the ammonium, respectively.

3.3.2 Nitrogen uptake rates

Ammonium and nitrate uptake rates (V) of *S. robusta* showed limited evidence of saturation over the range of concentrations tested. DIC was marginally lower for a Michaelis-Menten model fit to the overall response (i.e. assuming the same response for each N source) than for the equivalent linear model, i.e. with concentration as the sole factor (Table 3). Resulting estimates of V_{max} (µM N gDW^{-1} h^{-1}) and K_s (µM N), however, were unrealistically high (both > 3 000) given that the maximum concentration tested was ~ 300 µM N and typical values for these parameters are ~ 100 – 200 or below (Kang, et al., 2013; Rees, 2003). We therefore did not accept the uptake kinetics parameters from the Michaelis-Menten model but instead used the linear model (Figure 5) to calculate substrate affinity. DIC did not support the inclusion of the interaction term between N source and concentration in the linear model (Table 3), and the coefficient for the interaction term was effectively zero (mean 0.01, 95 % HDI: −0.22 – 0.24), demonstrating that the slope of the response, i.e. affinity, was similar for both N sources. The model without the interaction term showed that affinity was 0.89 (95 % HDI: 0.77 – 1.00). Uptake rates were similar for both N sources over the range of concentrations tested (Figure 5), with 95 % HDIs of the difference between N sources containing zero (mean difference nitrate – ammonium: −12.7, 95 % HDI −33.8 – 10.5). The maximum uptake rates observed occurred at the highest N concentrations, with uptake of ammonium reaching 340 µM N gDW^{-1} h^{-1}, and nitrate 280 µM N gDW^{-1} h^{-1}. Tissue N of the specimens from which material was excised for use in the uptake experiment was (mean ± s.e. for 2 samples): 2.40 ± 0.01 % DW.

Table 11. Comparison of DIC for Michaelis-Menten curve fit and linear models of nitrogen (N) uptake rate for N sources ammonium and nitrate. *Model selected by DIC

Model	DIC
Michaelis-Menten – separate curve per N source	359.6
Michaelis-Menten – single overall curve	357.6*
Linear – N source x concentration	361.6
Linear – N source + concentration	359.3
Linear – concentration	358.7

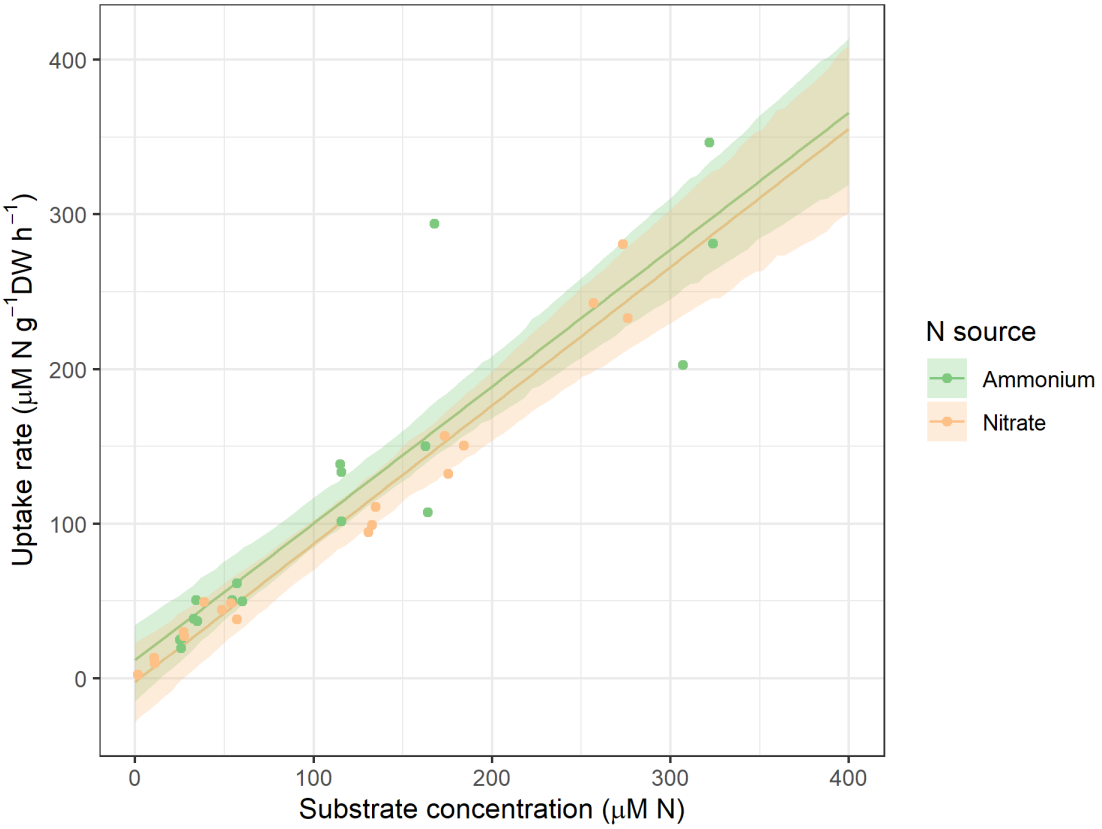

Figure 10. Uptake rates of *Solieria robusta* for Ammonium and Nitrate. Line shows linear model fit with shading indicating 95 % HDI of the fit. Points show data (n = 3 replicates per treatment combination)

3.3.3 Explant production

SGR of both tip and stem *S. robusta* explants increased with increasing enrichment solution (ES) strength, measured as equivalent nitrate-N concentration, but the increase was greater

with PES than VSM (Figure 6), with DIC selecting the model including the 2-way interaction term between ES type and strength (supplementary material). Overall, the greatest SGR of both explant types was achieved in full strength PES, with SGR of tip explants (mean and 95 % HDI) being 2.5 (1.3 – 3.6) and of stem explants being 2.2 (0.9 – 3.4) % d^{-1} in this enrichment solution.

Epiphytes were observed on only two tip explants, one each in half and full strength VSM, while 9 of 16 stem explants had visible epiphytes, including all stem explants in quarter strength VSM. The logistic model selected by DIC showed that frequency of epiphyte occurrence varied with ES type and with explant source contingent on ES strength (supplementary material). Epiphyte occurrence was more common with VSM for both explant types, while epiphytes were observed more frequently in lower ES strength for stem explants and at higher ES strength for tip explants. Stem explants in full strength ES of either type were noted to have particularly heavy epiphyte growth, which would have contributed to their final mass and hence calculated SGR. Epiphytic growth was not examined microscopically but appeared to primarily consist of filamentous red and brown algae, with a small amount of *Ulva* spp. The majority of explants (32 of 40) survived. There was no clear effect of any treatment on losses, with 1 – 2 specimens not surviving within each treatment combination.

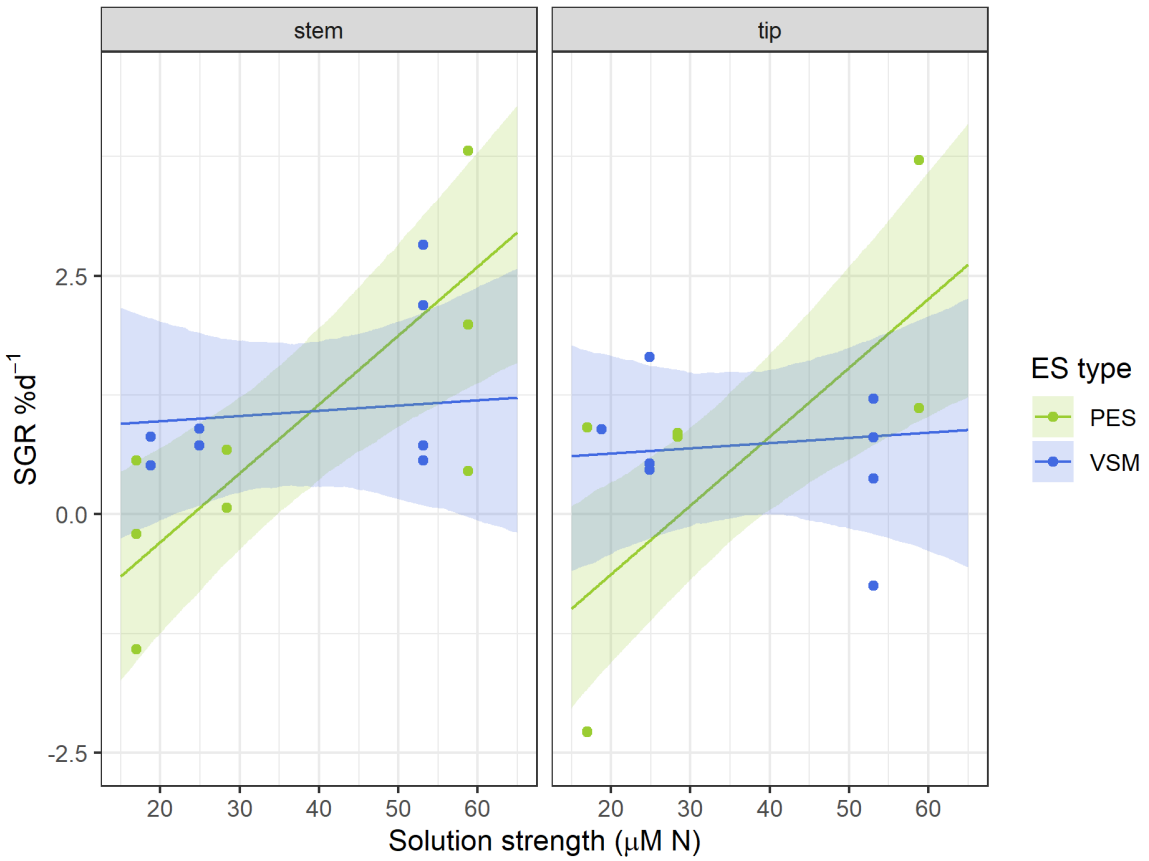

Figure 11. SGR (% d^{-1}) of *S. robusta* tip and stem explants grown in two enrichment solution (ES) types: Provasoli enrichment solution (PES) and Von Stosch medium (VSM). Line shows fitted model with shading indicating 95 % HDI of the fit. Points show SGR for each explant (n = 4 replicates per treatment combination). Note that SGR results are not shown for explants that did not survive.

4 Discussion

4.1 Suitability for IMTA of the four species investigated

Solieria robusta has the best potential for development as an aquaculture species in southern Australia of the four Rhodophyta species investigated, and could be applied to nutrient remediation, including in IMTA with existing fish aquaculture. *Solieria robusta* was fastest growing in the initial species comparison experiment, and, while *G. australe* removed the most N over four weeks, *S. robusta* has the potential to remove more N than *G. australe* over longer cultivation periods because its faster growth will result in greater biomass

accumulation. The cultivation period of farmed red seaweeds is typically in the range of 2 – 3 months (Ask and Azanza, 2002; Titlyanov and Titlyanova, 2010). Extrapolations of our calculations of N removal, using the modelled final N content and average SGR of each species, show that, for the same starting biomass, *S. robusta* N removal would exceed that of *G. australe* for any cultivation period ≥ 46 days.

The best growth rates we achieved for *S. robusta* (~ 4 – 5 % d^{-1}) compare favorably to published values for related species, such as farmed *Kappaphycus* spp. which have SGRs of 2 – 6 % d^{-1} (Ask and Azanza, 2002), while the SGR for *G. australe* (~ 3 % d^{-1}) was at the lower end of that reported for experimental culture of other Gelidiales of 3 – 7 % d^{-1} (Friedlander, 2008; Ganesan, et al., 2011). To assess if cultivation is commercially feasible, growth rates need to be considered in conjunction with product yield and value. Agar yield of *G. australe* has not been quantified, but given their growth rates, the typical agar yield of other Gelidiales that have been investigated for cultivation is too low for farming to be commercially viable (Friedlander, 2008). The ι-carrageenan yield of *S. robusta* (as assessed by Chiovitti, et al., 1999) compares favourably to that of commercially farmed *Eucheuma* spp., which have seasonally varying yield typically between 25 and 55 % (Azanza and Sa-a, 1990).

For species used in IMTA, the value of N remediation should also be taken into account, but given that effective N removal also depends on the extractive species having adequate growth rate, faster growing species are preferred (Barrington, et al., 2009; Neori, et al., 2004). Growth rates of *Pt. lucida* and *Pl. angustum* were both < 2 % d^{-1} in the initial experiment, indicating that they are less likely to be commercially viable for aquaculture unless further studies develop new methods to increase growth rates or they are identified as a source of high value bioproducts. Despite their relatively high N contents, the nutrient remediation potential of

these species is limited by their slow growth rates. Our further investigations therefore focused on *S. robusta.*

4.2 Temperature, light and nutrient responses

The thermal tolerance of *S. robusta* also demonstrates the suitability of this species for aquaculture in temperate Australia, and helps to inform suitable culture periods. Growth of seaweeds typically increases with temperature to a plateau at an optimal level, followed by a sharp decline once a critical temperature is reached (Eggert, 2012), as we observed in our temperature response experiment. We found that *S. robusta* grew faster than *G. australe* at temperatures greater than 14 °C, and had a greater maximum growth rate and high temperature tolerance than *G. australe.* The upper limit for *G. australe* growth was ~ 21 °C, with specimens kept at higher temperature bleaching and starting to disintegrate by the end of the 4-week experiment.

In the light and ammonium addition experiment, we found that *S. robusta* grew over a range of PAR levels although with a trend to better performance at 136 µE m^{-2} s^{-1} PAR than higher (> 250 µE m^{-2} s^{-1} PAR) light levels. Seaweeds typically show increasing growth and photosynthetic performance with light availability to an optimum above which damage to the photosystem results in decreased growth and effective quantum yield (Hanelt and Figueroa, 2012; Hurd, et al., 2014). Optimum PAR varies between and within seaweed species and reflects adaptation to local conditions (Borlongan, et al., 2017; Hanelt and Figueroa, 2012). Light and nutrient often have interactive effects on seaweed growth, photosynthetic performance and N storage (Brown, 1995; Endo et al., 2016; Lapointe, 1981; Lapointe and Tenore, 1981; Shivji, 1985), but we did not observe this in our experiment, possibly due to the relatively limited range of combinations tested.

Solieria robusta specimens accumulated tissue N when grown with added N in the initial species comparison experiment and at the higher level of ammonium addition in the light and ammonium experiment, likely incorporating N in photosynthetic pigments. Red seaweeds increase their phycoerythrin and phycocyanin content with increased N availability (e.g. Carmona, et al., 2006; Gal-Or and Israel, 2004; Lignell and Pedersén, 1987). These pigments absorb in the red and blue spectra respectively, and are likely to be responsible for the differences in specimen colour that we observed between ammonium treatments. Our observation that specimens under lower light within each ammonium treatment also showed darker colour with more blue/red is also consistent with expected effects of light intensity on photosynthetic pigments. Seaweeds generally increase their photosynthetic pigment content with decreasing light availability, e.g. with increasing depth, and excess light may damage or destroy pigments leading to bleaching at high irradiance (Hanelt and Figueroa, 2012; Hurd, et al., 2014).

Growth and photosynthetic performance of *S. robusta* also increased in the high ammonium addition treatment. The optimum ammonium level for *S. robusta* cultivation is, however, unresolved because growth was not assessed over a sufficient range of concentrations. SGR in the ammonium addition experiment was lower than that achieved in the species comparison and temperature experiments. Nutrient limitation can restrict seaweed growth (Roleda and Hurd, 2019) and may have affected the results due to the small culture volume relative to specimen biomass leading to depletion of available N between media exchanges. Seaweed tissue N content reflects nutritional content (Harrison and Hurd, 2001), and nutrient limitation may also have prevented specimens in the low ammonium addition treatment from increasing tissue N. We supplied ammonium weekly at equivalent fluxes expected around fish

farms, but, in the field, it is highly unlikely that *S. robusta* would become nutrient limited, due to the water volume per unit biomass and continual water exchange. Our results nonetheless demonstrate that *S. robusta* can accumulate available additional N, and its growth would benefit from the increased nutrient supply around fish farms.

A lack of saturation kinetics, as observed in our nutrient uptake rate experiment, can occur in red seaweeds that are N limited. A linear response to N concentration can arise, even at concentrations > 500 µM, when N-limited specimens perform surge uptake (Harrison and Hurd, 2001; Smit, 2002). Tissue N in the specimens used for our uptake experiment was 2.4 % DW, which was higher than the tissue N level of specimens in our other experiments, but may still be below the optimum tissue N for this species. The tissue N level indicative of nutrient limitation (= critical N level), varies between seaweed species, but is typically between 0.7 and 3.2 % (Harrison and Hurd, 2001).

The critical N level for a seaweed species can be determined by measuring the growth rate and tissue N of specimens grown under a range of N concentrations, and finding the tissue N at which growth plateaus (Harrison and Hurd, 2001). Our results do not allow determination of critical tissue N for *S. robusta* because our focus was examining growth at N concentrations likely to be experienced by seaweeds during cultivation around SA fish farms, and we did not investigate growth or tissue N with greater levels of N addition. While we also could not determine V_{max} from our uptake rate data, *S. robusta* demonstrated uptake rates > 200 µM N g^{-1}DW h^{-1}, which is above the 100 µM N g^{-1} DW h^{-1} considered useful for seaweeds applied to IMTA (Kang, et al., 2013). Given that N concentrations around fish farms in SA are likely to be ≤ 12 µM (Middleton, et al., 2013; Tanner and Volkman, 2009), the data provided by the uptake experiment allow calculation of uptake rates in an applicable concentration

range for modelling the influence of *S. robusta* cultivation on dissolved N in SA fish farming regions. Most seaweeds demonstrate affinity of < 2 for nitrate, and many also show affinity < 2 for ammonium, with higher ammonium affinity than this occurring predominantly in species adapted to eutrophic conditions (Rees, 2003). The affinity of *S. robusta* for both ammonium and nitrate of 0.89 is within this typical range, and is greater than that of several other seaweeds with IMTA potential (0.12 – 0.52; Kang, et al., 2013).

4.3 Informing suitable sites and cultivation periods

Temperature, light and nutrient response data from our experiments is useful for informing suitable sites and seasons for seaweed cultivation. Water temperatures in south-western Spencer Gulf, where most fish aquaculture in SA currently occurs, range between 14 and 20 °C seasonally, while other aquaculture zones within Spencer Gulf experience an annual temperature range of approximately 12 to 24 °C (Tanner and Volkman, 2009). The temperature range providing good growth of *S. robusta*, defined as ≥ 80 % of maximal SGR (= 3.2 % d^{-1}) following Eggert (2012), was 16.4 – 24.0 °C, suggesting that cultivation of this species from late spring through summer and autumn should be feasible throughout much of Spencer Gulf. The temperature range providing good growth (≥ 2.2 % d^{-1}) of *G. australe* was 12.4 – 21.0 °C, demonstrating that *G. australe* has a similar breadth of thermal tolerance to *S. robusta* but is adapted to a cooler temperature range. This temperature response suggests potential suitability for *G. australe* cultivation in southern Spencer Gulf, although its growth rates may be too low to achieve commercial viability (Friedlander, 2008). Summer temperatures in Spencer Gulf are, however, near to the upper limit for *G. australe,* therefore if cultivated in this area, production could be severely impacted by ocean warming and/or

marine heatwaves, an important consideration for longer-term success of seaweed aquaculture (Chung, et al., 2017).

Light availability in seaweed cultivation is affected by water depth and season, and depth can be adjusted to suit the species being cultivated (Buschmann, et al., 2008; Handå, et al., 2013; Hwang, et al., 2007). Only relative PAR measurements have been recorded in Spencer Gulf, but these and modelling of the light climate demonstrate a seasonal pattern of light availability, with winter irradiance being approximately one quarter of that in summer (Tanner and Volkman, 2009). Port Lincoln is at a similar latitude (35°S), and experiences comparable average monthly insolation to Adelaide (Bureau of Meteorology data, http://www.bom.gov.au/climate/data/), where the underwater light climate is well characterised (Collings, et al., 2006). Based on subsurface irradiation from Adelaide metropolitan waters and the modelled diffuse attenuation in Port Lincoln (~ 0.2 year-round) (Collings, et al., 2006; Tanner and Volkman, 2009), the optimum depth for *S. robusta* culture in southern Spencer Gulf is likely to be around 3 m in spring/autumn, 5 – 6 m in summer, and 2 m in winter. This would, however, depend on interactive effects of light and temperature, which have not been explored for this species.

Seasonal patterns in nutrient availability also occur in Spencer Gulf, due to changes in stocking density and fish feeding regimes, as well as natural processes (Middleton, et al., 2013; Tanner and Volkman, 2009). The highest N concentrations (primarily as ammonium) occur in late autumn-winter (May – Jul) in the tuna farming zone (Middleton, et al., 2013; Tanner and Volkman, 2009), and in spring (August – October) in kingfish farming areas (Middleton, et al., 2013). Cultivation over spring or autumn in relevant areas would therefore maximise N availability to seaweeds and provide the greatest nutrient remediation benefits.

Data on *S. robusta* temperature, light and nutrient responses could be incorporated into mechanistic models (e.g. Radiarta, et al., 2011; Westmeijer, et al., 2019) to assist in identifying the most suitable sites for *S. robusta* cultivation. The N storage capability of *S. robusta* can also be used to inform the biomass of cultivated seaweed needed offset nutrient inputs from a given farmed fish biomass. Based on the growth rate and N content of *S. robusta* recorded in the species comparison experiment, and assuming a cultivation period of 90 days, an initial biomass of 900 tonnes of seedlings of this species would be needed to completely mitigate the annual N outputs of 1000 tonnes of kingfish production, or 400 tonnes of tuna (= 200 tonne N, Fernandes, et al., 2007; Fernandes and Tanner, 2008). A cultivation 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 be needed grow sufficient *S. robusta* to offset 1000 tonnes of kingfish production using this system, but further research would be needed to determine if this type of culture system and planting density is suitable for this species and for the chosen farm location.

4.4 Seedstock production

Seedstock production will be important for developing seaweed aquaculture in Australia, because regulatory frameworks are unlikely to permit expansion of wild harvest (Roos, et al., 2018). Explant production techniques additionally facilitate mass production of seedlings with desirable phenotypic traits such as growth rates or disease resistance (Yong, et al., 2011; 2014). We found that explant production methods based on those used for commercial Solieriaceae are applicable to *S. robusta*. Although full-strength PES is detrimental for some

red seaweeds (de Paula, et al., 2001; Harrison and Berges, 2005), our results support that PES is a better medium for cultivation of Solieriaceae than VSM (Yong, et al., 2011; 2014).

The effect of PES on explant performance varies depending on the specific formulation used (Berges, et al., 2001; Harrison and Berges, 2005) and on frequency of addition, with pulse application being more beneficial than continuous supply (de Paula, et al., 2001). We used PES with the modifications recommended by Berges, et al. (2001), and pulse application (three times weekly) of both ES types. VSM contains some additional metal salts that are not included in PES; these salts may be detrimental to some seaweeds, leading to poorer growth performance (Yong, et al., 2011; 2014).

The greater occurrence of epiphytes on explants cultured in VSM may be due to VSM favouring growth of opportunistic algae over that of *S. robusta* explants. More frequent and heavier epiphytes on stem than tip explants may be due to stems harbouring more microscopic contaminants than tips, or to tip but not stem explants being able to out-compete opportunistic algae given suitable culture conditions. Segments with apical cells often have lower levels of contamination and potential for greater growth than other segment types (Kawai, et al., 2005; Yong, et al., 2014). While SGR was similar for both explant types in our experiment, it is likely that the apparent SGR of stem explants was influenced by epiphyte mass, while SGR of tip explants more accurately reflected mass accumulation of those explants.

Refinement of explant production methods for *S. robusta* should include exploration of additional methods for cleaning and preventing epiphytic growth; and light, temperature, salinity, pH and explant density conditions to maximise explant growth. The scarcity of

epiphytes on tip explants, however, suggests that using apical fragments is likely to provide the best explant performance. Given that cultivation conditions have not been optimised and that stock material was not specifically selected, the growth rate of *S. robusta* explants (SGR > 2 % d^{-1}) in our experiment is promising.

4.5 Conclusions

Solieria robusta demonstrated faster growth than the other investigated species in our initial experiments, and our additional investigations of this species support that it is suitable for aquaculture and has characteristics favourable for use in N remediation. *Solieria robusta* can be propagated from cuttings, demonstrates a promising growth rate, suitably high uptake rates for both ammonium- and nitrate-N, and the ability to accumulate and store tissue N. The data inform light and temperature parameters for growing *S. robusta*, which will help to identify suitable areas, depths and seasons for its cultivation. Data on tissue N and uptake rates can be incorporated into biogeochemical models to assess the influence of seaweed cultivation on dissolved N, determine the seaweed biomass required to offset N inputs from fish farming, and identify locations and seasons where seaweed cultivation would have greatest impact on N levels. Future work should prioritise refinement and upscaling of seedstock production methods to produce sufficient biomass for field cultivation trials.

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Supplementary material

Supplementary results for tissue N data analysis (section 3.1).

Table S1. Mean differences between parameter estimates for species: GA = *Gelidium australe,* PA = *Plocamium angustum,* PL = *Pterocladia lucida*, SR = *Solieria robusta* from Bayesian models of tissue nitrogen in samples pre- and post-nutrient addition. Estimates are mean difference between coefficients and 95 % HDIs of differences. * Significant difference based on 95 % HDIs.

Difference between species:	**Pre-nutrient addition**	**Post-nutrient addition**
PA – PL	0.35 (0.10 – 0.58)*	0.18 (−0.07 – 0.42)
PA – SR	0.35 (0.11 – 0.58)*	−0.04 (−0.27 – 0.21)
PA – GA	−0.82 (-1.05 – -0.57)*	−1.10 (−1.36 – −0.86)*
PL – GA	0.00 (−0.24 – 0.22)	1.04 (0.55 – 1.55)*
PL – SR	1.17 (0.92 – 1.39)*	1.27 (0.77 – 1.77)*
GA – SR	1.17 (0.93 – 1.41)*	1.16 (0.65 – 1.63)*

Table S2. Mean differences between parameter estimates for pre- and post- nutrient addition samples for each species: GA = *Gelidium australe,* PA = *Plocamium angustum,* PL = *Pterocladia lucida*, SR = *Solieria robusta* from Bayesian models of tissue nitrogen. Estimates are mean difference between coefficients and 95 % HDIs of differences. * Significant difference based on 95 % HDIs.

Species	**Difference between pre- and post- nutrient addition samples:**
GA	0.88 (0.66 – 1.11)*
PA	0.37 (0.00 – 0.74)
PL	0.14 (−0.24 – 0.53)
SR	1.42 (1.05 – 1.81)*

Table S3. Mean differences between estimates of tissue N increase for species: GA = *Gelidium australe,* PA = *Plocamium angustum,* PL = *Pterocladia lucida*, SR = *Solieria robusta* from Bayesian models of tissue nitrogen. Estimates are mean difference between coefficients and 95 % HDIs of differences. * Significant difference based on 95 % HDIs.

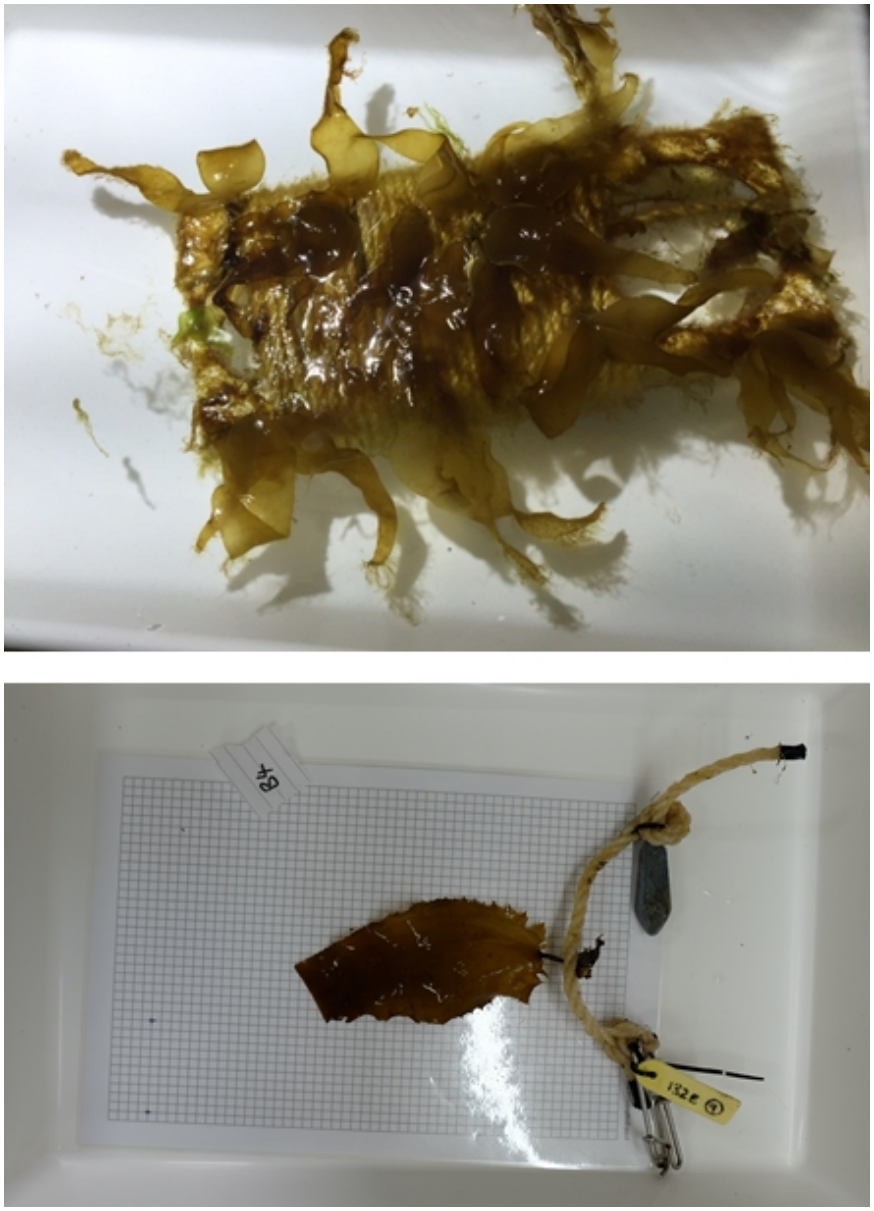
Comparison between species:	**Difference in N increase**
PA – PL	−0.16 (−0.48 – 0.14)
PA – SR	−0.39 (−0.72 – −0.09)*
PA – GA	−0.28 (−0.58 – 0.05)
PL – GA	0.23 (−0.06 – 0.54)
PL – SR	0.12 (−0.20 – 0.41)
GA – SR	−0.11 (−0.39 – 0.22)

Supplementary results for *Solieria robusta* explant production data analysis (section 3.3.3)

Table S4. DIC results comparing models of specific growth rate (SGR) and epiphyte occurrence for *Solieria robusta* explants with factors: Segment (tip or stem), enrichment solution (ES), and nitrogen (N) concentration. ΔDIC is the difference in DIC from the selected model for each response. A more complex model was only selected over a simpler model where DIC was reduced by 2 or more.

Factors included	DIC	ΔDIC
SGR		
Segment x ES x N	149	30
Segment + ES + N + Segment:ES + Segment:N + ES:N	124	5
Segment + ES + N + Segment:N + ES:N	123	4
Segment + ES + N + Segment:ES + ES:N	123	4
Segment + ES + N + Segment:ES + Segment:N	134	15
Segment + ES + N + ES:N*	119	0
Segment + ES + N	135	16
Epiphytes		
Segment x ES x N	36.9	0
Segment + ES + N + Segment:ES + Segment:N + ES:N	38.2	1.3
Segment + ES + N + Segment:N + ES:N	36.8	−0.1
Segment + ES + N + Segment:ES + ES:N	38.6	1.7
Segment + ES + N + Segment:ES + Segment:N	41.8	4.9
Segment + ES + N + Segment:ES	41.3	4.4
Segment + ES + N + Segment:N*	36.9	0
Segment + ES + N + ES:N	41.2	4.3
Segment + ES + N	40.9	4.0

Chapter 5. Hatchery production and nutrient remediation potential of the kelp *Ecklonia radiata*



Top: *Ecklonia radiata* seedlings growing on a seed collector. Below: young sporophyte of *Ecklonia radiata* being photographed for the hatchery cultivation experiment.

Statement of authorship to go here

Statement of Authorship

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Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Obtained funding for the project, provided advice on experimental design and statistical analysis, assisted with review and editing of the manuscript		
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Hatchery production and nutrient remediation potential of the kelp *Ecklonia radiata*

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Abstract

There is limited wild harvest and no established seaweed aquaculture in Australia, but farming native seaweeds would help to meet the growing demand for seaweed products. Seaweed farming in Australia for nutrient remediation is also of interest to permit sustainable expansion of fish farms. *Ecklonia radiata* (Laminariales), a brown seaweed native to Australia, has potential for commercialisation for human consumption and products including alginates, laminarin, and fucoidan. We investigated methods for hatchery production of *E. radiata* because seedstock production is an important step for establishing cultivation, and nutrient responses as an indicator of suitability for nutrient remediation. Vegetative gametophyte cultivation and string seeding using methods adapted from other Laminariales were successful for *E. radiata*. Provasoli enrichment solution with or without germanium dioxide was beneficial for gametophyte cultivation, with 22 °C and illumination of ~ 20 µE m^{-2} s^{-1} providing suitable conditions. Nitrate was a better nitrogen (N) source than ammonium for hatchery cultivation of sporophytes, with best growth achieved between 50 and 60 µM nitrate-N. Uptake of N by *E. radiata* did not display saturation kinetics over the tested concentrations, with similar uptake rate and affinity for ammonium and nitrate. N uptake rate and affinity compared favourably to other seaweed species used for nutrient remediation. Successful hatchery production of *E. radiata* provides the foundation for developing this species for aquaculture, and its N responses demonstrate suitability for nutrient remediation applications.

1 Introduction

Brown seaweeds (Ochrophyta: Phaeophyceae) are widely utilised for food and their secondary metabolites (Holdt and Kraan, 2011; Lorbeer, et al., 2013; Smit, 2004; Thomas and

Kim, 2011; White and Wilson, 2015). Kelps (Laminariales) are the predominant seaweeds grown for food, and with increasing global seaweed consumption, aquaculture of kelps is expanding (Buschmann, et al., 2017; McHugh, 2003; Skrzypczyk, et al., 2018; White and Wilson, 2015). Kelps are also utilised as sources of alginates, fertilisers, stock feed, biofuels, and high value extracts for cosmetics, nutraceuticals and medicines (Buschmann, et al., 2017; Gupta and Abu-Ghannam, 2011; Holdt and Kraan, 2011; Smit, 2004; Thomas and Kim, 2011).

Australia, and in particular southern Australia, has a highly diverse seaweed flora with high endemism (Phillips, 2001). Australia's seaweed flora could also be utilised as a source of food, hydrocolloids, and other extracts, and has the potential to yield novel bioactive compounds (Kirkendale, et al., 2010; Lee, 2010; Lorbeer, et al., 2013; Roos, et al., 2018). Several native Australian seaweeds are palatable and nutritious, having favourable fatty acid profiles and high protein and fibre content (Skrzypczyk, et al., 2018). While the potential value of Australia's seaweed resources has been recognised, commercial utilisation has been minimal. Australia is a net importer of seaweed products and has only a small industry based around beach-cast harvest and very limited wild harvest (Lee, 2010; Lorbeer, et al., 2013; Roos, et al., 2018). Despite its rich seaweed flora, Australia has only two Laminariales species: *Ecklonia radiata* (C. Agardh.) J. Agardh, a common canopy forming species of Australia's temperate reef systems (Connell and Irving, 2008), and *Macrocystis pyrifera* (Linnaeus) C. Agardh 1820, which has a limited distribution in Australia, being restricted to Tasmanian coasts and a small area of the south-eastern mainland (Womersley, 1987). Utilisation of native kelp in Australia must therefore involve one of these two species, with *E. radiata* likely to be the better candidate based on its wide geographic range.

Known in Australia as common or golden kelp, *E. radiata* is one of the main components of the beach-cast harvest in southern Australia used for production of plant fertiliser and animal feed (Lorbeer, et al., 2013), but it also has potential for commercialisation as a source of higher value products. *Ecklonia radiata* has a good nutritional profile and is palatable for humans (Skrzypczyk, et al., 2018), provides a similar yield of natural anti-oxidants to *Laminaria* and *Saccharina* spp. and other antioxidant-rich plants (Charoensiddhi, et al., 2015), and has prebiotic activity (Charoensiddhi, et al., 2017). *Ecklonia radiata* is also a good source of fucoidan, a bioactive polysaccharide; laminarin, a plant growth promotor; and alginates, hydrocolloids used as gelling agents in food products and for a range of industrial applications (Lorbeer, et al., 2015a; b; 2016).

Seaweed aquaculture is essential to the development of an Australian seaweed industry, because regulatory frameworks are unlikely to allow expansion of wild and beach-cast harvests (Lee, 2010; Roos, et al., 2018). Development of seaweed aquaculture in Australia is also desirable to mitigate nutrient inputs, including from fish aquaculture (Wiltshire, et al., 2015). This type of strategic co-culture, termed integrated multi-trophic aquaculture (IMTA) is growing globally and has economic and environmental benefits (Barrington, et al., 2009; Neori, et al., 2004; Troell, et al., 2003). Cultivation of *E. radiata* is likely to be feasible using methods and technologies applied for commercially farmed Laminariales elsewhere (Kirkendale, et al., 2010; Wiltshire, et al., 2015); limited experimental cultivation of *E. radiata* in New Zealand, and of other *Ecklonia* species (*E. cava* and *E. stolonifera*) in Korea, has been successful (Hwang, et al., 2009; Hwang, et al., 2012; Neill, et al., 2009). Ongoing wild collection for seedstock is unlikely to be permitted under Australian legislative frameworks, therefore nursery seedstock production is important for industry development (Roos, et al., 2018). Our

research therefore focused on hatchery production methods of *E. radiata,* and we additionally considered nutrient responses with respect to IMTA applications.

In common with several farmed Laminariales, mature *E. radiata* sporophytes produce spores in sori located along the central blade, with spores developing into microscopic gametophytes (Mohring, et al., 2013; Novaczek, 1984c; Womersley, 1987). Sporulation in *E. radiata* can be induced using the standard method applied for farmed kelp: allowing blade sections with sori to dehydrate and then re-immersing them in seawater (Mohring, et al., 2013; Neill, et al., 2009; Wiltshire, et al., 2015). Farming Laminariales typically involves seeding spores directly onto string or rope, or vegetatively culturing gametophytes in flasks for seeding onto rope (Edwards and Watson, 2011; Redmond, et al., 2014; Sahoo and Yarish, 2005). Seeded ropes are maintained in nursery conditions until young sporophytes develop to a stage suitable for out-planting (Sahoo and Yarish, 2005; Titlyanov and Titlyanova, 2010).

The performance of kelp early life stages during nursery cultivation is influenced by several factors that should be considered in applying methods to a novel species. Light, temperature and nutrients are important for seaweed growth and development, with optima varying between species and life stages (Hurd, et al., 2014). Germanium dioxide (GeO_2) is commonly added during cultivation of gametophyte and early sporophyte stages to inhibit diatom growth (Forbord, et al., 2012; Kerrison, et al., 2019; Shea and Chopin, 2007), but is detrimental to gametophytes of some kelps (Shea and Chopin, 2007). Nutrient supplementation with either full- or half-strength Provasoli Enrichment solution (PES) is usually applied for gametophyte cultivation and initial seeding of kelps (Edwards and Watson, 2011; Flavin, et al., 2013; Redmond, et al., 2014; Sahoo and Yarish, 2005). Provision of supplementary nutrient during later stages of hatchery culture can improve seedling performance in

subsequent out-planting (Rößner, et al., 2014). A range of string substrates are used in kelp cultivation, and the type of string used also influences the growth and survival of sporophytes (Kerrison, et al., 2017; Kerrison, et al., 2019).

Reproduction, gamete development and early sporophyte growth of *E. radiata* have been described (Jennings, 1967; Kirkman, 1981), and effects of light, temperature and nutrients on gametophyte and sporophyte performance investigated under conditions relevant to natural populations (Bearham, et al., 2013; Mabin, et al., 2013; Mohring, et al., 2014; Mohring, et al., 2013; Novaczek, 1984b; Staehr and Wernberg, 2009). *Ecklonia radiata* gametophytes perform best at ~18 – 22 °C (Mabin, et al., 2013; Mohring, et al., 2014) and photosynthetically active radiation (PAR) of ~ 17 – 42 µE m^{-2} s^{-1} (Novaczek, 1984c, b), with no difference in performance observed between natural and depleted nitrate levels (Mabin, et al., 2013). Sporophyte growth increases with temperature to an optimum at ~ 22 – 23 °C (Bearham, et al., 2013; Kirkman, 1981; Staehr and Wernberg, 2009), with higher PAR, especially > 20 µE m^{-2} s^{-1}, encouraging faster growth (Bearham, et al., 2013; Novaczek, 1984a). In natural populations, sporophyte growth is sometimes limited by nutrient availability (Bearham, et al., 2013). This information provides a guide to conditions likely to be suitable for *E. radiata* cultivation, but data on the effects of nutrient supplementation for gametophytes or sporophytes are lacking. Optimal conditions for vegetative gametophyte cultivation may vary from those for gametophyte development under natural conditions, hence, temperature and light responses for vegetative growth of gametophytes need to be assessed.

To assist development of techniques for hatchery production of *E. radiata,* we therefore investigated: (1) light, nutrient and GeO_2 addition conditions for vegetative gametophyte cultivation; (2) performance of sporophytes seeded on different string types; and (3)

responses to nutrient supplementation of laboratory grown *E. radiata* sporophytes. *Ecklonia radiata* nutrient uptake kinetics were also investigated because this species may be a suitable candidate for IMTA in southern Australia (Wiltshire, et al., 2015). Data on nutrient uptake dynamics and growth responses will assist in incorporating N removal by seaweeds into biogeochemical models, further elucidating the suitability of *E. radiata* for IMTA, and informing optimal nutrient addition for hatchery cultivation.

2 Methods

2.1 Gametophyte production

Adult sporophytes of *E. radiata* were collected at O'Sullivan Beach on January 28th 2015, February 5th and April 26th, 2016 (South Australia: latitude and longitude: -35.1196, 138.4674), wrapped in wet paper towel and placed in an insulated container with a small amount of seawater from the collecting location. After < 1h transport, a version of the Laminariales cultivation protocol (as described in Neill, et al., 2009; Sahoo and Yarish, 2005) was used to obtain spores. Clean sections of the central blade with fertile tissue were selected, rinsed with filtered seawater and wiped with 90 % ethanol before being desiccated in dark, humid conditions at ambient temperature for one hour.

2.2 String seeding

For seeding onto string, desiccated fertile blade sections from 4 adult sporophytes, each comprising ~ 20 cm^2 of visible sori, were placed in filtered seawater in an 80 L plastic tub. Gentle agitation was applied periodically over four hours. A water sample was taken to confirm the presence of zoospores. Seed collectors comprised 8 m of 4 mm diameter string wound onto frames made from 30 cm lengths of rectangular PVC drainpipe with circular

cut-outs on each of the wider sides (following Edwards and Watson, 2011). Collectors with three types of string: polypropylene, polyethylene and nylon, with three replicates of each type, were submerged into the zoospore slurry for 30 minutes. Once seeded with zoospores, collectors were gently transferred to individual 100 L conical-bottom tanks filled with filtered seawater with full strength PES added (Berges, et al., 2001; Harrison and Berges, 2005). The seeded collectors were maintained in these tanks for 18 weeks. The tanks were housed in a constant environment room at 18 °C, with 50 µE m^{-2} s^{-1} illumination from cool white LED lamps on a 12 h light : 12 h dark cycle, and gentle aeration applied after the first 10 days. Approximately half the tank volume was exchanged twice weekly, with full strength PES added with each water exchange.

At the end of the experimental period, all visible seedlings were gently removed from the string collectors. The number of seedlings per collector was recorded, and the thallus length (as per Mabin, et al., 2013) of each seedling was measured. Ten randomly selected seedlings from each collector were gently patted dry with paper towel and weighed to the nearest 0.001 g.

Initial data exploration showed that seedling counts on collectors were over-dispersed with respect to the Poisson distribution that is otherwise appropriate for count data (Zuur, et al., 2013). Count data were therefore analysed using a negative binomial model in a Bayesian framework, as detailed in section 2.7, to assess the effect of string type on seedling count. To assess the effect of string type on length and weight of seedlings, Bayesian linear mixed models were run, with collector as a random effect.

2.3 Gametophyte vegetative culture

For gametophyte culture, desiccated fertile blade sections were cut into approximately 5 cm^2 pieces and placed into a 1 L beaker containing filtered autoclaved seawater. After 2 hours the zoospore suspension was strained through 50 µm Nitex® plankton netting to remove the sporophyte tissue. The zoospore suspension was placed on a magnetic stirrer and 10 mL transferred to each of 60 x 200 mL conical flasks by pipette while the suspension was continually stirred to maintain zoospores in suspension.

For the first experiment, the 60 flasks were randomly assigned to three media treatments, with 20 flasks per treatment. Media treatments comprised 200 mL sterile filtered natural seawater with either no nutrient addition, addition of half-strength PES (PES/2) (Berges, et al., 2001; Harrison and Berges, 2005), or addition of PES/2 and GeO_2. In the latter treatment, an initial dose of 200 µL saturated GeO_2 solution (following West, 2005) was added when flasks were first filled and subsequent media exchanges were supplemented with 50 µL saturated GeO_2 solution.

The flasks were suspended in tubs in 20 fibreglass aquaria that acted as water baths to maintain temperature at 18 °C; aquaria were housed in a controlled environment room. Each aquarium contained three flasks, one of each media treatment: seawater, seawater + PES/2 and seawater + PES/2 + GeO_2, and was randomly assigned to one of four PAR treatments, with 5 replicate aquaria per PAR level. Lighting was supplied by cool-white LED lamps and each aquarium was covered with layers of red cellophane to provide red light at four PAR levels (mean ± SE, n = 5): 1.9 ± 0.3, 16.1 ± 0.9, 33.0 ± 1.3 and 48.6 ± 1.7 µE m^{-2} s^{-1}. PAR levels are reported in results as the nearest whole number, i.e. 2, 16, 33 and 49 µE m^{-2} s^{-1}. A diagram of the experimental set up is provided in Figure 1. Red light was used because this promotes

vegetative growth of gametophytes while preventing fertile development (Edwards and Watson, 2011; Redmond, et al., 2014). Light was provided using a 16 h light : 8 h dark cycle. Gentle aeration was provided to each flask to keep the gametophytes in suspension.

Media renewal was carried out three times a week as follows. A sterilized spatula was used to scrape the bottom and the side of each flask to remove any adhering gametophytes. Gametophytes were then allowed to settle for 2 hours with no aeration, and approximately 50 % of the medium was gently poured off, flasks were left a further 2 hours to allow gametophytes to settle again, then as much of the old media as possible was poured off. Flasks were then refilled with sterile seawater, with PES and GeO_2 added to the relevant treatments. After 46 days culture, the gametophyte abundance in each flask was assessed using haemocytometer counts.

For the second experiment, the effects of temperature were assessed on gametophytes grown in full-strength PES, with or without GeO_2, or half-strength PES. Gametophytes were maintained in 60 flasks, with 20 flasks per treatment. Treatments comprised 200 mL sterile filtered natural seawater with either PES/2, PES, or PES + GeO_2. Saturated GeO_2 solution was added at 200 µL initially and 50 µL with each medium exchange. As per the first experiment, one flask of each medium was placed into a tub suspended in one of 20 aquaria in a controlled environment room, so that each aquarium contained three flasks. Submersible heaters in the aquaria maintained the water temperature, with five aquaria randomly assigned to each of four temperature treatments (mean ± SE, n = 5): 15.6 ± 0.1, 18.0 ± 0.5, 20.2 ± 0.5, and 22.1 ± 0.3 °C. Temperature treatments are referred to by the nearest whole degree in results, i.e. as 16, 18, 20 and 22 °C. All aquaria were covered with red cellophane providing PAR (mean ± SE for n = 20 tanks) of 48.7 ± 1.6 µE m^{-2} s^{-1}. A diagram of the experimental set up is provided in

Figure 2. Flasks were maintained as per the first experiment, and gametophytes were counted after 38 days culture.

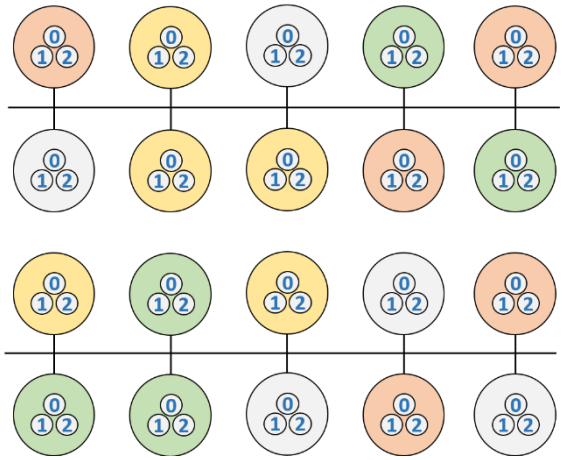

Figure 12. Diagram of experimental set up for gametophyte cultivation experiment 1. Larger circles represent aquaria, which were arranged in four rows, and small circles represent flasks within each aquarium. Numbers represent nutrient treatments: 0 – nil, 1 – PES/2, 2 – PES/2 + GeO_2 and colours represent PAR level: green - 2, yellow – 16, orange – 33, light grey - 49 µE m^{-2} s^{-1}.

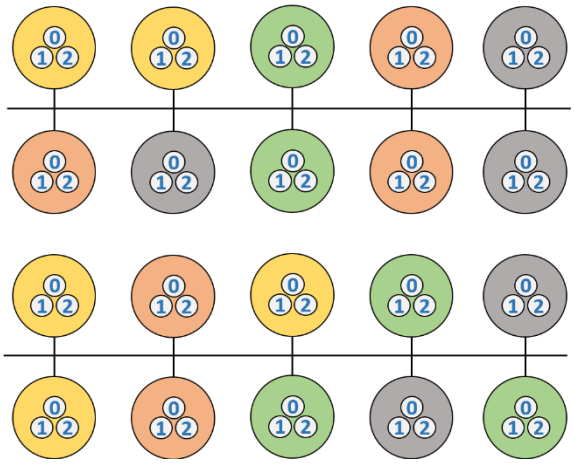

Figure 13. Diagram of experimental set up for gametophyte cultivation experiment 2. Larger circles represent aquaria, which were arranged in four rows, and small circles represent flasks within each aquarium. Numbers represent nutrient treatments: 0 – PES/2, 1 – PES, 2 – PES + GeO_2 and colours represent temperature: grey 16, green 18, yellow 20 and orange 22 °C.

Gametophyte counts in the first experiment were over-dispersed, while counts in the second experiment fitted a Poisson distribution. Counts from the first experiment were therefore

analysed using a negative binomial mixed model, with light level and media type as fixed effects, and tank as a random effect. Counts from the second experiment were analysed with a Poisson mixed model, with temperature and media type as fixed effects and tank as a random effect. Analyses were conducted in a Bayesian framework as described in section 2.7.

2.4 Nutrient responses in hatchery cultivation

Juvenile *E. radiata* sporophytes were collected from O'Sullivan beach on June 19th 2019 and transferred to 20 L glass aquaria in a controlled environment room. Specimens selected for use in the experiment were identified as early stage plants following Kirkman (1981), and were either stage 1 (single blade with no laterals), or early stage 2 (small lateral protuberances).

Aquaria were maintained at 20.0 ± 0.8 °C with LED lighting (Fluval 3.0 plant spectrum) providing PAR of 112.7 ± 4.4 µE m^{-2} s^{-1} with a 12 h light : 12 h dark cycle with lighting changes at 08:00 and 20:00. Water was circulated within each aquarium by submersible pumps (AquaOne 101PH) and gentle aeration was applied. Aquaria were randomly assigned to one of eight nitrogen (N) treatments, with three replicates per treatment. Treatments comprised a daily dose of 1, 2, 5 or 10 mL of a 0.2 M stock solution of ammonium, provided as NH_4Cl, or nitrate, provided as $NaNO_3$. Stock solutions each contained phosphate (P, as KH_2PO_4) with a 10:1 N:P ratio.

Sporophytes were assigned randomly to aquaria and acclimated for two weeks prior to the experiment start. During acclimation, aquaria were supplied with constant flow-through filtered natural seawater at (mean ± SE for n = 24 tanks) 4.1 ± 0.1 Lh^{-1} with no nutrient dosing. Following acclimation, nutrients were added daily to each aquarium by peristaltic dosing

pumps (Aquatronica ACQ450). A single dose of the required volume was applied to each aquarium one hour into the lighting period at 09:00 daily and the seawater supply to the aquaria was suspended from 07:00 – 17:00 to allow seaweed to take up nutrient, with flow over the remaining 14 hours providing an average daily water exchange of approximately 3x the total volume of each aquarium. Sporophytes were maintained under experimental conditions for 5 weeks. Epiphytic algae were wiped from aquarium surfaces weekly and removed by siphoning out a minimal volume of water from each at the start of the water exchange period to minimise the impact of water removal on nutrient levels. Water samples for N concentration (water N) analysis (detailed in section 2.6) were collected once per week from each aquarium, approximately one hour after addition of the daily nutrient dose.

Sporophytes were photographed on 5 mm graph paper at the start and end of the experiment. Initial and final thallus blade areas (i.e., excluding stalk and holdfast) were determined from photographs using FIJI/ImageJ (Schindelin, et al., 2012) and used to calculate specific growth rate (SGR) assuming exponential growth, i.e.: SGR = 100 x $\ln(A_t - A_0)/t$, where A_t = final area, A_0 = initial area, and t is time in days. Samples for analysis of tissue N were taken from the blade of specimens immediately prior to the initial and after the final photographs.

Effective quantum yield of PSII photochemistry (Genty, et al., 1989) was calculated for each specimen based on fluorescence values taken three hours into the lighting period on the last day of the experiment using a wireless waterproof Pulse Amplitude Modulated (PAM) fluorometer (Classic Fluorometer, Aquation Pty Ltd, Australia), following Maxwell and Johnson (2000). Immediately prior to photographing at the end of the experiment, rapid light curves (RLCs) were generated for each specimen using the RLC program of the PAM

fluorometer. The RLC program involved an initial fluorescence measurement taken prior to light exposure, and eight measurements with increasing PAR exposure over the range 2 – 300 µE m^{-2} s^{-1}. PAR exposures were of actinic light applied for 10 s at each level, followed by the saturating flash (~ 2 000 µE m^{-2} s^{-1}) used for fluorescence measurement.

We initially examined whether specimen growth responses fit the Droop equation (Droop, 1968; 2003), which relates growth to concentration of a limiting nutrient. Attempts to fit data to the Droop equation using the *nls* package in *R* (Ritz and Streibig, 2008) did not converge. Visualisation of results indicated that growth responses displayed non-linearity, but not of the rectangular hyperbolic form of the Droop equation. We therefore instead compared linear models and generalised additive models (GAM), each using either water N or tissue N as a predictor, using Akaike's Information Criterion, AIC (Arnold, 2010; Burnham, et al., 2011). Water N was a better predictor for SGR than tissue N, with AIC being lower for GAM (supplementary material). GAM was therefore used with water N fitted as a smooth effect. The effects of N source and water N were tested by comparing a model of the overall water N response for both species with one that also included a term for the N source difference, using AIC and examining the approximate significance of smooth terms within the selected model (Wood, 2017). 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, 2017) in *R*. The effect of N source, dose and water N on plant tissue N at the end of the experiment was analysed using a linear model fit as described in section 2.7.

Photosynthetic-irradiance (PE) curve parameters: light saturated photosynthetic rate (P_s), optimum irradiance (E_{opt}), and initial slope of the PE curve (α), were determined using the *R* package *phytotools* (Silsbe and Malkin, 2015). Curves were fitted to the equation of Eilers and

Peeters (1988), using an irradiance normalised model with quantum efficiency of photosynthesis (Φ_{PSII}) data as recommended by Silsbe and Kromkamp (2012).

Bayesian linear models were used to examine the effects of N source, water N and TN_{final} on P_s, E_{opt}, α, and Φ_{PSII} as described in section 2.7.

2.5 Nutrient uptake rates

Material of *E. radiata* for determination of nutrient uptake rates was taken from additional sporophytes collected from O'Sullivan beach on June 19th 2019 and maintained in the controlled environment room for two weeks under the same conditions used for acclimation of sporophytes for the cultivation experiment (see section 2.4). We applied the multiple flask method (Harrison, et al., 1989) using excised blade sections (following Kang and Chung, 2018; Li, et al., 2007; Sato, et al., 2016; Wang, et al., 2012). Specimens were cleaned by wiping with cotton-fibre gauze, and a single circular disc of tissue with a diameter of approximately 40 mm was cut from the middle section of the blade of each specimen with a sterile scalpel. Discs had mean fresh weight (± s.e., n = 24) of 2.01 ± 0.04 g and were placed into separate flasks containing 200 mL low-nutrient artificial sea water (Sigma s9883) with salinity of 36. Flasks were maintained in a culture cabinet (Climatron 520-DL) under lighting of 100 µE $m^{-2}s^{-1}$ PAR at 20 °C for two hours prior to nutrient addition to allow recovery from cutting, and during the uptake period. Flasks were randomly assigned to nutrient treatments, which comprised N of 10, 25, 50 and 100 µM as either ammonium (from NH_4Cl) or nitrate ($NaNO_3$), with three replicate flasks per treatment. Phosphorus (as KH_2PO_4) was added in a 10:1 N:P ratio to avoid P limitation. Four flasks containing low-nutrient artificial seawater and 50 µM N + 5 µM P, comprising two with added ammonium and two with nitrate, were maintained as controls with no seaweed tissue. Water samples of 50 mL for N analysis were taken from each flask

after addition of nutrient and mixing, and then after one hour. Tissue N was determined for each disc after the uptake experiment. Nutrient analysis methods are described in section 2.6.

Uptake rates (V) were determined as: $V = (M_0 - M_t) / (t \times DW)$, where M_0 and M_t are the moles of N at time 0 and t, calculated from concentration x volume at each time, t is the time interval and DW the seaweed dry weight. Because we used only four N levels, we did not have sufficient resolution to fit data to Michaelis-Menten curves to assess uptake kinetics.To examine if uptake rates were linear over the range of concentrations used, we compared models using nutrient level as a factor (ANOVA design) to linear models using initial substrate concentration as a continuous covariate. The concentration used in linear models was the actual concentration measured in initial water samples, which differed from the nominal treatment concentration in several flasks. Models were fitted in JAGS, using the deviance information criterion (DIC) to determine whether the linear or ANOVA fit was more parsimonious, i.e. whether responses showed evidence of non-linearity, indicative of saturation. For both ANOVA and linear models, we compared fit with and without an interaction term between N concentration and N source using DIC to determine if uptake rates were different between N sources. Where a linear response is observed, the slope of the response indicates the affinity for the substrate, with the interaction term being used to assess any difference in affinity between N sources. See section 2.7 for details of the JAGS methods used.

2.6 Nutrient analyses

Samples for tissue N content were frozen, freeze-dried overnight and then ground to a fine powder using a Fritsch stainless steel ball mill. A 100 mg aliquot was analysed on a LECO

Truspec CNS Elemental Analyser (LECO, St Joseph, MI, USA). Water nutrient samples were kept frozen until analysis on a Thermo Scientific™ Aquakem™ for ammonium levels above 3 µM and nitrate levels above 15 µM, with lower level samples analysed by flow injection analysis (FIA) on a Lachat QuickChem 8000 Automated Ion analyser. Ammonium ($NH_3 + NH_4^+$) was determined using the indophenol blue method (Lachat, 2003a) in both cases. Nitrate was determined using the sulphanilamide method using hydrazine reduction for the Aquakem™ or a cadmium reduction column for FIA (Lachat, 2003b).

2.7 Bayesian methods

Bayesian analyses were conducted using Markov Chain Monte Carlo (MCMC) simulations obtained by running each model in JAGS v. 4.3.0 (Plummer, 2017). In each case, uninformative priors were used. Specifically, diffuse normal priors (mean 0 and precision 0.0001) were used for the estimate of covariate effects in linear models, a uniform (0,20) prior was used for the estimate of size of the negative binomial distribution in the seedling count analysis, and uniform (0,100) priors were used for standard deviation estimates of fixed effects in linear models and of random effects in mixed models. Each analysis used three chains. Linear and Poisson models used 40 000 iterations for burn-in, followed by 10 000 iterations thinned at a rate of 10, while negative binomial models used 50 000 iterations for burn-in and 50 000 iterations, thinned at a rate of 50, for estimation. For all analyses, therefore, estimates were based on 3 000 simulations. JAGS was run using the *R2jags* package (Su and Yajima, 2015) in *R* (R Core Team, 2019). Convergence was assessed using the Gelman-Rubin convergence statistic, and confirmed by visual inspection of trace, density and autocorrelation plots generated using the *mcmcplots* package (McKay Curtis, 2015). The importance of interaction terms involving factors was assessed by comparing the deviance information criterion (DIC)

between nested models with and without the interaction. Hypothesis testing was conducted following Kruschke (2014). Differences between factor levels were considered significant where 95 % highest density intervals (HDIs) of the difference between their posterior parameter estimates did not contain zero, and continuous predictors were considered significant where 95 % HDIs of the relevant coefficients did not contain zero. HDIs were calculated using the *HDinterval* package (Meredith and Kruschke, 2018).

3 Results

3.1 Growth on seeded ropes

Gametophyte performance did not vary between string types, with seedlings reaching a similar weight on each string type (Figure 1). There was a trend for polyethylene string collectors to have fewer seedlings of greater length, but differences were not significant (Figure 1, Table 1). The limited number of replicates and variable nature of the data, particularly the overdispersed counts, provided low statistical power.

Table 12. Mean differences between parameter estimates for string types: PE (Polyethylene), PP (Polypropylene), Ny (Nylon) from Bayesian models of seedling count, length and weight. Estimates are mean difference between coefficients and 95 % HDIs of differences. Note that a negative binomial model was used for count data, hence the coefficient difference is on the log scale (i.e. exponent of coefficient indicates multiplicative difference).

Difference between string types:	Regression coefficient for term:		
	Seedling count	Length	Weight
PE − PP	−1.03 (−2.62 – 0.85)	8.75 (−15.44 – 34.55)	0.022 (−0.136 – 0.176)
Ny − PP	−0.20 (-1.82 – 1.67)	-6.30 (-28.48 – 16.42)	−0.027 (−0.167 – 0.109)
PE − Ny	−0.83 (−2.46 – 0.99)	15.05 (−9.15 – 40.81)	0.049 (−0.101 – 0.209)

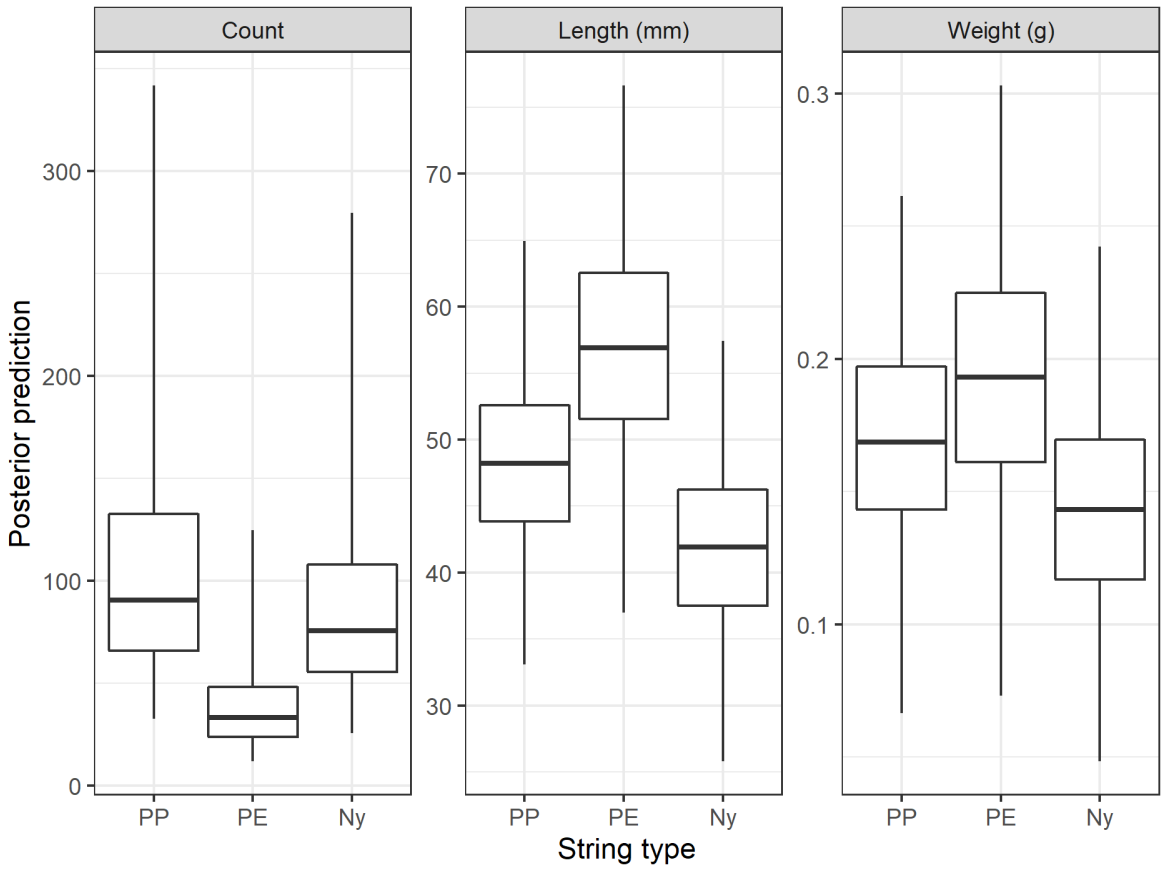

Figure 14. Posterior predictions of seedling count, length and weight for string types: PE (Polyethylene), PP (Polypropylene), Ny (Nylon) from Bayesian models. Boxes show mean and interquartile range, with whiskers showing 95 % HDIs of the posterior predictions.

3.2 Gametophyte cultivation

Gametophyte counts in the first vegetative cultivation experiment were higher in treatments with PES/2 added than in unmodified seawater, and similar between PES/2 treatments with and without addition of GeO_2 (Table 2; Figure 2). Counts were not different between PAR levels, although there was a tendency for lower counts in the lowest PAR level, especially with PES added. DIC, however, selected the model without the media type x light interaction (ΔDIC −20.9 for the model with no interaction term). For the second experiment, DIC selected the model without the interaction of temperature and media type (ΔDIC −7.1). Counts generally increased with temperature, and were higher at 22 than 18 °C, with other temperatures intermediate (Table 2; Figure 2). Within each temperature, there was a trend for higher

counts in full strength PES with or without GeO_2 compared with half strength PES, but differences were not significant (Table 2).

3.3 Sporophyte nutrient responses

Sporophyte growth (SGR based on area) was significantly affected by water N (approximate significance of smooth term p = 0.004), and while the smooth term describing the difference between responses for the two N sources was not significant (p = 0.09), AIC provided support for inclusion of this term (ΔAIC = −2.5 for model with N source difference term). Given the non-significant smooth term and relatively small difference in AIC for the model including a different response by N source, we examined predictions of models both with and without this term. The model including the separate N source term showed that when grown with added nitrate, SGR of *E. radiata* sporophytes increased with water N concentration to a maximum of 2.1 % d^{-1} at a water N of approximately 60 µM nitrate-N, declining slightly at high water N, while, with added ammonium, SGR generally declined with increasing water N, with a slight decline over the range 0 – 30 µM ammonium-N, before declining more steeply (Figure 3). The model including only the overall N response showed optimum SGR of 1.6 % d^{-1} at a water N of approximately 50 µM (Figure 3).

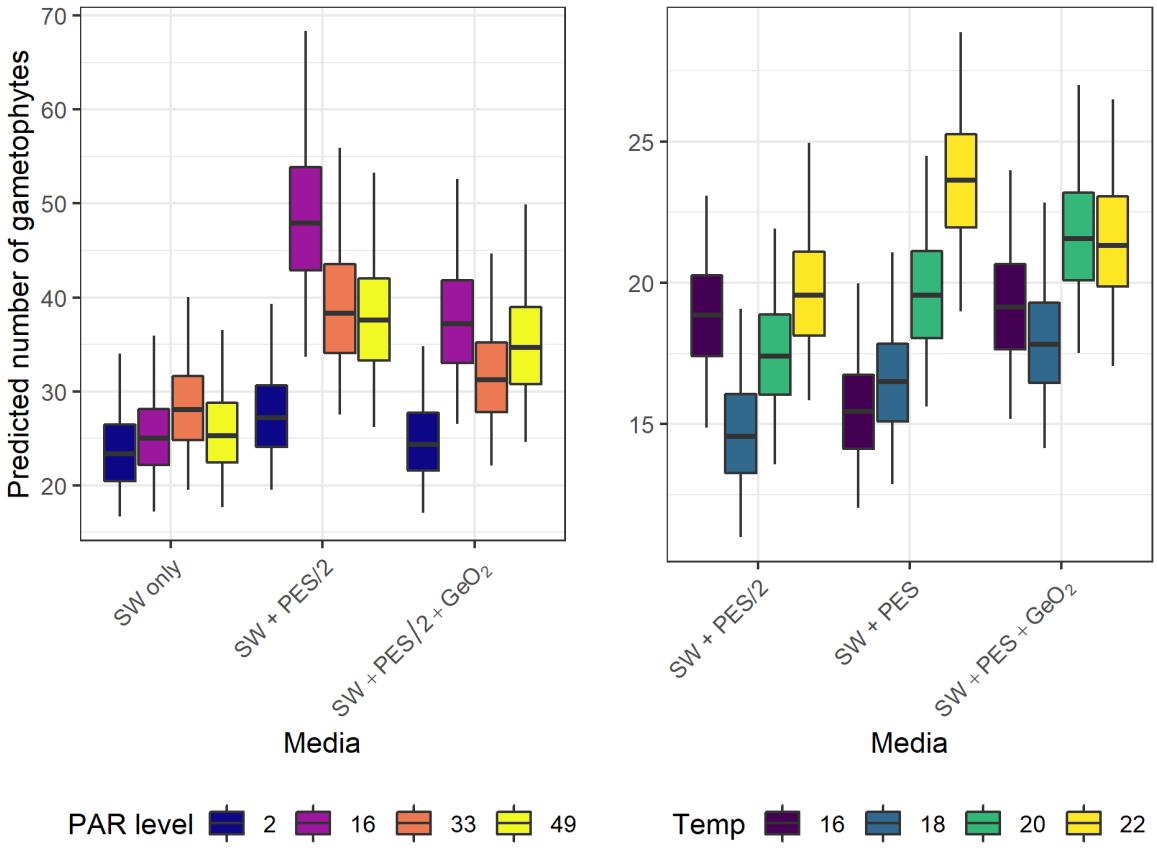

Figure 15. Posterior predictions from Bayesian models of gametophyte counts with media type and left: PAR (μE m^{-2} s^{-1}) or right: temperature (°C). Boxes show mean and interquartile range, with whiskers showing 95 % HDIs of the posterior predictions.

The initial N content of sporophytes (mean ± SE for n = 24 plants) was 1.14 ± 0.06 % DW, while final N varied between treatments. DIC selected the model with no interaction between N source and dose (ΔDIC = −3.1). Final tissue N was higher with increasing dose (slope: 0.098, 95 % HDI 0.063 – 0.135) but with no effect of N source (mean difference ammonium − nitrate: −0.028, 95 % HDI −0.280 – 0.192). Tissue N of sporophytes decreased over the five week experimental period at the lower levels of N addition, with final N (mean ± SE for n=6) being 0.68 ± 0.08 % DW in tanks with the lowest (1 mL) N dose, 0.78 ± 0.08 % DW with 2 mL dose,

1.21 ± 0.18 % DW with 5 mL dose and 1.55 ± 0.08 % DW at the highest level of N addition (10 mL dose).

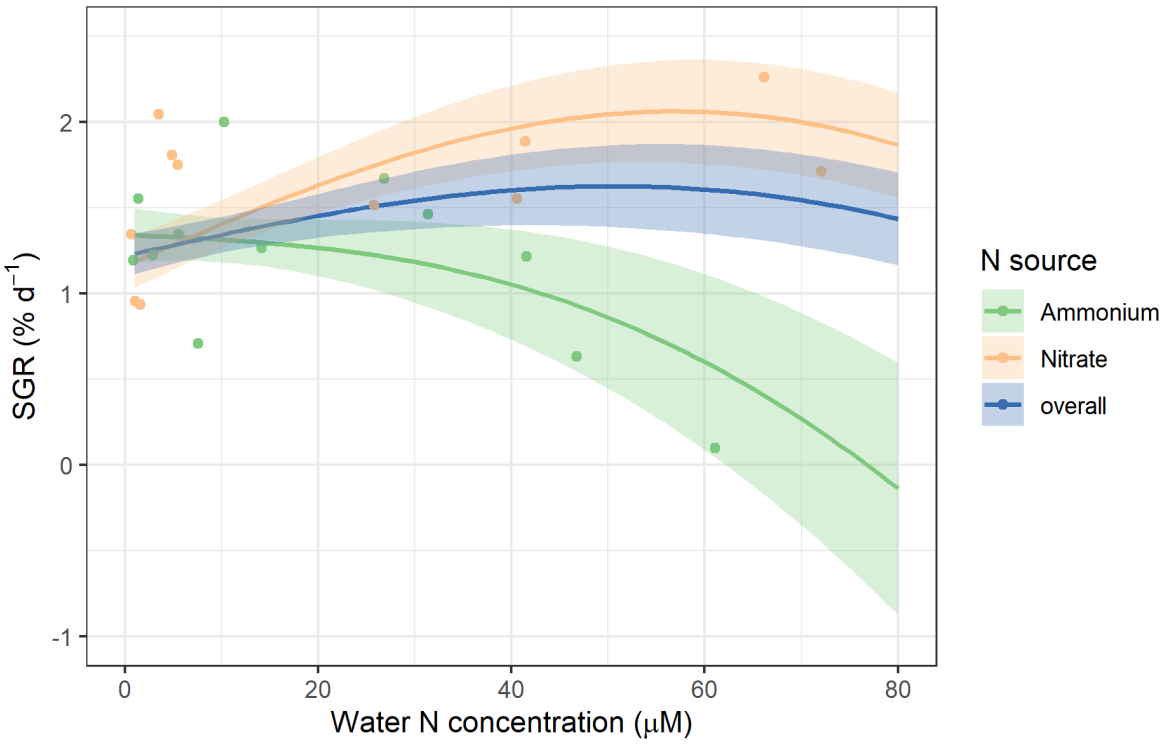

Figure 16. Specific growth rate (SGR) in blade area (points) by nitrogen (N) concentration for *Ecklonia radiata* and GAM predictions (lines, with shaded area showing 95 % confidence interval) of fitted models with and without N source (ammonium and nitrate) as a factor. The model including N source illustrates differences in response with N source, and the model with N source illustrates the overall response of SGR to N concentration.

Water N was a better predictor for quantum efficiency of photosynthesis (Φ_{PSII}) than was tissue N (Table 3), and DIC was lowest for the model including an interaction term, but with ΔDIC of only −0.8, indicating minimal support for inclusion of this term. The 95 % HDIs of the interaction term (−0.11 – 0.37) also indicated it was not significant. Trends in the predicted response from this model were, however, similar to those of SGR, i.e., for Φ_{PSII} to increase with water N for nitrate (slope: 0.11, 95 % HDI −0.11 – 0.32), but to be similar or decline slightly with water N for ammonium (slope: −0.03, 95 % HDI −0.10 – 0.05). The model without

an interaction term showed that water N tended to have a small but not significant positive effect on Φ_{PSII} (slope: 0.05, 95 % HDI −0.02 – 0.12). This model showed no difference for the effect of N source on Φ_{PSII} (mean difference ammonium – nitrate: −0.01, 95 % HDI −0.07 – 0.06). Tissue N was a better predictor for differences in PE curve parameters than water N, with DIC selecting the model without an interaction term in each case (Table 3). Optimum irradiance (E_{opt}) was higher for sporophytes grown in nitrate than ammonium, and for plants with higher tissue N (Table 4). Other parameters were similar between N sources and for tissue N; maximum photosynthetic rate tended to increase with tissue N but the 95 % HDIs of the estimate of this slope included zero.

Table 13. Mean differences between parameter estimates of gametophyte counts for media treatments and PAR levels from Bayesian models. Estimates are mean difference between coefficients and 95 % HDIs. * indicates HDIs do not contain zero, i.e. parameter estimates are considered different.

Difference between media types:	Count	Difference between media types:	Count
PES/2 − nil	0.38 (0.18 – 0.58)*	PES − PES/2	0.13 (−0.01 – 0.28)
PES/2 + GeO_2 − nil	0.22 (0.02 – 0.41)*	PES + GeO_2 − PES/2	0.06 (−0.09 – 0.20)
PES/2 − PES/2 + GeO_2	0.16 (−0.04 – 0.35)	PES + GeO_2 − PES	−0.06 (−0.20 – 0.08)
Difference between PAR levels:		Difference between temperatures:	
33 − 49	0.01 (−0.36 – 0.43)	18 − 16	−0.08 (−0.30 – 0.18)
16 − 49	0.12 (−0.23 – 0.51)	20 − 16	0.09 (−0.14 – 0.31)
2 − 49	−0.25 (−0.64 – 0.16)	22 − 16	0.19 (−0.04 – 0.41)
33 − 16	−0.11 (-0.49 – 0.28)	18 − 20	−0.18 (−0.42 – 0.06)
33 − 2	0.26 (−0.14 – 0.66)	18 − 22	−0.27 (−0.52 – −0.05)*
16 − 2	0.37 (−0.04 – 0.75)	20 − 22	−0.10 (−0.31 – 0.13)

Table 14. Comparison of DIC for models to predict photosynthetic parameters: quantum efficiency (Φ_{PSII}), initial slope (α), optimum irradiance (E_{opt}) and saturating photosynthetic rate (P_s), with predictors: nitrogen (N) source (difference nitrate – ammonium). *Model with lowest DIC for each response variable.

	Model DIC for response:			
Model terms	Φ_{PSII}	α	E_{opt}	P_s
N source x tissue N	−78.1	−96.0	376.6	303.9
N source + tissue N	−79.1	−95.6*	374.4*	301.7*
N source x water N	−75.8*	−99.2	381.1	304.8
N source + water N	−76.6	−97.9	382.5	305.6

Table 15. Effect of tissue nitrogen and N source (difference nitrate – ammonium) on photosynthetic-irradiance curve parameters: initial slope (α), optimum irradiance (E_{opt}) and saturating photosynthetic rate (P_s). *Different to zero based on 95 % HDI.

	Parameter estimate mean and 95 %HDI for:	
Response	Tissue N	N source
α	0.04 (−0.02 – 0.10)	0.03 (−0.02 – 0.08)
E_{opt}	11.5 (3.1 – 19.1)*	12.4 (6.2 – 19.2)*
P_s	3.64 (−0.04 – 7.29)	−1.16 (−4.44 – 1.78)

Uptake rates (V) of *E. radiata* were linear over the range of concentrations tested (Figure 4), i.e. did not demonstrate evidence of saturation for either nitrate or ammonium , with linear models providing a better fit to the data than ANOVA models as assessed by DIC (Table 5). Substrate affinity was therefore determined from the regression slope of V on N concentration from the linear model. DIC did not support the inclusion of the interaction term between N source and concentration in the linear model (Table 5), and the coefficient for the interaction term was close to zero (0.02), with 95 % HDI containing zero (−0.13 – 0.17), demonstrating that the slope of the response, i.e. affinity, was similar for both N sources. The model without the interaction term showed that affinity was 0.50 (95 % HDI: 0.43 – 0.57). Uptake rates were similar for both N sources over the range of concentrations tested (mean

difference nitrate – ammonium: 1.65, 95 % HDI −3.68 – 7.12). The maximum uptake rates were at the highest N concentrations, with uptake of ammonium reaching 101 µM N g^{-1} h^{-1}, and nitrate 89 µM N g^{-1} h^{-1}. The tissue N content of specimens used for the uptake experiment was (mean ± SE for n = 16) 1.08 ± 0.05 % DW. There was no change in ammonium or nitrate concentration in control flasks over the experimental period.

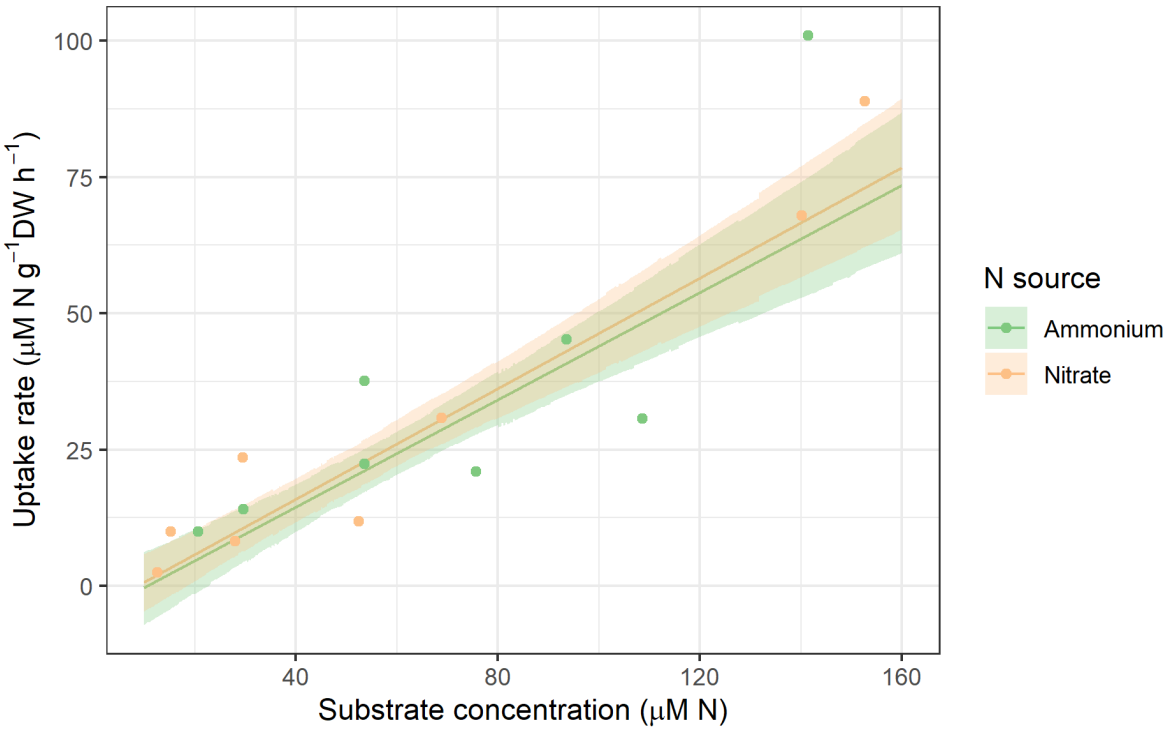

Figure 17. Uptake rates (points) and fitted linear model (lines, with shaded area showing 95 % HDI) of ammonium and nitrate uptake for *Ecklonia radiata* by initial substrate concentration.

4 Discussion

Our experiments demonstrate the feasibility of hatchery propagation for the common kelp *E. radiata*, providing an important foundation for developing this species for aquaculture. Methods that are used for commercially cultivated Laminariales can be successfully applied

to *E. radiata* for production and vegetative cultivation of gametophytes, and for seeding onto string collectors.

Table 16. Comparison of DIC for ANOVA (using N concentration as a categorical factor) and linear models (using N concentration as a continuous covariate of nitrogen (N) uptake rate for N sources ammonium and nitrate. *Model selected by DIC.

Model	DIC
ANOVA –N source x concentration	417.6
ANOVA – N source + concentration	412.6
Linear – N source x concentration	359.3
Linear – N source + concentration	356.5*

We found that nutrient addition was beneficial for vegetative gametophyte cultivation, resulting in greater gametophyte abundance than unmodified seawater. Full or half-strength PES, with or without GeO_2, was suitable. While these media treatments were not clearly different, the highest gametophyte counts tended to occur in full strength PES without GeO_2 addition, therefore this would be the recommended medium to use unless diatom growth is problematic, in which case, GeO_2 may be applied. GeO_2 is sometimes detrimental to kelp gametophytes (Shea and Chopin, 2007), but addition of GeO_2 for ~ 1 week during initial cultivation can suppress diatoms while avoiding potential toxicity (Kerrison, et al., 2015). GeO_2 addition following West (2005) was tolerated by *E. radiata* gametophytes over 38 – 46 days cultivation, although in our study GeO_2 addition did not appear to be necessary, because diatom growth was not observed in the cultivation flasks.

We found all light levels tested to be suitable, although the trend to lower gametophyte counts in the lowest PAR treatment (2 µE m^{-2} s^{-1}) suggests this light level may be insufficient for long term cultivation. For vegetative cultivation under red light, a PAR of 5 – 20 µE m^{-2} s^{-1} is recommended for gametophytes of other Laminariales (Edwards and Watson, 2011;

Redmond, et al., 2014) and, while clear differences were not demonstrated in our case, we recorded highest counts at 16 µE m^{-2} s^{-1}, suggesting a similar PAR level to other Laminariales is suitable for *E radiata* gametophyte cultivation. For development of *E. radiata* gametophytes under full spectrum light, Novaczek (1984b, c) found that a PAR range of 17 – 42 µE m^{-2} s^{-1} was optimal, with little difference in gametophyte performance over this range. This additionally suggests that a PAR of ~ 20 µE m^{-2} s^{-1} is suitable, with slightly higher illumination being acceptable but not necessary.

We found that 22 °C was better for *E. radiata* gametophyte cultivation than < 20 °C. This is warmer than the temperature applied for gametophyte cultivation of other Laminariales (typically < 15 °C, e.g. Edwards and Watson, 2011; Flavin, et al., 2013; Redmond, et al., 2014), but reflects that *E. radiata* is adapted to warmer temperatures than other kelps, with a distribution that extends to warm temperate regions (Mohring, et al., 2014; Novaczek, 1984b). The thermal optimum for *E. radiata* gametophytes varies across its latitudinal range, increasing with *in situ* water temperature up to ~ 23 °C for gametophytes obtained from plants in the warmest regions (Mohring, et al., 2014). Our plants were collected in Adelaide, South Australia, in the middle of the latitudinal range for *E. radiata.* Mohring, et al. (2014) found that 18 – 20 °C was the thermal optimum for *E. radiata* gametophytes from Adelaide, but also identified that gametophyte performance was strongly linked to *in situ* conditions at small spatial scales and to short-term temperature patterns. Our gametophytes may therefore have been sourced from a population adapted to a local microclimate or recent warmer conditions. Our cultivation method also varied from that applied by Mohring, et al. (2014) who grew gametophytes attached to substrates under full spectrum lighting. Overall,

however, temperatures suitable for growth and development of gametophytes under natural conditions were also suitable for vegetative cultivation of *E. radiata* gametophytes.

Vegetative cultivation of gametophytes permits year-round production (Flavin, et al., 2013; Redmond, et al., 2014), but also facilitates seeding strings by spraying with a gametophyte suspension, which can produce more even coverage of sporophytes than direct seeding (Edwards and Watson, 2011). We used direct seeding in our string seeding experiment, which preceded the gametophyte cultivation experiments. Seeding of *E. radiata* was successful, but sporophytes were patchily distributed on our collectors. Using vegetative gametophyte cultivation and the spray method for string seeding following Edwards and Watson (2011) may provide more even sporophyte coverage. Sporophyte development and growth occurred on each of the three string types that we tested, although our sample size was too low to discern if any of the string types tested was better for *E. radiata* seeding or growth. All string types tested have been used for cultivation of Laminariales and appear suitable for use with *E. radiata,* but the trend to higher counts of seedlings on polypropylene string, with very similar average seedling mass to other string types, suggests it may be preferred. It should be noted, however, that performance of seeded sporophytes on string under nursery conditions does not always reflect performance after out-planting, because bioadhesion of sporophytes to string will affect losses in the field (Kerrison, et al., 2019). Polyvinyl alcohol (PVA) substrates demonstrate better bioadhesion than untreated polypropylene or polyamide, but surface treatment of the other string types can improve their bioadhesion (Kerrison, et al., 2019). We did not test PVA because we focused on three primary string types identified as in use for Laminariales culture, which did not include PVA. Kerrison et al. (2017, 2019) demonstrated that additional string types and pre-treatments are worth investigating.

In hatchery cultivation, nitrate was a better N source than ammonium for *E. radiata*, promoting increased growth to an optimum at around 60 µM nitrate-N. The difference in response between N sources was minimal at concentrations up to 20 µM, but at N concentrations > 40 µM, SGR was always greater for the sporophytes grown in nitrate than ammonium, with the greatest differences observed at the highest water N concentrations.

Better performance of *E. radiata* grown with added nitrate was reinforced by these specimens having higher optimum irradiance values than those grown with ammonium. Many seaweeds show better growth with ammonium than nitrate as an N source, but ammonium is toxic to some algae at higher concentrations, e.g. > 25 µM for sensitive species (Berges, et al., 2001; Harrison and Hurd, 2001; Kevekordes, 2001; Roleda and Hurd, 2019), leading to reduced growth. The difference in growth between N sources for *E. radiata* may be caused by ammonium toxicity at higher concentrations. Nitrate is also a better N source than ammonium for growth of the brown seaweed *Sargassum hemiphyllum* in hatchery cultivation (Han, et al., 2018). Comparisons of N sources for Laminariales hatchery growth are scarce, but some Laminariales uptake nitrate preferentially over ammonium (Ahn, et al., 1998; Xu, et al., 2011), although others show more rapid uptake of ammonium (Braga and Yoneshigue-Valentine, 1996; Sato and Agatsuma, 2015). While nitrate appears to be a better N source than ammonium for *E. radiata* hatchery growth where nutrients are added to levels typically higher than those found in nature, the ammonium concentration around SA fish farms is ≤ 12 µM N (Middleton, et al., 2013; Tanner and Volkman, 2009), within the range where growth performance is similar with either N source and lower than the concentration where adverse impacts of ammonium occurred.

In contrast to other studies (Han, et al., 2018; Hanisak, 1990; Harrison and Hurd, 2001; Pedersen and Borum, 1996; 1997) we did not find a strong relationship between growth rate and tissue N, although there was a trend for increasing growth with higher tissue N for both N sources. The Droop equation (Droop, 1968; 2003) relates growth to tissue concentration of a limiting nutrient, and nutrient response growth data are often fitted to this equation to predict critical and optimal tissue N for seaweeds (Han, et al., 2018; Harrison and Hurd, 2001; Lemesle and Mailleret, 2008; Pedersen and Borum, 1996; 1997). The growth-tissue N relationship is, however, only applicable under equilibrium conditions (Lemesle and Mailleret, 2008), which may not have occurred in our experiments.

In our treatments with lower levels of N addition, tissue N of sporophytes decreased over the experimental period, suggesting they were using stored N for growth. Conversely, specimens grown at the highest added N concentration showed increased tissue N over the five-week experiment. In contrast to unicellular algae and ephemeral seaweeds, large brown seaweeds can maintain growth over periods of low nutrient availability by accumulating and storing nutrients, particularly N, when available, and then utilising it as required (Harrison and Hurd, 2001; Pedersen and Borum, 1996; 1997).

In our experiment water N may have been a better predictor of growth because aquaria where measured water N concentrations were higher were those where N supply was excess to that required by *E. radiata*. This excess N resulted in tissue N accumulation while facilitating high growth rates. Specimens in treatments with lower water N concentration were nutrient limited and utilised stored N, and were therefore unable to achieve growth rates as high as in treatments without N limitation. Had we continued the experiment for longer, it is likely that tissue N would have reached equilibrium and demonstrated a clearer relationship to growth.

A lack of fit to the Droop equation, however, could also have resulted from the decline in growth of *E. radiata* at higher ammonium-N concentrations, rather than the plateau in growth expected. We did find that plants with higher tissue N showed higher optimum irradiance values, indicating an ability to utilise higher irradiance levels. Other photosynthetic parameters did not demonstrate clear patterns with either water or tissue N, or N source, although there was a general trend towards better photosynthetic performance at high N levels in all measures. This suggests that additional tissue N was incorporated into photosynthetic pigments, and, over longer-term growth, these plants are likely to perform better than those with lower tissue N.

N uptake rates of *E. radiata* were linear over the range tested, and we determined affinity from the linear model. A lack of evidence of saturation suggests samples used in the uptake experiment were N limited and hence were performing surge uptake (Harrison and Hurd, 2001; Smit, 2002). The average tissue N of these specimens was 1.08 % DW, while sporophytes grown with added N accumulated tissue N content up to > 1.5 % DW. The tissue N content that reflects N limitation in *E. radiata* could not be derived from our data due to a lack of a clear relationship between tissue N and growth, but this critical N level, which is species specific, is typically in the range 0.7 – 3.2 % DW (Harrison and Hurd, 2001). The calculated affinity is useful for characterising N uptake at low concentrations, including in the range likely to be experienced in the vicinity of SA fish farms (Middleton, et al., 2013; Tanner and Volkman, 2009). The affinity calculated for *E. radiata* in our experiments (0.50) did not vary between N sources, and is similar to that of other Laminariales, e.g. *Laminaria abyssalis* demonstrated affinity of 0.36 for nitrate and 0.43 for ammonium (Braga and Yoneshigue-Valentine, 1996), first-year class *Laminaria groenlandica* has affinity 0.32 for nitrate and 0.35

for ammonium (Harrison, et al., 1986), and *Ecklonia cava* has affinity of 0.38 for ammonium (Kang, et al., 2013). The range of uptake rates we observed for *E. radiata* is also within that observed for other Laminariales (Ahn, et al., 1998; Braga and Yoneshigue-Valentine, 1996; Harrison, et al., 1986; Kang, et al., 2013), and a range of other seaweeds that may be applied for IMTA (Kang, et al., 2013).

Hatchery production and string seeding are feasible for *E. radiata*. In combination with production feasibility, the ability of *E. radiata* to accumulate available N in tissue biomass, and comparable N affinity and uptake rates to other cultivated brown seaweeds, demonstrates that this species is suitable for aquaculture and nutrient remediation. Future research should focus on upscaling and further refining gametophyte and hatchery production and seeding methods to generate seedstock for field grow out experiments.

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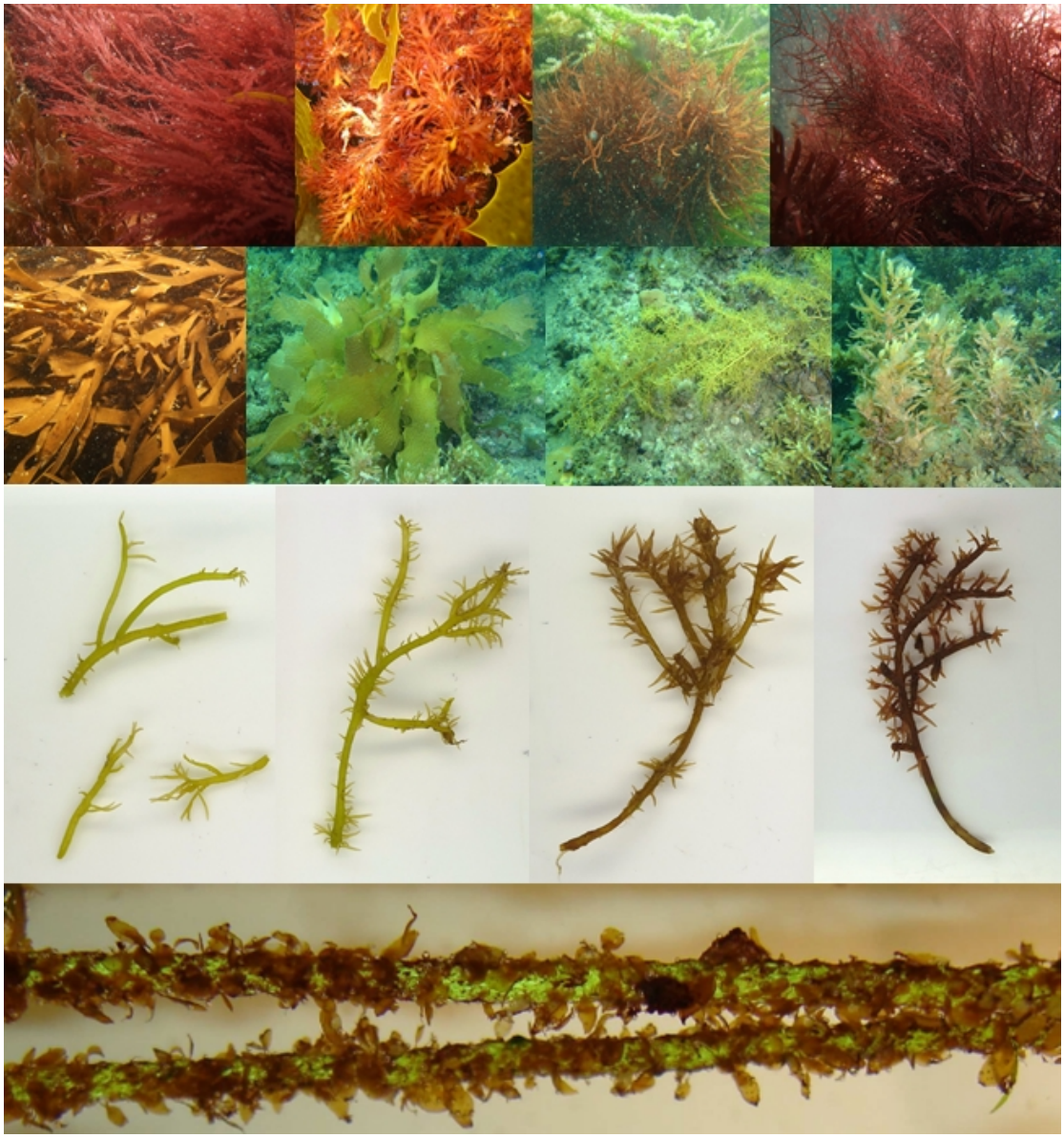
Supplementary material

Table S1. Comparison of AIC for linear and generalized additive models (GAM) of *Ecklonia radiata* growth using water or plant tissue nitrogen (N) and N source (nitrate or ammonium) as predictors. GAM smoothers denoted as s(term). *Model selected by AIC

Model	**AIC**
Linear	
N source x Tissue N	49.4
N source + Tissue N	47.5
N source x Water N	48.2
N source + Water N	46.3
N source + Water N + Tissue N	46.1
GAM	
s(Tissue N) + s(N source)	36.2*
s(Water N) + s(N source)	49.4
s(Water N) + s(Tissue N) + s(N source)	37.2

Chapter 6. General discussion



Top: The eight candidate species reds: *Plocamium angustum, Pterocladia lucida, Solieria robusta, Gelidium australe*; browns: *Scytothalia dorycarpa, Ecklonia radiata, Cystophora subfarcinata, Sargassum linearifolium.* Middle: *Solieria robusta* specimens grown under different light and ammonium-N addition levels (see Chapter 4). Bottom: *Ecklonia radiata* sporophytes seeded onto string (see Chapter 5).

1 Overview

Growing demand for seaweed products and the potential application of seaweeds for nutrient mitigation are driving interest in the development of seaweed farming in Australia (Lee, 2010; Lorbeer, et al., 2013; Roos, et al., 2018), including in integrated multi-trophic aquaculture (IMTA) systems. My research into eight candidate native Australian seaweeds for offshore IMTA has identified the red seaweed *Solieria robusta* (Solieriaceae, Gigartinales, Rhodophyta) and the brown seaweed *Ecklonia radiata* (Lessoniaceae, Laminariales, Phaeophyceae) as the most suitable species of those investigated. My research also provides information on methods for cultivation and seed stock production of these two species, and data on their nitrogen (N) responses that will assist in incorporating N removal by seaweeds into biogeochemical models to optimise IMTA applications.

2 Identifying native Australian seaweeds for aquaculture

A literature review, liaison with potential end users and international researchers, and initial surveys and field collections, identified eight candidate seaweeds for aquaculture in South Australia (SA) (Chapter 1). These eight species comprised four brown seaweeds: *Ecklonia radiata*, *Cystophora subfarcinata, Sargassum linearifolium,* and *Scytothalia dorycarpa*; and four red seaweeds: *Gelidium australe, Pterocladia lucida*, *Solieria robusta,* and *Plocamium angustum*.

The suitability for cultivation of these eight candidate species was investigated in an initial field trial, off Adelaide, SA (Chapter 2). Results from this trial were considered in combination with laboratory investigations of reproduction in brown seaweeds (Chapter 2) and growth and N removal performance of red seaweeds under simulated fish farm conditions (Chapter

4). Species distribution modelling (SDM) was applied to assess the relative environmental suitability of existing aquaculture zones in SA for each species (Chapter 3).

The initial field trial and initial laboratory investigations identified two red seaweeds that showed promising growth in the field (*G. australe*) or laboratory (*So. robusta*), and two brown seaweeds that showed potential for cultivation in the field and for reproduction in the laboratory (*E. radiata* and *C. subfarcinata*). These four species were then used in an on-farm field trial on a Yellowtail Kingfish farm near Port Lincoln, SA (Chapter 2). Temperature responses of *G. australe* and *So. robusta* were also investigated in a laboratory experiment to determine their relative growth performance over a temperature range relevant to Spencer Gulf (Chapter 4).

Aside from limited experimental cultivation of *E. radiata* in New Zealand (Neill, et al., 2009), the initial field trial reflects the first attempt at offshore cultivation of any of the eight candidate species, and the first trial of at-sea seaweed cultivation in southern Australia. The fish farm field trial was the first cultivation of seaweed in Australia in proximity to fish aquaculture. Limitations on available locations for these trials and of seaweed biomass meant that I could test only a few of the parameters likely to affect seaweed performance (e.g. depth, cultivation periods, distance to fish cages, planting density).

Given the lack of prior knowledge of suitable cultivation methods or conditions for any of the assessed species, it is likely that environmental conditions were sub-optimal, especially given the marine heatwave that was experienced over the summer period of the initial field trial (Bureau of Meteorology, 2014; Roberts, et al., 2019). The trials used seaweed material collected from the wild, with no pre-selection of specimens for desirable characteristics or

assessment of nutritional status. The results therefore demonstrate the relative feasibility for cultivation of each species, and provide some information on seasonality of growth, but do not reflect the performance that would be obtained with an optimised cultivation system.

The field trials were impacted by herbivory and fouling, which are recognised problems in seaweed aquaculture (Titlyanov and Titlyanova, 2010; Troell, et al., 2009), but manipulation of stocking density (Ask and Azanza, 2002; Titlyanov and Titlyanova, 2010), strategic timing of out-planting (Troell, et al., 2009), selection of appropriate farm sites (Abreu, et al., 2009; Ask and Azanza, 2002; Neill, et al., 2009) and farm management practices (Ask and Azanza, 2002; Troell, et al., 2009) can alleviate these issues. The initial field trial demonstrated that cultivation technology adapted from other farmed species is likely to be suitable, but better performance may be obtained through site selection, improving farming systems, identification and production of strains with desirable characteristics, and refinement of hatchery grow-out to enhance seedling survival and growth.

Investigations of suitable sites, out-planting times and planting density for seaweed cultivation are therefore important, but, to permit these investigations, sufficient seaweed biomass is required. Production of seed stock will be a critical step in developing aquaculture of Australian native seaweeds (Roos, et al., 2018), hence much of my research focused on propagation, particularly for the brown seaweeds, which typically do not regrow from cuttings. The ability to reliably reproduce the brown seaweeds was an important consideration in the selection of species for further research, especially given minor differences between the brown seaweeds in the initial field trial, with the exception of *Sc. dorycarpa*, which performed poorly (Chapter 2).

The SDM results also showed that at least some southern Spencer Gulf aquaculture zones were likely to be suitable for each of the brown seaweeds, although with limited areas suitable for *Sc. dorycarpa* (Chapter 3). Of the other three brown seaweeds, reproduction was more feasible for *E. radiata* and *C. subfarcinata* than *Sa. linearifolium*. The highly seasonal growth cycle in *Sa. linearifolium*, which, in common with many *Sargassum* spp., sheds spent reproductive branches in summer (Womersley, 1987), would also limit the potential cultivation period for this species.

Ecklonia radiata demonstrated better growth than *C. subfarcinata* over July-August in the initial field trial, but there was no clear difference between these species in the fish farm trial (Chapter 2). Seeding onto string was, however, only successfully achieved for *E. radiata* (Chapter 5); zygotes of *C. subfarcinata* that settled onto string did not develop (Wiltshire, et al., 2015). Selection of *E. radiata* as the best candidate brown seaweed was also supported by growing interest in commercialisation of this species due to its suitability as food and as a source of bioproducts (Charoensiddhi, et al., 2015; Charoensiddhi, et al., 2017; Lorbeer, et al., 2013; Skrzypczyk, et al., 2018; Winberg, et al., 2011). The field and SDM results suggest that *C. subfarcinata* and *Sa. linearifolium* may also be feasible to cultivate in southern Spencer Gulf; if these species are to be farmed, however, further research is needed to develop methods for their reproduction and string seeding.

For the red seaweeds, the ability to regrow from cuttings was demonstrated by all species in the initial field trial (Chapter 2) and the initial laboratory experiment comparing the four red species with nutrient added to simulate fish farm conditions (Chapter 4). There were, however, clear differences in growth rates between species, with *Pt. lucida* and *Pl. angustum* consistently having lower growth (< 2 % d^{-1}) than *So. robusta,* which grew best in the

laboratory with specific growth rate (SGR) > 5 % d^{-1}, and *G. australe*, which grew best in the initial field trial with SGR > 3 % d^{-1}. The fish farm field trial suggested greater potential for high growth rates in *So. robusta* than *G. australe* (Chapter 2), and in the laboratory investigation of temperature responses of these species, *So. robusta* grew better than *G. australe* at temperatures > 14 °C, and showed greater tolerance for temperatures > 20 °C (Chapter 4).

The SDM results (Chapter 3) showed that *So. robusta* had the highest relative habitat suitability of all eight species throughout Spencer Gulf, and in particular was the most suitable species of all the candidate seaweeds for northern Spencer Gulf, where warmer temperatures occur than in the southern gulf (Nunes and Lennon, 1986; Petrusevics, 1993). SDM results demonstrated generally low environmental suitability of Spencer Gulf aquaculture zones for *Pt. lucida* and *Pl. angustum*, while some parts of southern Spencer Gulf showed potential suitability for *G. australe.* In combination, these results show that *So. robusta* is likely to be more suitable for cultivation that *G. australe* over a large part of Spencer Gulf, while *Pt. lucida* and *Pl. angustum* are less suitable for cultivation than the other red species.

Solieria robusta has more potential commercial uses than *G. australe;* while *G. australe* is a known agar producer (Gordon-Mills, et al., 1990), the agar yield of Gelidiales is usually too low for aquaculture to be commercially viable at their typical growth rates of ~ 3 – 7 % d^{-1} (Friedlander, 2008). *Solieria robusta* produces carrageenan (Chiovitti, et al., 1999), with yield comparable to commercially farmed Solieriaceae, and other bioproducts that may be suitable for high value applications (Ara, et al., 2002; Khanzada, et al., 2007), and is additionally an edible species (Novaczek, 2001; Tito and Liao, 2000).

My research demonstrated that *E. radiata* and *So. robusta* are the best candidate species of those investigated for aquaculture, but this does not exclude other seaweeds as being suitable. The focus of my research was identifying and investigating species that may be applied to IMTA with existing fish aquaculture in SA, hence, only species with native ranges including southern Spencer Gulf and with expected suitability for offshore cultivation were considered. *Ecklonia radiata* and *So. robusta* are both widely distributed around southern Australia and are likely to be suitable for cultivation in other areas, not just Spencer Gulf. Species that occur in other regions, however, may also be suitable for cultivation within their natural ranges. For example, canopy forming brown seaweeds that occur around south-eastern Australia, e.g. giant kelp *Macrocystis pyrifera* (Laminariales) and bull kelp *Durvillea* spp. (Fucales), may be good candidates for cultivation in that region. Aquaculture of *Macrocystis pyrifera* is being developed in Chile, where this species is commercially harvested (Camus, et al., 2018).

Species that occur in Spencer Gulf but which are generally restricted to sheltered conditions were also not considered as suitable candidates for IMTA on SA fish farms due to the relatively exposed location of these farms, but some of these species may be suitable for inshore or land-based cultivation, including in IMTA. For example, *Ulva* spp. (Ulvales, Chlorophyta) and *Porphyra* spp. (Bangiales, Rhodophyta) have good potential for cultivation and nutrient removal in land-based recirculating systems (Winberg, et al., 2011). Additionally, with the focus of my research being suitable species for nutrient mitigation, ease of cultivation and N removal ability of the candidate seaweeds were primary concerns, with commercial value being a secondary consideration. A species identified as having high value or potential for

commercialisation may warrant additional effort to develop methods for its cultivation, even if it is, at least initially, more difficult to cultivate than the species identified here.

3 Investigating novel seaweeds for aquaculture

My research highlights both some of the barriers that will be encountered and important considerations in developing novel species for aquaculture. Legislation in Australia is unlikely to allow the expansion of wild harvesting activities (Roos, et al., 2018), hence it is crucial to develop methods for seedstock production. Field trials are needed to test and refine cultivation, but should be large-scale to most accurately assess performance (Troell, et al., 2009). Such trials require availability of sufficient biomass, and are logistically challenging. Other research can be used to inform suitable sites or conditions for cultivation, allowing design of appropriate field trials to validate findings, and minimising effort spent on investigation of areas or conditions that are less likely to be suitable. Upscaling seed stock production can assist by providing biomass for field trials, reducing the need for wild collection, and, in the longer term, facilitating mass production of strains with desirable characteristics.

3.1 Seed stock production

Farming of Laminariales typically involves seeding spores onto string or rope, with gametophytes developing on the string, becoming fertile and reproducing, and sporophytes then developing on the same string substrate (Flavin, et al., 2013; Sahoo and Yarish, 2005; Titlyanov and Titlyanova, 2010). Gametophyte cultivation prior to seeding can provide seedstock year-round and facilitate strain selection (Edwards and Watson, 2011; Flavin, et al., 2013; Li, et al., 1999; Redmond, et al., 2014; Sahoo and Yarish, 2005).

My research demonstrated that spores could be obtained from *E. radiata* (Chapter 2) and seeded directly onto string, and that vegetative gametophyte cultivation is also feasible (Chapter 5). I determined suitable media, light and temperature conditions for vegetative gametophyte cultivation of *E. radiata*: nutrient addition of either full or half strength Provasoli enrichment solution (PES) was beneficial, and addition of germanium dioxide, a diatom inhibitor, was tolerated.

The illumination range suitable for *E. radiata* gametophyte cultivation was similar to that of other Laminariales, but *E. radiata* gametophytes performed best at temperatures warmer than are suitable for other Laminariales. This result was not surprising given that the native range of *E. radiata* is in warmer climates than its farmed relatives. I found that light and temperature levels suitable for vegetative gametophyte cultivation were similar to the optimal conditions for natural gametophyte development in this species. This demonstrates that while methods for related farmed species may be applied to Australian seaweeds, consideration should be given to the natural conditions in which each species occurs, because optimal conditions for cultivation are likely to reflect local adaptation. For a species with a wide latitudinal range, such as *E. radiata*, physiological optima may vary between material sourced from different areas (Mabin, et al., 2013; Staehr and Wernberg, 2009).

I found that addition of N in hatchery cultivation promoted faster growth of *E. radiata* sporophytes (Chapter 5). Effects of hatchery fertilisation on subsequent success of out-planted *E. radiata* sporophytes, however, has not yet been assessed. The ideal substrate for *E. radiata* seeding is also not established. Polyethylene, polypropylene or nylon appear suitable; these are the main string types used in aquaculture currently, but other substrates such as polyvinyl alcohol that may be suitable have not been tested. Seeding methods may

also be improved by applying vegetative gametophyte cultivation followed by spray seeding (Edwards and Watson, 2011), or pre-treating string to improve bioadhesion (Kerrison, et al., 2019).

In contrast to brown seaweeds, many red seaweeds can be grown from cuttings. Micropropagation methods are being developed for important farmed species to facilitate mass production of seedlings with desirable traits (Reddy, et al., 2008; Yong, et al., 2014).

Solieria robusta grew from cuttings in the field using methods adapted from other Solieriaceae (Chapter 2), and cuttings displayed promising growth rates in laboratory experiments (Chapter 4). Micropropagation via explant production was also feasible for *So. robusta*, and could be used to generate seedstock; tip explants grown in full-strength PES performed best overall (Chapter 4). I grew explants under temperature and light conditions that were within the optimal range for growth of this species from other laboratory work (Chapter 4), but additional investigation could further improve methods for explant production. For example, the addition of plant growth regulators can be beneficial for micropropagation of red seaweeds, including Solieriaceae (Hurtado, et al., 2009; Yokoya and Handro, 2002; Yunque, et al., 2011), and explant performance is also affected by pH, carbon supply and culture density (Baweja, et al., 2009; Yunque, et al., 2011).

3.2 Determining suitable sites and conditions for cultivation

For existing farmed or other well-studied species, knowledge of suitable environmental conditions for growth can be combined with spatial data to identify potential sites for aquaculture (e.g. Falconer, et al., 2016; Radiarta, et al., 2011; Silva, et al., 2011; Snyder, et al., 2017; Zhang, et al., 2017). Light and temperature responses of *E. radiata* have been studied (Bearham, et al., 2013; Mabin, et al., 2013; Staehr and Wernberg, 2009), but not in an

aquaculture context, and relevant data were lacking for the other candidate species. I applied two approaches to assist in identifying the best potential aquaculture sites and conditions for the species under consideration: correlative SDM to determine relative environmental suitability of existing Spencer Gulf aquaculture zones for each species (Chapter 3); and laboratory investigations of light, temperature and nutrient responses for selected red species (Chapter 4).

While SDM is an established method of predicting species occurrence and habitat suitability for a range of purposes (Elith and Leathwick, 2009; Robinson, et al., 2011), it has only relatively recently been applied to aquaculture site selection (e.g. Falconer, et al., 2016; Linhoss, et al., 2016). Methods for SDM are evolving, and there is growing recognition that default modelling methods are not always the most appropriate, especially for applications were model transferability and interpretability are important (Halvorsen, et al., 2015; Radosavljevic and Anderson, 2014; Syfert, et al., 2013; Warren and Seifert, 2011), such as aquaculture site selection. I found that forward selection under the maximum likelihood interpretation of the maximum entropy (maxent) SDM method (Halvorsen, 2013; Halvorsen, et al., 2015; Mazzoni, 2016) produced the most parsimonious models, and that these models retained high predictive performance as assessed by a range of metrics (Chapter 3). These models help to inform which zones may be most suitable for seaweed aquaculture, and assist in guiding future research and industry development.

Aquaculture zones in northern Spencer Gulf appear generally unsuitable for cultivation of the candidate brown seaweeds, but showed good suitability for *So. robusta.* In southern Spencer Gulf, several aquaculture zones are likely to be suitable for cultivation of brown seaweeds, especially the zones between Port Lincoln and Port Neill in the south-western gulf, and these

zones are also probably suitable for *So. robusta* cultivation (Chapter 3). Predictions could also be generated for areas other than Spencer Gulf to identify additional regions that may be suitable for each species. Response curves generated by SDM can indicate suitable environmental conditions, and apparent optima, for each species (Marcelino and Verbruggen, 2015).

Temperature was an important predictor of suitability for most species in my models, and temperature is likely to be an important driver of seaweed occurrence and performance across latitudinal gradients (Bearham, et al., 2013; Mabin, et al., 2013; Martínez, et al., 2018; Yesson, et al., 2015). SDM illustrates the average annual temperature range suitable for long term occurrence of the modelled species, but does not provide information on seasonal growth patterns. Spring is expected to be the best season for cultivation of temperate seaweeds (Titlyanov and Titlyanova, 2010), and best growth of the candidate seaweeds was achieved in spring during both the initial and fish farm trials (Chapter 2). Native populations of *E. radiata* also show best growth in spring, with higher temperatures (>21 °C) leading to reduced growth of this species in summer, and low light availability restricting growth at other times (Bearham, et al., 2013).

I obtained further data on temperature responses of *So. robusta* and *G. australe* in the laboratory experiment (Chapter 4) that assessed growth of both species across the range of temperatures (12 – 25 °C) likely to be experienced seasonally within Spencer Gulf (Nunes and Lennon, 1986; Petrusevics, 1993). The generalized additive model (GAM) fit to data from this experiment could be applied to predict seasonal growth responses to temperature for *So. robusta* (or *G. australe*) to assist in identifying suitable locations and cultivation periods, although it should be noted that light availability is also likely to influence seasonal

performance. For *So. robusta*, temperatures are likely to be suitable for cultivation throughout Spencer Gulf from spring through summer and autumn.

Light (measured as photosynthetically active radiation, PAR) responses of *So. robusta* were investigated in a separate laboratory experiment that also assessed growth and photosynthetic performance of this species with two levels of added ammonium-N (Chapter 4). Growth, photosynthetic performance and tissue N content were all enhanced at the higher level of ammonium addition. *Solieria robusta* growth did not display clear differences with light, but photosynthetic performance was reduced under the two higher intensities tested, suggesting that best longer-term growth is likely to be achieved at PAR < 250 µE m^{-2} s^{-1}.

In Spencer Gulf, light availability in winter is approximately a quarter of that in summer (Tanner and Volkman, 2009). The depth of suspended cultivation systems could be varied seasonally to achieve a suitable PAR throughout the year. The optimum depth for *So. robusta* cultivation in southern Spencer Gulf is likely to be around 3 m water depth in spring/autumn, 5 – 6 m in summer and 2 m in winter. Further research is required, however, to assess whether light and temperature have interactive effects in the field.

4 Applying seaweeds to integrated multi-trophic aquaculture

In IMTA systems, seaweeds are used to remove and store nutrients (Buchholz, et al., 2012; Chopin, et al., 2001; Neori, 2008). Seaweeds incorporate nutrients into biomass as they grow, and many also accumulate additional nutrients in photosynthetic pigments or internal nutrient pools (Buschmann, et al., 2008; Carmona, et al., 2006; Corey, et al., 2013; Kang, et al., 2013; Ribeiro, et al., 2012; Zhou, et al., 2006). The most effective species for IMTA have relatively high growth rates, but also an ability to accumulate additional available nutrients (Buschmann, et al., 2008; Chopin, et al., 2001; Kang, et al., 2013; Ribeiro, et al., 2012). For

seaweeds used in recirculating or effluent treatment systems, high uptake rates and removal efficiency are also important (Carmona, et al., 2006; Chopin, et al., 2001; Corey, et al., 2013; Kang, et al., 2013). Uptake rates also influence the efficacy of seaweeds at intercepting and removing nutrients over finer spatial and temporal scales in at-sea IMTA (Chopin, et al., 2001; Kang, et al., 2013; Neori, et al., 2004). Knowledge of uptake rates is also required to parameterise dynamic biogeochemical models of at-sea IMTA (e.g. Broch, et al., 2013; Hadley, et al., 2015). These models assist in determining the best seasons and locations for seaweed aquaculture to optimise nutrient removal, and in assessing the effects of farmed seaweed on nutrient levels.

Dissolved N is usually the primary nutrient of concern in IMTA applications (Kang, et al., 2013), and this is the case in SA, where dissolved N is the limiting factor for environmentally sustainable expansion of fish aquaculture (Middleton, et al., 2013; Tanner, et al., 2007). I investigated the ability of the candidate seaweeds to sequester and store N during the field trials (Chapter 2) and in laboratory experiments of the red species (Chapter 4) and of *E. radiata* (Chapter 5). I also assessed N uptake rates of *So. robusta* and *E. radiata.*

I found that all the red seaweeds tested could accumulate tissue N when supplied with additional nutrient (Chapter 4). *Solieria robusta* had the lowest N content of the tested red species, but despite this, would be able to remove the most N over time due to its faster growth. Light intensity also affected tissue N of *So. robusta*, with specimens growing at lower PAR accumulating more N, probably due to incorporation into photosynthetic pigments (Chapter 4).

The tissue N of seaweeds indicates their nutritional history (Fong, et al., 1994). Seasonal differences in tissue N content were observed in the initial field trial (Chapter 2), likely reflecting variation in N availability throughout the year, although, as per the laboratory experiment, N content was typically higher for *G. australe* than *So. robusta.* Tissue N of the red species was also affected by cultivation method in the initial field trial, potentially due to differences in light availability between specimens tied to rope or contained within bags. Tissue N was only assessed for one set of *So. robusta* specimens from the fish farm trial, and was found to be relatively low in comparison to the tissue N of this species in other experiments. Minimal fish stocking of adjacent cages at the time of the trial may have resulted in nutrient limitation (Chapter 2).

The brown seaweeds *E. radiata* and *C. subfarcinata* demonstrated variation in tissue N over the initial field trial, but without one species having consistently higher N content (Chapter 2). When grown in the laboratory with added nutrient, *E. radiata* was able to accumulate additional tissue N (Chapter 5). Nitrate promoted better growth than ammonium for *E. radiata* when applied at higher concentrations, with ammonium toxicity possibly occurring at the higher applied concentrations. These concentrations were higher than those expected to occur during field cultivation.

Uptake rates of *So. robusta* (Chapter 4) and *E. radiata* (Chapter 5) increased linearly with N concentration over the ranges tested without showing evidence of saturation. Uptake kinetics could therefore not be fully determined, but substrate affinity, which demonstrates ability to take up nutrient at low concentrations, could be calculated. Given that the expected N concentration in the vicinity of SA fish farms is ≤ 12 µM, the data obtained allow modelling of uptake by either species in the range of N concentrations relevant for understanding the

effects of IMTA on environmental nutrient loads in SA. Affinity and uptake rates of both *So. robusta* and *E. radiata* did not vary between ammonium and nitrate N sources and compared favourably to those of other seaweeds applied to IMTA.

IMTA systems offer economic benefits to farmers in addition to improving environmental sustainability (Handå, et al., 2012; Petrell and Alie, 1996; Sanderson, et al., 2012; Sarà, et al., 2009; Troell, et al., 2003; Whitmarsh, et al., 2006), but farmers may be concerned that co-cultivation will enhance or facilitate disease or parasite transmission between species (Skar and Mortensen, 2007; Troell, et al., 2003). To address concerns about co-cultivation of seaweed and fish, I examined whether seaweed cultivation infrastructure would retain eggs of fish parasites (flukes) that are commercially relevant for aquaculture of yellowtail kingfish as part of the fish farm trial (Chapter 2).

Fluke eggs were found on seaweed infrastructure, with a greater frequency of occurrence on infrastructure located in-line with prevailing currents than offset, but the numbers of eggs recorded were very low. The co-location of seaweed and fish farming is therefore unlikely to impact fluke management on kingfish farms. Any potential impacts could be minimised by avoiding having seaweed infrastructure in-line with prevailing current, but the specific arrangement of seaweeds around fish cages should also consider seaweed performance and aim to maximise nutrient removal and seaweed growth while maintaining an acceptably low biosecurity risk.

5 Future research directions

My research has laid the foundation for developing offshore aquaculture of the seaweeds *So. robusta* and *E. radiata*, and for incorporating seaweeds into IMTA systems. The next steps

towards developing seaweed aquaculture and/or implementing IMTA in southern Australia would be:

1. Scaling up and improving hatchery production to produce the required seed stock for large-scale field trials and farming. My research has demonstrated suitable methods for hatchery production that would be feasible to apply at a larger scale, but suitable facilities for production would need to be established. Further refinement of these methods is likely to improve productivity. The success of out-planted hacthery-grown material also needs to be assessed, and should be an important consideration in refining hatchery methods. In the longer term, hatchery production can facilitate strain selection to improve growth and product quality of farmed seaweeds.

2. Identifying the most suitable locations for seaweed aquaculture. My research provides data on the environmental conditions and areas that are suitable for each species: from SDM results in both cases, supplemented with laboratory data from *So. robusta.* Exisiting data on *E. radiata* should also be considered in conjunction with my results for this species. Information on environmental suitability for seaweed growth should be combined with finer-scale environmental data to assist in identifying the most suitable potential sites. Site selection will also need to consider other aspects that are important for aquaculture, including logistics, spatial management, and potential impacts of farming on the environment. For IMTA applications, biogeochemical modelling that incorporates N removal by seaweeds will also assist in determining where seaweed aquaculture will be most effective at mitigating nutrient inputs.

3. Large-scale field trials. Once suitable sites have been identified and sufficient biomass can be produced, large-scale field trials should be used to validate seaweed performance in offshore aquaculture, and refine cultivation systems. Aspects to validate include depths for outplanting, and, for IMTA, the specific arrangement of seaweeds in relation to fish cages or farms. Additional important factors to assess will be: planting density, timing of out-planting and harvesting, effects of hatchery conditions on seaweed performance, and the ideal string or rope substrate for *E. radiata.* To develop aquaculture of *So. robusta*, cultivation systems that permit automation of planting and harvesting, e.g. based on the tube-net system of Góes and Reis (2011), need to be investigated, because systems requiring manual labour will not be commercially viable in Australia. Any identified concerns about seaweed aquaculture impacting health or biosecurity of fish or other farmed species in IMTA should also be investigated.

4. Commercialisation of seaweed products. Both *E. radiata* and *So. robusta* are edible, and produce compounds for which there are existing markets (e.g. hydrocolloids) and bioactives with the potential for high value applications. Further research on the yield, quality and biological activities of these seaweed products will assist in commercialising these species and in identifying new potential uses and markets. Where seaweeds are applied in IMTA, the value of nutrient remediation should also be recognised. The value of seaweed production, including seaweed products and nutrient removal, should be quantified for inclusion in viability assessments and business planning. The primary application for each species should be considered in refining cultivation systems, since product yield and quality may not be optimised by

the same conditions that maximise seaweed growth. Strain selection is a longer-term goal, but should also consider product yield and quality in addition to seaweed growth.

6 Overall conclusions

The native seaweeds *So. robusta* and *E. radiata* were identified as being the most suitable of the eight species investigated for aquaculture in Spencer Gulf. Both species are feasible to propagate and cultivate using methods adapted from farmed relatives (Solieriaceae and Laminariales, respectively). Vegetative gametophyte cultivation can be applied to *E. radiata*, and micropropgation via explant production is viable for *So. robusta*. These methods provide the foundation for scaling up seed stock production of either species, minimising the requirement to harvest wild biomass for seed stock production, and facilitating strain selection.

Both *So. robusta* and *E. radiata* can accumulate tissue N when surplus nutrient is available, and both species demonstrate N uptake rates and affinities that are suitable for IMTA. Data generated from laboratory experiments and by SDM will assist in identifying the most suitable sites and conditions for cultivation of these seaweeds, and in guiding further research. Growth of *So. robusta* and *E. radiata* in field cultivation was demonstrated, but further larger-scale field trials will be needed to assess seaweed performance in potential farm sites and to refine cultivation systems.

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