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Selection of temperate *Ulva* species and cultivars for land-based cultivation and biomass applications

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ABSTRACT

Green seaweeds from the cosmopolitan genus *Ulva* are targets for land-based aquaculture and a diverse range of biomass applications, but are not currently cultivated in Aotearoa New Zealand. Therefore, the objective of this study was to identify target species and cultivars of *Ulva* as a first step towards establishing land-based cultivation of seaweed in Aotearoa New Zealand. We isolated 24 cultivars of *Ulva* from natural populations in the Bay of Plenty region of New Zealand. We compared growth and biomass productivities of 18 of these cultivars, either in their original collection morphology (e.g., blade/filamentous) and/or in cluster morphology where possible as a result of induced formation of free-floating germling clusters. Specific growth rates and biomass productivities of multiple cultivars in small-scale laboratory cultures were high (>20% day^{-1} and >8 g dry weight (DW) m^{-2} day^{-1} respectively), with biomass increases of 5 to 8-fold per week in the fastest growing cultivars. However, there was significant variation in growth and biomass productivity among cultivars of each morphology type. Biomass productivities were highest for cultivars WB2 (blade, 7.5 g DW m^{-2} day^{-1}), SW9 (blade cluster, 9.4 g DW m^{-2} day^{-1}), SW8 (filamentous, 7.8 g DW m^{-2} day^{-1}), and SW6 (filamentous cluster, 9.8 g DW m^{-2} day^{-1}). Growth rates and biomass productivities were consistently higher for cluster compared to non-cluster morphologies for each morphology type (e.g. filamentous or blade), demonstrating that clusters are a viable option to enable free-floating cultivation of filamentous species of *Ulva*. These results confirm the suitability of *Ulva* as a target for intensive land-based aquaculture in Aotearoa New Zealand. The significant inter-cultivar variation found in the current study further highlights the importance of sampling widely and focusing on cultivar rather than species selection when identifying targets for cultivation.

1. Introduction

Green seaweeds from the cosmopolitan genus *Ulva* are targets for a diverse range of applications. There is a long history of the consumption of *Ulva* as a high value human food product, for example as “aonori” in Japan [1], or in soup and salad preparations in Europe [2]. *Ulva* is commercially cultivated as an aquaculture feed product for abalone in South Africa [3] and as food grade production in Europe [4,5]. The suitability of *Ulva* as a feed supplement for a wide range of other animals including shrimp [6,7], fish [8–10], broiler chickens [11], and livestock [12,13] has also been demonstrated. *Ulva* has been targeted for agricultural products such as liquid extracts [14] and compost [15]. *Ulva* is also suitable as a feedstock for bioenergy applications [16–18] and nutraceuticals [19]. *Ulva* can be cultivated in waste water from aquaculture or other industries to bioremediate excess nutrients [20–22]. There is also increasing interest in developing *Ulva* biorefineries for the integrated production of multiple products [23–27].

The use of *Ulva* for any of these applications requires the selection of appropriate target species to provide a consistent and reliable source of biomass. Several criteria should be considered when selecting target species. It is essential for target species to have high areal biomass productivities as most applications require large amounts of biomass [28,29]. Target species should occur locally to minimise any biosecurity concerns associated with the use of non-endemic species [29,30]. A broad geographical distribution is also desirable to enable the use of the same target species in multiple locations. Finally, the chemical

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composition of the biomass is important as this can determine suitability for end-product applications.

Although numerous studies have assessed the suitability of various species of *Ulva* for a range of biomass applications, most have only tested a single species using cultures established from biomass opportunistically collected from local sites (e.g., [12,14,16,20,22]). However there is considerable variation among species in growth rates [29,31,32], metabolic characteristics [33] and chemical composition [19]. For example, specific growth rates of four species of *Ulva* in a laboratory study ranged from 3 to 29% day^{-1} [29], and proportions of the monosaccharide rhamnose in sulfated polysaccharide ulvan from 20 species of *Ulva* range from 5 to 92 mol% [19]. Furthermore, growth rates can also vary substantially between cultivars of *Ulva* from the same species [29,31,33]. These findings highlight the importance of comparing multiple species and cultivars of *Ulva* when selecting targets for biomass applications.

Commercial cultivation of seaweed in Aotearoa New Zealand does not currently occur [34]. However, land-based aquaculture and seaweed cultivation have been identified as target areas to help achieve ambitious goals for growing the aquaculture sector in this country [35]. Therefore, as a first step towards establishing land-based cultivation of seaweed in Aotearoa New Zealand, the objective of this study was to identify target species and cultivars of *Ulva* for land-based cultivation and biomass applications. Multiple species of seaweed may be suitable for land-based cultivation. However we focused on *Ulva* due to its broad distribution and common occurrence in coastal habitats in New Zealand [36], the established ability of this genus to maintain continuous high productivities over long timeframes in land-based cultivation systems [37,38], and the suitability of *Ulva* biomass for a broad range of applications [3]. We focused on the Bay of Plenty region of New Zealand as aquaculture is a key focus of the region's economic development strategy [39]. The specific aims of the study were to 1) survey coastal environments across the Bay of Plenty to determine which species of *Ulva* occur in this region; 2) determine which species and cultivars of *Ulva* can be maintained in free floating cultures; 3) quantify the growth and productivity of these cultivars; and 4) analyse the chemical composition of potential target cultivars of *Ulva* to determine their suitability for biomass applications.

2. Methods

2.1. Sample collection

Twenty-four samples of *Ulva* with blade or filamentous morphologies were collected from intertidal environments across the Bay of Plenty region in Aotearoa New Zealand between November 2018 and March 2019 (Table 1) under Ministry for Primary Industries University of Waikato Special Permit 560. The amount of material collected for each sample varied. A single individual blade was collected for some samples, whereas other samples comprised multiple small individuals of either blade or filamentous morphologies. Samples were transported in water taken at the collection site back to the University of Waikato Coastal Marine Field Station, Tauranga, Aotearoa New Zealand. Each sample was gently brushed to remove any epiphytes and then placed in an individual plastic bucket filled with nutrient enriched filtered seawater (Cell-Hi F2P, Varicon Aqua Solutions UK, 0.1 g L^{-1}, 12.3 mg nitrate-N L^{-1} and 1.1 mg P L^{-1}) in a temperature and light controlled laboratory (12:12 light:dark cycle, 160 μmol photons m^{-2} s^{-1}, 18 °C). Culture medium was replaced once per week. Buckets were provided with aeration by a continuous stream of air entering through multiple inlets around the base of the buckets. Free floating stock cultures (tumble cultures) of each sample were established by scaling up the original biomass that was collected. Stock cultures were maintained under these conditions for at least three months prior to the start of experiments to allow acclimation to free-floating tumble culture and ensure that all algae were pre-exposed to identical conditions. In some cultivars we were able to induce the formation of free-floating germling clusters (hereafter referred to as clusters, Fig. 1), following the methods of Hiraoka & Oka [40]. These have been promoted as a way of enabling continuous, stable production of biomass, particularly of species with filamentous morphologies, in high density tank cultures [40]. It was possible to induce formation of clusters in seven of the nine cultivars with blade morphologies and 8 of the 10 cultivars with filamentous morphologies. Where possible, stock cultures of cluster morphologies were established for each cultivar and maintained under the same conditions as described above.

Table 1
List of *Ulva* species sampled, cultivar ID, Genbank accession number, collection date and location, and the morphology of samples at the time of collection. Morphology tested specifies the morphology (blade, filament, blade cluster or filament cluster) that was tested for each cultivar in growth trials.

Species	Cultivar	Accession number	Collection date	Location	GPS	Collection morphology	Morphology tested
Ulva australis	BO1	MW250831.1	22/03/2019	Bowentown	−37.463684, 175.990069	Blade	Not tested
Ulva prolifera	BO2	MW250830.1	22/03/2019	Bowentown	−37.463684, 175.990069	Filamentous	Cluster
Ulva compressa	EB1	MW250829.1	15/03/2019	Opape	−37.972440, 177.421272	Filamentous	Cluster
Ulva rigida	EB2	MW250806.1	16/03/2019	Ruakakoakoa	−37.542300, 178.075908	Blade	Cluster
Ulva sp. *B*[a]	KA1	MW250827.1	01/04/2019	Karewa Island	−37.530483, 176.133344	Blade	Cluster
Ulva sp.[b]	OH1	–	08/04/2019	Ohope	−37.954694, 177.024031	Filamentous	Not tested
Ulva australis	OH2	MW250826.1	08/04/2019	Ohope	−37.954442, 177.024343	Blade	Not tested
Ulva compressa	OH3	MW250825.1	08/04/2019	Ohope	−37.954348, 177.024229	Filamentous	Filamentous, cluster
Ulva australis	OH4	MW250824.1	08/04/2019	Ohope	−37.953632, 177.025257	Blade	Cluster
Ulva australis	OM1	MW250823.1	22/03/2019	Omokoroa	−37.632943, 176.053579	Blade	Not tested
Ulva flexuosa	OM2	MW250822.1	22/03/2019	Omokoroa	−37.632943, 176.053579	Filamentous	Cluster
Ulva compressa	OM3	MW250821.1	22/03/2019	Omokoroa	−37.632943, 176.053579	Filamentous	Filamentous
Ulva sp. *B*	SW2	MW250820.1	19/10/2018	Otumoetai	−37.665027, 176.155470	Blade	Blade
Ulva sp. B	SW5	MW250819.1	28/10/2018	Mt Maunganui	−37.632634, 176.186598	Blade	Blade, cluster
Ulva compressa	SW6	MW250818.1	08/11/2018	Sulphur point	−37.659539, 176.166428	Filamentous	Filamentous, cluster
Ulva prolifera	SW7	MW250817.1	06/04/2019	Maketu	−37.756864, 176.444327	Filamentous	Cluster
Ulva ralfsii	SW8	MW250805.1	06/04/2019	Maketu	−37.758258, 176.437397	Filamentous	Filamentous
Ulva sp. B	SW9	MW250815.1	23/04/2019	Sulphur Point	−37.659230, 176.167999	Blade	Blade, cluster
Ulva australis	WB1	MW250814.1	22/03/2019	Waihi Beach	−37.394014, 175.940240	Blade	Not tested
Ulva australis	WB2	MW250813.1	22/03/2019	Waihi Beach	−37.394014, 175.940240	Blade	Blade, cluster
Ulva intestinalis	WK1	MW250812.1	08/04/2019	Whakatane	−37.946594, 177.008488	Filamentous	Cluster
Ulva intestinalis	WK2	MW250811.1	08/04/2019	Whakatane	−37.945175, 177.010179	Filamentous	Not tested
Ulva intestinalis	WK3	MW250810.1	08/04/2019	Whakatane	−37.941992, 177.011376	Filamentous	Cluster
Ulva australis	WK4	MW250809.1	08/04/2019	Whakatane	−37.939782, 177.012484	Blade	Blade, cluster

[a] Recorded as *Ulva* sp. 1 in Heesch et al. [36].
[b] Sample did not successfully amplify.

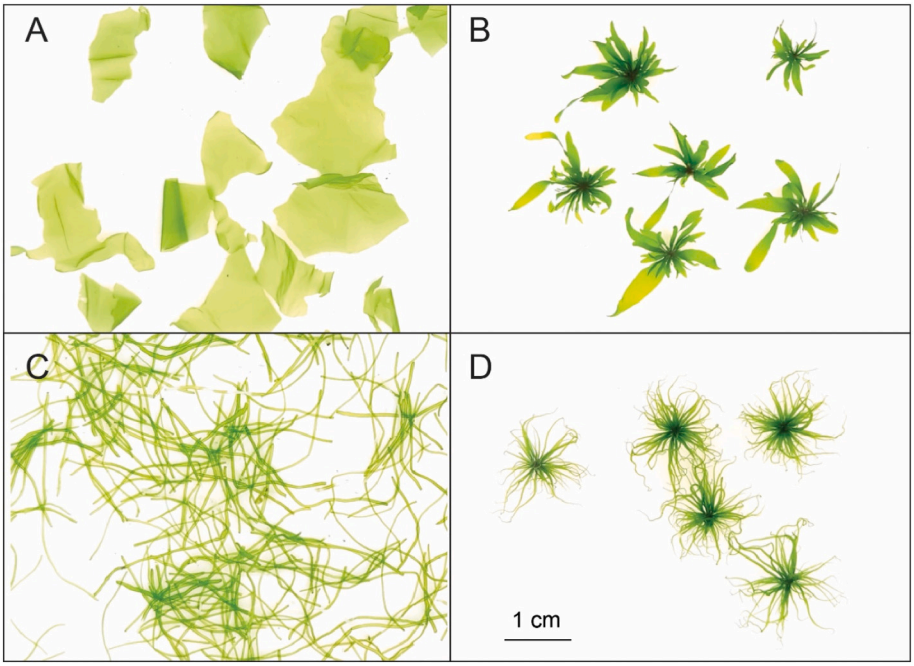

Fig. 1. Examples of (A) blade (cultivar SW5), (B) blade cluster (cultivar SW5), (C) filamentous (cultivar SW8) and (D) filamentous cluster (cultivar SW6) morphologies tested in growth trials.

2.2. Species identification

Due to the well-established difficulties associated with identifying *Ulva* specimens to species level using morphological and cytological characteristics [41], samples were identified using DNA barcoding. DNA was extracted using the Chelex method of Goff and Moon [42]. The *rbc*L locus was amplified and sequenced from the *Ulva* specimens using primers SHF1/SHR4, as in Heesch et al. [36]. Sequences were trimmed and assembled using Geneious Prime 2020.2.3, and the consensus sequences were compared with sequences in GenBank using BLAST (https://blast.ncbi.nlm.nih.gov/). *Ulva* sequences were aligned with *rbc*L sequences from a range of *Ulva* taxa including representative sequences from taxa previously recorded from New Zealand, taking into account recent updates to *Ulva* taxonomy and species assignments [31,43,44]. Ten sequences from *Umbraulva*, *Ulvaria* and *Gemina* taxa were included as outgroups. A maximum likelihood (ML) phylogenetic tree was estimated using PhyML 3.3.20180214 ([45], implemented in Geneious Prime) under the GTR+I+G model of sequence evolution, with support estimated under the SH-like approximate likelihood ratio test (aLRT, [46]). The tree was visualised using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

2.3. Growth trials

Growth trials were conducted on 18 of the 24 cultivars that were collected. Cultivars BO1, EB1, OH1, OH2, WK2 and WB1 were excluded from trials as they did not survive or grow well in culture over the three-month period prior to the start of experiments. Growth trials were conducted on cultivars in their original collection morphology (e.g., blade or filamentous) and in cluster morphology where possible. For some cultivars we were able to maintain stock cultures of biomass only in the original collection morphology or only in cluster morphology, while for other cultivars we were able to maintain stock cultures of biomass in both the original collection morphology and in cluster morphology. We conducted growth trials on nine cultivars in blade morphology, five cultivars in filamentous morphology, seven cultivars in blade cluster morphology, and eight cultivars in filamentous cluster morphology (Table 1). We were able to test four cultivars in both blade and blade cluster morphologies (SW5, SW9, WB2 and WK4) and two cultivars in both filamentous and filamentous cluster morphologies (OH3 and SW6).

Three replicate cultures of each cultivar/morphology combination were grown in batch cultures in 5 L plastic buckets filled with nutrient enriched filtered seawater (Cell-Hi F2P, Varicon Aqua Solutions UK, 0.1 g L^{-1}, 12.3 mg nitrate-N L^{-1} and 1.1 mg P L^{-1}) and maintained under the same conditions as described in Section 2.1. Replicate cultures were arranged in a randomised block design and the position of individual buckets within each block was rotated every one to two days. Cultures were stocked at a rate of 0.5 g fresh weight (FW) L^{-1}. As stock cultures of each cultivar contained a mix of different sized filaments or blade fragments, the biomass used to stock experimental replicates was cut to a standardised size at the start of the experiment. Cultures with blade morphologies were cut into discs with a 12 mm diameter using a hole punch; cultures with filamentous morphologies were cut to a length of 30–50 mm using a craft knife. The length and width of individual cluster branches continually increase with growth. Therefore, the size of cluster morphologies was standardised by beginning experiments when the length of branches had reached a target size of 10–20 mm. Clusters used to stock replicate cultures for each cultivar were seeded at the same time, therefore all individuals within each culture were the same age.

Cultures were harvested, spun to remove excess water, and weighed every 7 days. Following harvesting, stocking density was reset by restocking 0.5 g FW L^{-1} of the harvested biomass back into each replicate culture. All remaining harvested biomass was dried for later analysis. This process was repeated a further two times, providing for a total of three consecutive harvests. Buckets were washed and culture water was replaced with fresh nutrient enriched filtered seawater at each harvest. The excess biomass from each replicate culture at each harvest was weighed to determine the FW, then dried in an oven at 60 °C for at least 36 h and then reweighed to determine the dry weight:fresh weight (DW:FW) ratio for each individual culture for each week of growth. Specific growth rates (SGR) were calculated for each replicate for each harvest using the equation SGR (% day^{-1}) = $\mathrm{Ln}(B_f / B_i) / T * 100$, where B_f and B_i are the final and initial algal biomasses (g FW) and T is the number of days in culture. Biomass productivity (g DW m^{-2} day^{-1}) was calculated for each replicate for each harvest using the equation P =

$([(B_f - B_i) * DW{:}FW] / A) / T$, where B_f and B_i are the final and initial algal biomasses (g), *DW:FW* is the dry weight to fresh weight ratio, A is the area (m^2) of culture tanks and T is the number of days in culture.

2.4. Chemical composition

To provide a general indication of the suitability of cultivars for end product applications, biomass samples from the final harvest of the nine best performing cultivars across all morphologies (e.g. blade or blade cluster and filamentous or filamentous cluster) were analysed for carbon, hydrogen, nitrogen, sulfur and ash content. All samples were harvested at the same time of the day. All analyses were conducted by OEA Labs UK following standard methodology as described in Glasson et al. [23].

2.5. Statistical analysis

All data are presented as the mean ± S.E. Repeated measures permutational Analysis of Variance (PERMANOVA) was used to test for differences in the SGR, the biomass productivity and the FW:DW ratio between cultivars and harvests (both fixed factors), and replicates (random factor nested within cultivars) for each morphology type [47]. For the six cultivars where we were able conduct growth trials in both the original collection morphology and in cluster morphology, we used PERMANOVA to test for differences in the overall SGR and biomass productivity between cultivars and morphologies (both fixed factors). As we only analysed chemical composition in nine cultivars from the final harvest, PERMANOVA was also used to test for differences in the carbon, hydrogen, nitrogen, sulfur and ash content between cultivars (fixed factor), without the inclusion of morphology or harvest as factors. All analyses were conducted in Primer v7 (Primer-E Ltd., UK) using Euclidean distance resemblance matrices, 9999 unrestricted permutations of raw data and a Type III sum of squares [47]. Several cultivars were excluded from analyses as they either did not grow or went reproductive and released spores during the course of the three-week experiment. These cultivars were OM1, OH4, KA1 and EB2 (all blade non-cluster morphology) and WK1 (filamentous non-cluster morphology).

Fig. 2. Maximum likelihood phylogram showing the relationships of sequences from this study (bolded) to selected *Ulva* sequences from GenBank. Support values (aLRT) are shown above branches. Names associated with clades containing sequences from this study are shown to the right of each clade; GenBank accession numbers are appended to each sequence. The names of unidentified *Ulva* species from New Zealand follow those used in the New Zealand Threat Classification System, http://nzcts.org.nz.

3. Results

3.1. Species identification

Amplification and sequencing were successful for all but one sample, meaning that we were able to assign species names to 23 of the 24 samples we collected. The results of the phylogenetic analysis are shown in Fig. 2. Designations of undescribed species follow that of the New Zealand Threat Classification system (https://www.doc.govt.nz/about-us/science-publications/conservation-publications/nz-threat-classification-system/). Identifications of most samples to species level were unequivocal, however *Ulva* sp. KA1 (MW250827.1) was resolved on a long branch, reflecting several substitutions with respect to other sequences in the *Ulva* sp. B clade. This sequence was short in length (850 bp) and contained a number of unresolved nucleotides. On balance we consider this is most likely to be a variant of *Ulva* sp. B, despite the long branch length. *Ulva* sp. B has not yet been formally described to our knowledge. This taxon was sequenced as '*Ulva* sp. 1' by Heesch et al. [36] who found this species to be widespread throughout New Zealand. In their study it was frequently collected from both open coasts and embayments. The identifications of all samples are given in Table 1. In total, we found three species with blade morphologies (*U. australis*, *U. rigida* and *Ulva* sp. B) and five species with filamentous morphologies (*U. compressa*, *U. flexuosa*, *U. intestinalis*, *U. prolifera*, and *U. ralfsii*). The most common blade species was *U. australis* (6 cultivars found at 5 locations), and the most common filamentous species was *U. compressa* (4 cultivars found at 4 locations).

3.2. Growth trials

Average specific growth rates ranged from 5.9 to 37.3% day^{-1} across all cultivars, morphologies and harvests (Fig. 3). For each morphology type, specific growth rates varied significantly among cultivars, however the relative performance of cultivars varied among harvests, as indicated by significant cultivar × harvest interaction effects (Table 2). For blade morphologies, WB2 had the highest specific growth rate in the first and third harvest, but SW9 had the highest specific growth rate in the second harvest. For blade cluster morphologies and filamentous morphologies, SW9 and SW8 respectively had the highest specific growth rate in all three harvests. For filamentous cluster morphologies, SW6 had the highest specific growth rate in the first and second harvest, but SW7, EB1, OM2 and SW6 all had similarly high specific growth rates in the third harvest. Across all harvests, the cultivars with the highest specific growth rates were SW9 for blade morphologies (20.6 ± 0.5% day^{-1}) and blade cluster morphologies (22.1 ± 0.7% day^{-1}), SW8 for filamentous morphologies (30.9 ± 0.6% day^{-1}), and SW6 for filamentous cluster morphologies (32.6 ± 1.2% day^{-1}).

Average biomass productivity ranged from 2.4 to 11.5 g DW m^{-2} day^{-1} across all cultivars, morphologies and harvests (Fig. 4). There were significant differences in biomass productivities among cultivars for each morphology type, however the relative performance of cultivars varied among harvests as indicated by significant cultivar × harvest interaction effects (Table 2). For blade morphologies, biomass productivities were highest for WB2 in the first harvest, SW9 in the second harvest, and WB2, SW9 and WK4 all had similarly high biomass productivities in the third harvest. For blade cluster morphologies, SW6 had the highest biomass productivities in the first and second harvest, and both SW6 and KA1 had similarly high biomass productivities in the third harvest. For filamentous morphologies, SW8 had the highest biomass productivities in all three harvests. For filamentous cluster morphologies, SW6 and OM2 both had similarly high biomass productivities in the first harvest, SW6 had the highest biomass productivity in the second harvest and SW7 had the highest biomass productivity in the third harvest. Across all harvests, the cultivars with the highest biomass productivities were WB2 for blade morphologies (7.5 ± 0.5 g DW m^{-2} day^{-1}), SW9 for blade cluster morphologies (9.4 ± 0.2 g DW m^{-2} day^{-1}), SW8 for filamentous morphologies (7.8 ± 0.4 g DW m^{-2} day^{-1}), and SW6 for filamentous cluster morphologies (9.8 ± 0.5 g DW m^{-2} day^{-1}).

Across all harvests and cultivars, average specific growth rates and

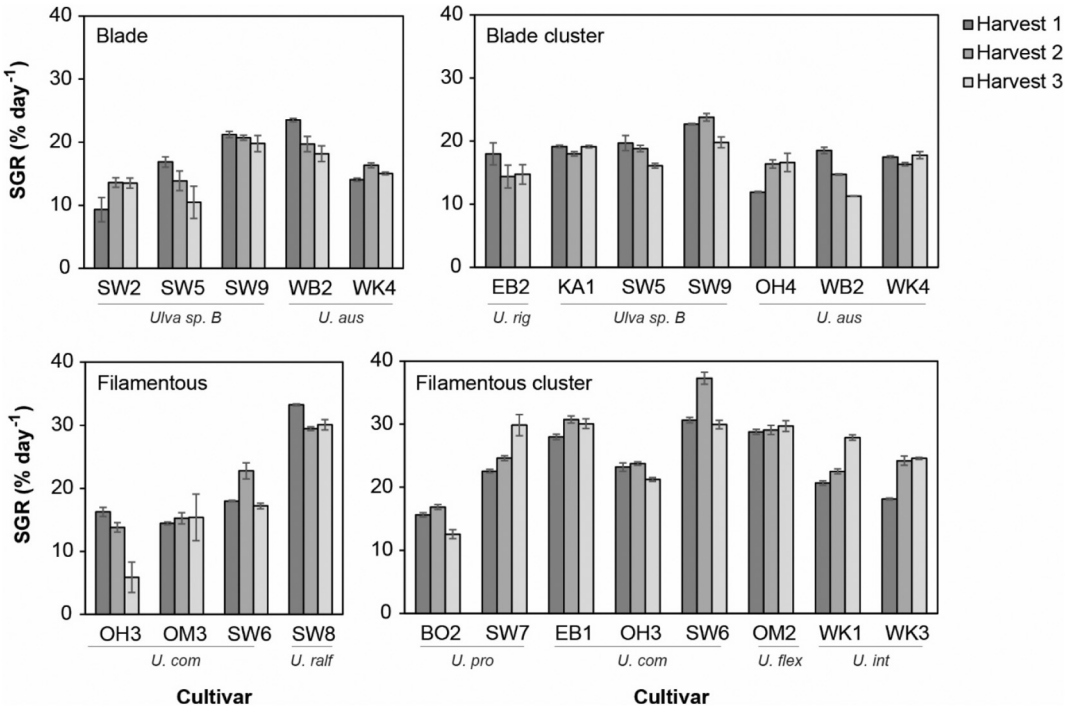

Fig. 3. Mean (±S.E.) specific growth rates (SGR, % day^{-1}) of *Ulva* cultivars with blade, blade cluster, filamentous and filamentous cluster morphologies over three consecutive harvests (n = 3 cultures). Species identifications are indicated below cultivar code. Species abbreviations: *U.* sp. B – *U.* sp. B; *U. aus* – *U. australis*; *U. rig* – *U. rigida*; *U. com* – *U. compressa*; *U. ralf* – *U. ralfsii*; *U. pro* – *U. prolifera*; *U. flex* – *U. flexuosa*, *U. int* – *U. intestinalis*.

Table 2
Results of permutational analyses of variance (PERMANOVAs) testing the effects of cultivar (Cu), harvest (Ha) and replicates (nested within cultivars, Re(Cu)) on specific growth rate (SGR), biomass productivity and FW:DW ratios on cultivars of *Ulva* with varying morphologies. Pseudo F (F) and P values are presented.

Morphology	Variable	Effect	df	F	P
Blade	SGR	Cu	4	24.0	<0.001
		Ha	2	4.2	0.028
		Re(Cu)	10	2.1	0.073
		Cu × Ha	8	5.5	<0.001
		Res	20		
	Biomass productivity	Cu	4	19.5	<0.001
		Ha	2	29.7	<0.001
		Re(Cu)	10	1.0	0.508
		Cu × Ha	8	5.6	<0.001
		Res	20		
	DW:FW	Cu	4	127.4	<0.001
		Ha	2	18.9	<0.001
		Re(Cu)	10	1.4	0.252
		Cu × Ha	8	2.8	0.031
		Res	20		
Blade cluster	SGR	Cu	6	38.3	<0.001
		Ha	2	5.9	0.006
		Re(Cu)	14	0.5	0.894
		Cu × Ha	12	4.8	<0.001
		Res	28		
	Biomass productivity	Cu	6	80.2	<0.001
		Ha	2	25.5	<0.001
		Re(Cu)	14	0.4	0.956
		Cu × Ha	12	3.7	0.002
		Res	28		
	DW:FW	Cu	6	207.5	<0.001
		Ha	2	3.9	0.032
		Re(Cu)	14	1.4	0.227
		Cu × Ha	12	8.2	<0.001
		Res	28		
Filamentous	SGR	Cu	3	91.1	<0.001
		Ha	2	7.2	0.006
		Re(Cu)	8	1.1	0.391
		Cu × Ha	6	4.7	0.006
		Res	16		
	Biomass productivity	Cu	3	66.7	<0.001
		Ha	2	11.2	0.001
		Re(Cu)	8	1.3	0.321
		Cu × Ha	6	6.1	0.002
		Res	16		
	DW:FW	Cu	3	320.6	<0.001
		Ha	2	1.6	0.255
		Re(Cu)	8	0.4	0.884
		Cu × Ha	6	8.1	<0.001
		Res	16		
Filamentous cluster	SGR	Cu	7	181.7	<0.001
		Ha	2	48.5	<0.001
		Re(Cu)	16	1.3	0.258
		Cu × Ha	14	18.9	<0.001
		Res	32		
	Biomass productivity	Cu	7	57.2	<0.001
		Ha	2	1.3	0.266
		Re(Cu)	16	0.7	0.803
		Cu × Ha	14	13.5	<0.001
		Res	32		
	DW:FW	Cu	7	320.6	<0.001
		Ha	2	1.5	0.245
		Re(Cu)	16	0.4	0.879
		Cu × Ha	14	8.1	<0.001
		Res	32		

biomass productivities were highest in filamentous cluster morphologies (19.3 ± 0.7% day^{-1} and 8.2 ± 0.2 g DW m^{-2} day^{-1} respectively) compared to other morphology types. There was a significant effect of morphology (cluster vs non-cluster) on specific growth rates and biomass productivities. Both metrics were higher for cluster morphologies compared to non-cluster morphologies for five of the six cultivars where we were able to test both morphology types (Fig. 5, PERMANOVA: cultivar × morphology interaction effect: F = 20.99, df: 4,24, P < 0.001). There was no clear effect of species on specific growth rate or biomass productivity. For all four morphology types, there was considerable variation among cultivars of the same species, and cultivars from different species often had comparable performance. For example, for the filamentous cluster morphology, biomass productivity of SW7 (*U. prolifera*, 9.5 ± 0.4 g DW m^{-2} day^{-1}) was comparable to SW6 (*U. compressa*, 9.8 ± 0.5 g DW m^{-2} day^{-1}) and OM2 (*U. flexuosa*, 9.0 ± 0.3 g DW m^{-2} day^{-1}), but was 75% higher than BO2 (*U. prolifera*, 5.4 ± 0.3 g DW m^{-2} day^{-1}).

DW:FW ratios ranged from 0.09 to 0.31 across all cultivars, morphologies and harvests (Fig. 6). There were significant differences in DW:FW ratios among cultivars and between harvests for each morphology type, however these differences were not consistent, as indicated by significant cultivar × harvest interaction effects for all morphology types except filamentous cluster (Table 2). Across all harvests and cultivars, average DW:FW ratios were highest for blade cluster (0.25 ± 0.01) and blade morphologies (0.24 ± 0.01) and lowest for filamentous (0.16 ± 0.01) and filamentous cluster morphologies (0.18 ± 0.01). SW8 had the lowest average DW:FW ratio (0.10 ± 0.01), and WB2 (blade cluster) had the highest average DW:FW ratio (0.29 ± 0.01).

3.3. Chemical composition

Chemical composition of the biomass at the final harvest varied significantly among the 9 cultivars we analysed (Tables 3 and 4). Ash content ranged from 17.9 to 34.3% DW; carbon content ranged from 22.0 to 30.4% DW; hydrogen content ranged from 4.0 to 5.3% DW; nitrogen content ranged from 2.1 to 2.8% DW; and sulfur content ranged from 2.4 to 6.0% DW. Regardless of whether or not cultivars had a cluster morphology, ash content was higher overall in filamentous cultivars (30.8 ± 1.9% DW) compared to blade cultivars (19.6 ± 1.2% DW), but carbon, hydrogen and sulfur content were higher overall in blade cultivars (C: 28.3 ± 1.1% DW, H: 5.1 ± 0.1% DW; S: 5.2 ± 0.5% DW) compared to filamentous cultivars (C: 24.6 ± 1.2% DW; H: 4.4 ± 0.2% DW; S: 2.9 ± 0.2% DW). In contrast, nitrogen content was comparable between blade cultivars (2.4 ± 0.2% DW) and filamentous cultivars (2.4 ± 0.1% DW).

4. Discussion

A critical first step towards the development of land-based seaweed aquaculture is the selection of target species and cultivars [29]. Our survey of the Bay of Plenty region of Aotearoa New Zealand identified eight species of *Ulva* from 24 wild-collected samples, of which 75% were able to be maintained in free-floating cultures. Specific growth rates and biomass productivities of multiple cultivars in small-scale laboratory cultures were high (>20% day^{-1} and >8 g m^{-2} day^{-1} respectively), with biomass increases of 5 to 8-fold per week in the fastest growing cultivars. These growth rates are comparable to, or greater than, those recorded in controlled laboratory studies for commonly cultivated species of *Ulva*, e.g. *U. prolifera* (21% day^{-1}, [48]), *U. linza* (8% day^{-1}, [49]), *U. fasciata* (16% day^{-1}, [50]), *U. rigida* (9% day^{-1}, [51]). These results confirm the suitability of *Ulva* as a target for intensive land-based aquaculture in Aotearoa New Zealand.

Ulva sp. B and *U. compressa* had the highest growth rates and biomass productivities in our experiments and were therefore identified as the best performing species with blade and filamentous morphologies respectively. These species are two of the most common and widespread species of *Ulva* in Aotearoa New Zealand [36,52] and both were found at multiple sites in the current study. Their broad geographic distribution makes them ideal candidates to target for cultivation [29] as it will be possible to select local cultivars in different geographic regions. Additionally, both *Ulva* sp. B and *U. compressa* are thought to be either native to Aotearoa New Zealand or naturalised over 150 years ago [36,52]. The selection of native species as targets is important to minimise the risk of cultivated algae escaping and impacting on native biodiversity [28]. It

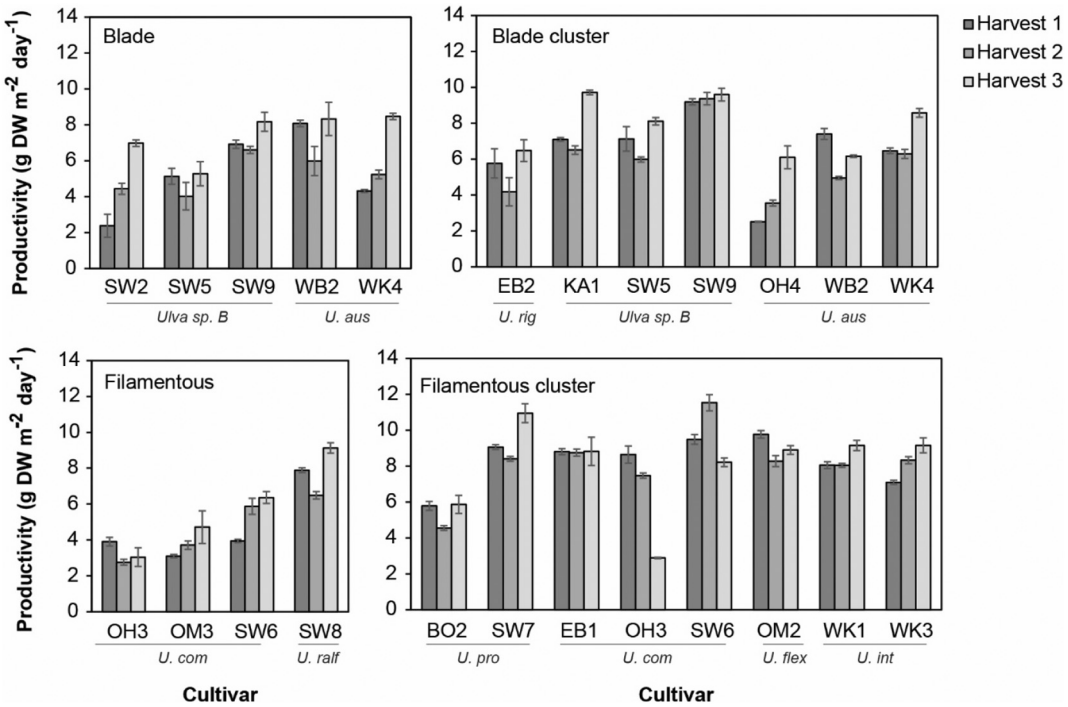

Fig. 4. Mean (±S.E.) biomass productivity (g DW m^{-2} day^{-1}) of *Ulva* cultivars with blade, blade cluster, filamentous and filamentous cluster morphologies over three consecutive harvests (n = 3 cultures). Species identifications are indicated below cultivar code. Species abbreviations: *U.* sp. B – *U.* sp. B; *U. aus* – *U. australis*; *U. rig* – *U. rigida*; *U. com* – *U. compressa*; *U. ralf* – *U. ralfsii*; *U. pro* – *U. prolifera*; *U. flex* – *U. flexuosa*, *U. int* – *U. intestinalis*.

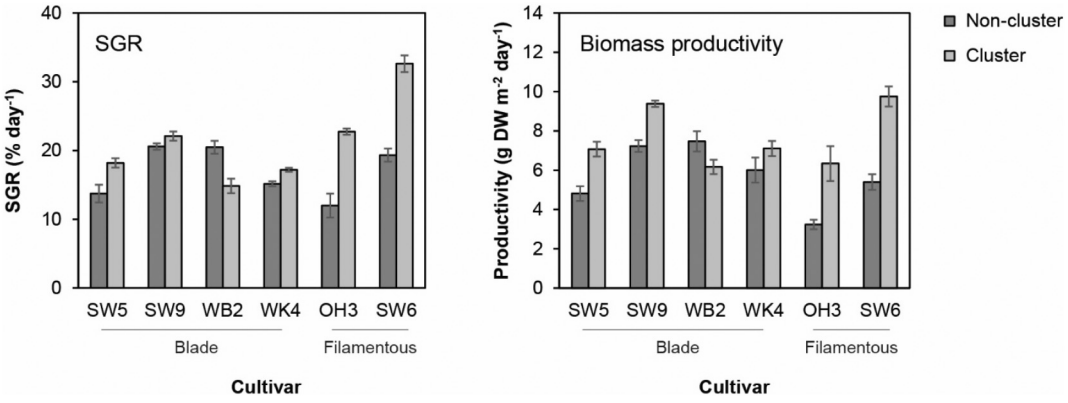

Fig. 5. Mean (±S.E.) specific growth rates (SGR, % day^{-1}) and biomass productivity (g DW m^{-2} day^{-1}) over the three harvests of *Ulva* cultivars tested in their original collection morphology (blade or filamentous) and in cluster morphology (n = 3 cultures).

also avoids potential legislative difficulties associated with farming invasive species [e.g., 34]. However, as our experiments only had limited replication at the species level, further trials testing a larger number of cultivars are required to confirm the superior performance of *Ulva* sp. B and *U. compressa*.

However, selection of targets at a species level does not account for significant variation in performance between cultivars. We found considerable variation in growth rates and biomass productivities among cultivars. Growth rates were 6-fold higher and biomass productivities were 4-fold higher in the fastest growing cultivars compared to the slowest growing cultivars in our experiments. Moreover, in some cases, variation in growth rates among cultivars of the same species was larger than variation among cultivars from different species. For example, average overall growth rates of SW9 were 50% higher than those of SW2 (both *Ulva* sp. B.), but were similar to WB2 (*U. australis*). Large variation in growth rates among cultivars has been reported previously for *Ulva* [29,31]. For example, growth rates ranged from 0.09 to 0.37 mg·mg^{-1} day^{-1} among 49 cultivars of *Ulva* [33]. However, most studies only test performance of a single species in response to varying environmental conditions (e.g., [48–50]), or compare a single cultivar of different species (e.g., [14,32]). The significant inter-cultivar variation found in the current study further highlights the importance of sampling widely and focusing on cultivar rather than species selection when identifying targets for cultivation [33]. Furthermore, these results demonstrate the potential for substantial and immediate gains in productivity through cultivar selection as opposed to longer term potential for gains through selective breeding.

It is unknown if the differences reported here in growth and biomass productivities among cultivars have a genetic basis (e.g., different genotypes) or are a result of phenotypic plasticity (e.g., same genotypes but different response to environmental conditions). Macroalgae often show high levels of phenotypic plasticity [53,54] and a recent study reported low levels of intra-specific genetic diversity for six species of *Ulva* [55]. However, differences in numerous traits, including growth,

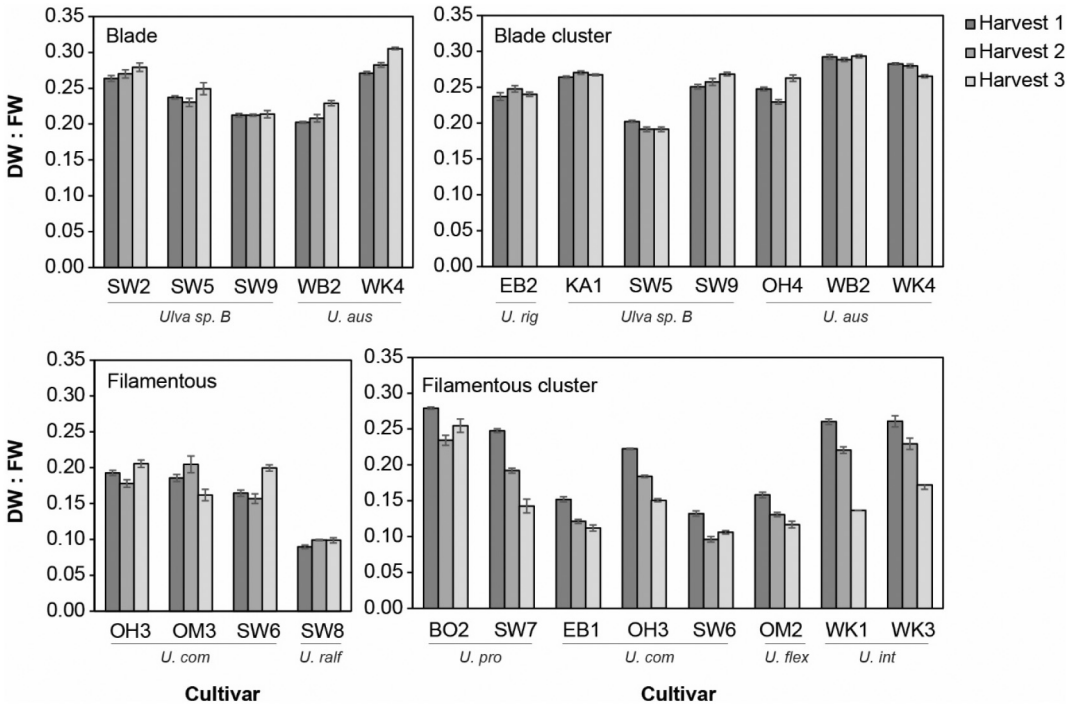

Fig. 6. Mean (±S.E.) dry weight: fresh weight (DW:FW) ratios of *Ulva* cultivars with blade, blade cluster, filamentous and filamentous cluster morphologies over three consecutive harvests (n = 3 cultures). Species identifications are indicated below cultivar code. Species abbreviations: *U.* sp. B – *U.* sp. B; *U. aus* – *U. australis*; *U. rig* – *U. rigida*; *U. com* – *U. compressa*; *U. ralf* – *U. ralfsii*; *U. pro* – *U. prolifera*; *U. flex* – *U. flexuosa*, *U. int* – *U. intestinalis*.

Table 3
Ash content (% DW) and ultimate analysis (carbon (C), hydrogen (H), nitrogen (N), and sulfur (S)) (% of DW) of 9 cultivars of *Ulva* with varying morphologies. Values are means (S.D.). N = 3. Data are reported on an "as received" basis.

Morphology	Cultivar	Ash	C	H	N	S
Blade	WB2	22.9	29.8	5.0	2.7	4.0
		(1.5)	(2.5)	(0.2)	(0.2)	(0.8)
Blade cluster	KA1	17.9	30.4	5.3	2.8	5.0
		(0.1)	(0.3)	(0.1)	(0.1)	(0.1)
	SW5	18.0	27.3	5.1	2.2	5.7
		(0.7)	(1.0)	(0.1)	(0.2)	(0.4)
	SW9	19.7	25.7	4.9	2.1	6.0
		(0.7)	(0.6)	(0.1)	(0.0)	(0.1)
Filamentous	SW8	34.3	22.0	4.1	2.1	3.5
		(1.8)	(1.2)	(0.1)	(0.1)	(0.1)
Filamentous cluster	EB1	31.7	25.2	4.5	2.6	2.4
		(0.4)	(0.58)	(0.1)	(0.3)	(0.2)
	OM2	26.6	25.4	4.3	2.4	3.2
		(7.6)	(0.9)	(0.1)	(0.1)	(0.1)
	SW6	35.4	22.2	4.0	2.5	2.4
		(3.0)	(2.0)	(0.2)	(0.1)	(0.1)
	SW7	26.3	28.3	4.9	2.2	3.0
		(3.9)	(1.2)	(0.1)	(0.2)	(0.1)

Table 4
Results of PERMANOVAs testing the effects of cultivar (Cu) on the carbon, hydrogen, nitrogen, sulfur and ash content of 9 cultivars of *Ulva*. Pseudo F (F) and P values are presented.

Variable	Effect	df	F	P
Carbon	Cu	8	14.6	<0.001
	Res	18		
Hydrogen	Cu	8	37.7	0.001
	Res	18		
Nitrogen	Cu	8	8.3	<0.001
	Res	18		
Sulfur	Cu	8	57.1	<0.001
	Res	18		
Ash	Cu	8	14.1	<0.001
	Res	18		

between green tide and non-green tide cultivars of *Ulva* appear to have a genetic basis [31]. There is also evidence that there is a genetic component to variation in growth and biomass productivity for cultivars of freshwater green macroalgae [56]. The relative contribution of genotypes (G) versus environmental factors (E) to a trait, and their interaction (G × E, also known as phenotypic plasticity), can be determined using common garden experiments where individuals from different populations are grown in a common environment [57]. While our experiments were not strictly designed as a common garden experiment, there were elements that were similar as cultivars were collected from different environments and then maintained under identical conditions for at least three months prior to the start of experiments. The persistence of significant differences among cultivars in growth rates and biomass productivities at the end of this period suggests that genetic factors are influencing these traits to some degree. However, further analysis using appropriate molecular markers or structured common garden experiments is required confirm this hypothesis.

For the first time, we compared the performance of cultivars in their collection morphology (e.g., blade or filamentous) to a cluster morphology. Clusters have been promoted as a way of enabling continuous stable production of biomass, particularly for species with filamentous morphologies, in high density tank cultures [40]. We found that a larger number of cultivars survived in culture in cluster morphology compared to blade or filamentous morphology. We also found that growth rates and biomass productivities were consistently higher for cluster morphologies compared to non-cluster morphologies for each morphology type (e.g. filamentous or blade). Filamentous species of *Ulva* are typically cultivated by seeding onto nets or ropes, and aside from a recently developed novel method of seeding onto free floating "bioballs" [58–60], there has been little exploration of the potential to cultivate filamentous species in free-floating cultures. Our results extend the findings of Hiraoka and Oka [40] and demonstrate that clusters are a viable option to enable free-floating cultivation of

filamentous species of *Ulva*. The consistently high performance of cluster morphologies reported here provides a strong rationale for further testing of their use in intensive land-based cultivation of *Ulva*.

There were significant differences among cultivars in the chemical composition of biomass at the end of the experiment, most strikingly in DW:FW ratios and ash content. DW:FW ratios were consistently lower for filamentous cultivars compared to blade cultivars, regardless of whether cultivars had a cluster morphology. This is most likely because water can get trapped within hollow filaments on harvest, even after spinning to remove excess surface water. Higher DW:FW ratios are desirable as these indicate a lower water content in the biomass, reducing inputs required to obtain dry biomass for processing [61]. Ash content was higher overall in filamentous cultivars compared to blade cultivars, regardless of whether cultivars had a cluster morphology. The higher ash contents are a result of higher water contents, and therefore content of inorganic compounds, in filamentous cultivars. High ash contents in the biomass can be favourable for animal feed applications as they can indicate the accumulation of elements such as calcium, magnesium, potassium and phosphorus which are essential minerals for farm animal nutrition [62]. Alternatively, low ash contents in the biomass can be favourable for bioenergy applications as inorganic compounds in the ash can impact bioenergy processes such as hydrothermal liquefaction (HTL) and biogas production [63,64]. Nitrogen contents ranged from 2.1 to 2.8% DW. Using a nitrogen-to-protein conversion factor of 5 [65], this equates to a crude protein content of 10.5–14% DW. This is on the lower end for species of *Ulva* (10–26% DW, [66,67]), but it may mean that the content of carbohydrates, in particular soluble fibres such as a the functional biopolymer ulvan, have a correspondingly higher concentration in the biomass [38]. However, it is possible to manipulate the chemical composition of *Ulva* by varying cultivation conditions and post-harvest processing treatments [24,62,68–70]. Additionally, cascading biorefinery processes can be used to sequentially and selectively extract various components from the biomass, enabling the production of a residual biomass enriched in target compounds [23]. This means that it is possible to manipulate the chemical composition of the biomass to target specific commercial applications. As a result, selection of target cultivars should primarily focus on growth and biomass productivities, with chemical composition a secondary consideration. Based on these specifications, cultivars such as WB2 (*U. australis*) and SW9 (*U.* sp. B) (both blade morphology) or SW8 (*U. ralfsii*) and SW6 (*U. compressa*) (both filamentous morphology) should be targeted for land-based cultivation in the Bay of Plenty region of Aotearoa New Zealand as they had the fastest growth rates and highest biomass productivities.

CRediT authorship contribution statement

Rebecca Lawton: Conceptualization; Investigation; Data curation; Formal analysis; Writing - original draft; Visualization; Project administration. **Judy Sutherland:** Investigation; Formal analysis; Visualization; Writing – original draft. **Christopher Glasson:** Conceptualization, Investigation, Writing – review and editing. **Marie Magnusson:** Conceptualization, Investigation, Writing - review & editing, Funding acquisition.

Declaration of competing interest

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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