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Promise and pitfalls of locally abundant seaweeds as biofilters for integrated aquaculture

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ABSTRACT

Seaweeds from the genus *Caulerpa* offer promise for bioremediation in integrated tropical aquaculture in northern Australia, as they are common on shallow reefs adjacent to where aquaculture is developing and their propagation is readily manipulated through fragmentation. Fragments of five varieties of *Caulerpa* had high growth rates (between 3 and 7% day^{-1}) and high nitrogen content (up to 3% dry weight/0.2% fresh weight for *Caulerpa taxifolia*) in tank-based culture. These attributes combined confirm the promise for *Caulerpa* in integrated aquaculture, especially as certain species (*Caulerpa lentillifera* and *Caulerpa racemosa*) are valuable products. However, this potential was not realised when *Caulerpa* spp. were cultured in an in situ aquaculture context. Only a limited proportion of fragments (13%, predominantly *C. taxifolia*) persisted during a 6 week in situ experiment in a flow-through settlement (treatment) pond from an 800 tonne year^{-1} fish production facility. Mean growth of persisting pond fragments (less than 0.3% day^{-1}) was much less than concurrent tank cultures (3–7% day^{-1}). The factor most strongly influencing pond culture was the negative (smothering) impact of blooming filamentous algae (*Cladophora* and *Chaetomorpha* spp.). Poor pond growth of *Caulerpa* was further substantiated in an additional test, determining that persistence and growth (or lack thereof) was independent of initial seeding size of fragments. These results suggest that *Caulerpa* culture will not be easily integrated into settlement ponds in tropical aquaculture. However, because some species of *Caulerpa* grew well in tank-based systems (*C. racemosa* grew at >7% day^{-1}) and others are capable of luxury uptake (*Caulerpa serrulata* and *C. taxifolia* almost doubled internal nitrogen in nutrient-rich water), *Caulerpa* species have application in bioremediation of intensive tank-based aquaculture and perhaps treated pond aquaculture effluent.

1. Introduction

Integrated plant–animal aquaculture has developed as an effective mechanism to reduce the level of nutrients in effluent through nitrogen and phosphorous absorption by plants or algae (LaPointe et al., 1976; Asare, 1980; Brown et al., 1999; Chopin et al., 2001; Neori et al., 2004; Lu and Li, 2006; Crab et al., 2007). This process is particularly important for intensive aquaculture, in which dissolved nitrogenous waste (especially ammonium) can accumulate to levels that limit farm output either directly through harming fish (Handy and Poxton, 1993; Metaxa et al., 2006) or indirectly through operational constraints on effluent release (Tacon and Forster, 2003; Carmona et al., 2006). Given the potential negative impacts of aquaculture effluent on natural systems (e.g. Sara, 2007), integrated plant–animal aquaculture is now prominent in environmental management solutions, and will be particularly important to land-based aquaculture with point-source effluent (Naylor and Burke, 2005; Neori et al., 2007).

Marine macroalgae (seaweeds) can effectively strip nutrients from aquaculture effluent prior to its release to the environment (Chopin et al., 2001; Neori et al., 2004). The characteristics that make seaweeds particularly effective as biofilters are high growth rates (which facilitate nitrogen acquisition), pliable reproductive modes and simple habitat requirements. Seaweeds typically have high growth rates in nutrient-rich aquaculture effluent (Neori et al., 2000; Nagler et al., 2003; Hernández et al., 2006; Pereira et al., 2006), as nutrients are no longer a limiting growth factor as they often are in nature (Lobban and Harrison, 1994). Furthermore, some algae have luxury uptake of nitrogen which is stored and accessed as required (Naldi and Wheeler, 1999; Harrison and Hurd, 2001). Luxury uptake limits the impact of variable nutrient loads on algae in nature and, importantly, will enhance nitrogen removal per unit weight of algae in nutrient-rich artificial systems. Seaweed propagation can also be manipulated, including the production and settlement of sexual and asexual propagules (e.g. for nori — *Porphyra* spp.: Chopin et al., 1999; Blouin et al., 2007) and the regeneration of asexually derived fragments (Buschmann et al., 1995; Correa et al., 1999). And as seaweeds grow on a variety of substrata, their culture can be adapted to cage, tank or pond-based systems (Buschmann et al., 1995, 1996; Nagler et al., 2003; Hernández et al., 2006; Matos et al., 2006; Hernández-González et al., 2007).

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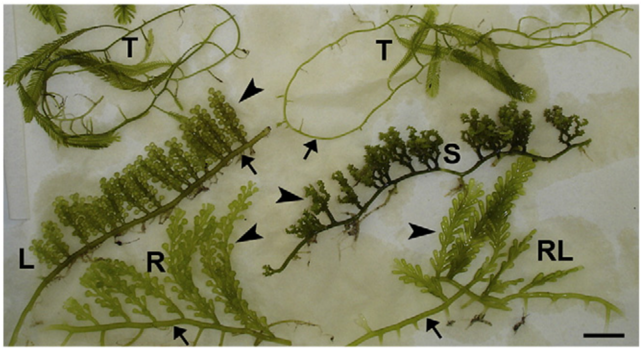

Fig. 1. Habit of *Caulerpa* fragments used in growth experiments. L = *C. lentillifera*, C = *C. racemosa*, RL = *C. racemosa* var. *laetevirens*, S = *C. serrulata*, T = *C. taxifolia*. Note: horizontal stolons (arrows) giving rise to upright fronds (arrow heads). Scale bar, 2 cm.

Despite many marine algae having characteristics amenable to bioremediation, their use is dominated by a small group of seaweeds, particularly *Porphyra*, *Gracilaria* and *Ulva* species (e.g. Chopin et al., 1999; Neori et al., 2000; Msuya et al., 2006; Blouin et al., 2007). These algae have been either selected for integrated feeding applications (using effluent-cultured algae to directly feed cultured animals, e.g. Neori et al., 2000; Troell et al., 2006) or for providing a valuable secondary product (Buschmann et al., 1996; Chopin et al., 1999). Integrated aquaculture has to date had a temperate focus with few examples for tropical regions (but see Nagler et al., 2003; Msuya et al., 2006). Because tropical aquaculture systems and algal biodiversity differ substantially from cold/temperate systems, a greater tropical focus is required to establish seaweed culture as integral to the expansion of tropical aquaculture (including in Australia; Mazur and Curtis, 2006).

In tropical north-eastern Australia – a region adjacent to the World Heritage listed Great Barrier Reef Marine Park – the two main products of aquaculture are pond-reared prawns (shrimp) and barramundi (Mazur and Curtis, 2006). However, suitable forms of integrated aquaculture are for the most part lacking. One reason for this is that the data required for incorporating seaweed culture into integrated operations have not been quantified, specifically the influence on algal growth of site specific conditions, such as temperature, light intensity and regime, and water quality. For this region of Australia appropriate algae for use in integrated aquaculture must also be local, a similar consideration was the premise for *Porphyra* cultivation in North America (Carmona et al., 2006). The use of local seaweeds is essential to environmental sensitivity and to promote aquaculture as a sustainable industry.

In the present study we test the use of local algae in integrated aquaculture, with an emphasis on identifying seaweeds with high growth rates, high nitrogen content and fragmentation as a mode of reproduction, the latter with the intent for a simple and perpetual propagation method. We selected five prominent members of the green algal genus *Caulerpa* from shallow reef habitats near Townsville, Australia, and quantified their growth in two separate tank-based seawater systems. We also examined growth and nitrogen content of these algae cultured in a settlement pond of a fish farm (intensively culturing barramundi, *Lates calcarifer*). This enabled us to draw comparisons between the relatively controlled tank-based systems and the variable environment of a tropical aquaculture pond, and provided an assessment of the relative importance of *Caulerpa* growth and nutrient acquisition for their role in bioremediation.

2. Materials and methods

2.1. Study organisms and field sampling

Caulerpa (Chlorophyta; Udotaceae) is a genus of siphonaceous algae comprised of close to 200 species (Guiry and Guiry, 2008; www.algaebase.org), some of which rely on asexual propagation via thallus fragmentation for their ecological success (Smith and Walters, 1999). Fragmentation and the subsequent attachment of *Caulerpa* spp. have facilitated its invasion of new habitats (Ceccherelli and Cinelli, 1999), suggesting that any local species of this genus could be particularly suitable for propagating in the settlement ponds of aquaculture facilities.

More than ten species of *Caulerpa* are found on the Great Barrier Reef, Australia (Cribb 1996; Benzie et al., 1997) and many are accessible on shallow inshore reefs. *Caulerpa* species were collected from Townsville (19° 15′ 52″ S, 146° 48′ 54″ E) and Magnetic Island (19° 10′ 25″ S, 146° 49′ 40″ E). Five common varieties with distinct morphologies (Fig. 1; *Caulerpa taxifolia*, *Caulerpa lentillifera*, *Caulerpa serrulata*, *Caulerpa racemosa* var. *laetevirens*, *C. racemosa* — previously *C. racemosa* var. *clavifera*, identified using Cribb (1996) with current nomenclature by Guiry and Guiry, 2008) were sampled from five different sites, two at Townsville (Kissing Point & The Strand) and three at Magnetic Island (Nelly Bay, Picnic Bay and Cockle Bay). We assessed the presence/absence of different *Caulerpa* species at these sites and live samples were returned to a closed seawater system prior to use in experiments.

2.2. Tank-based culture

To assess the growth and nitrogen content (two important variables for estimating nitrogen removal in integrated aquaculture) we ran two experiments concurrently under controlled conditions at James Cook University. Growth was quantified as the specific growth rate of algae in two separate but adjacent tank-based closed (recirculating) systems that differed in their water qualities. The level of nitrogen was more than 80 times higher in one system (high N, 1.4 mg/L; Table 1) compare to the other (low N, 0.017 mg/L). Variables for the closed-system experiment (means ± 1SD) were measured on five occasions over the 19 days experimental period (Table 1; the main form of nitrogen was nitrate due to microbial biofilters on both systems). Daily maximum surface irradiances (PAR) in the tanks were >200 µmol photons m^{-2} s^{-1}.

The experimental design comprised of four replicate tanks in each system (high and low N). Each tank contained two samples (fragments) of each of the five species of *Caulerpa*, which were randomly allocated a position on a submerged tray and secured by loosely fitted cable ties. Fragments of algae used were sub-samples of an individual of each species; a growing tip comprised of a section of stolon (horizontal runner) with at least five upright fronds (Fig. 1). We selected a generic type of fragment as opposed to standardising for initial mass across *Caulerpa* species because of the varied sizes and morphologies amongst the algae (Fig. 1). This meant that average initial size (mass) varied between the smaller, *C. taxifolia* (1.3 g) and *C. serrulata* (2.4 g) and larger morphologies, *C. racemosa* var. *laetevirens* (4 g), *C. lentillifera* (4.7 g), and *C. racemosa* (4.8 g). Growth was measured over 19 days of culture in both systems (high N and low N). Four new replicates of *C. serrulata* were added on the second day because of death (see Section 2.6).

2.3. Settlement pond (in situ) culture

Growth of the five species of *Caulerpa* was quantified in a settlement pond at Good Fortune Bay Fisheries Ltd (GFB), a barramundi farm at Guthalungra, Queensland (20° 1′ 46″ S, 148° 11′ 20″ E), approximately 160 km south of Townsville producing ~800 tonnes per annum. This farm

Table 1
Mean values (±1SD) of environmental variables for the culture of *Caulerpa* spp. under three experimental conditions

Environmental variable	Tank culture	Tank culture	Pond culture
	High N	Low N	In situ
Total N (mg L^{-1})	1.4 ± 0.02	0.017 ± 0.02	~1.8
Temperature (°C)	28.7 ± 0.6	27.8 ± 0.9	26.5 ± 3
Salinity (ppt)	36.26 ± 0.6	36.44 ± 0.6	37.50 ± 0.3
pH	8.14 ± 0.09	8.19 ± 0.04	8.46 ± 0.45

is a land-based flow-through system, pumping seawater from the nearby Great Barrier Reef and returning effluent water on a tidal frequency from a large (~1 ha) settlement pond. As water is not filtered prior to entering the farm, the propagules of many marine animals and plants have entered the settlement pond, essentially creating a marine mesocosm.

To determine whether the nutrient-rich pond water is suitable for culturing *Caulerpa*, we measured the growth of five species at seven different locations (blocks) on the periphery of the settlement pond (~5 m from the edge) over a 6 week period in the austral spring (between September and October 2006). Daily maximum surface irradiance (PAR) was ~2000 μmol photons m^{-2} s^{-1}. The design incorporated a control location in the inlet channel (i.e. one non-effluent block). Environmental variables (means±1SD) quantified over the 27 days during the experiment were as follows; temperature=26.5±3 °C, salinity=37.5±0.3 ppt, pH=8.46±0.45, and a maximum total nitrogen 1.80 mg L^{-1}.

As water is released from the settlement pond on a tidal frequency, and because we wished the algae to remain subtidal at all times, submerged trays (2 m×1 m plastic culture tray: Tooltech Pty Ltd, Australia) secured beneath PVC-floats were used as culture vessels. To ensure that an appropriate depth for culture of *Caulerpa* was used for the present study and for future culturing, algae were deployed at three different depths (PVC spacers separated the tray from the float by 4 cm, 15 cm and a maximum of 30 cm below the water surface). One tray per depth was deployed at each block. Two fragments of each *Caulerpa* species were placed on each tray in a random order and secured by loosely fitted cable ties.

2.4. Influence of seeding size and culture depth in pond culture

As a result of the widespread loss/death of experimental fragments in the initial pond experiment (see Results), we investigated the potential limitation of fragment size by repeating the experiment with similar sized fragments as well as a larger seeding size. For this experiment only two *Caulerpa* species were used (*C. taxifolia* and *C. lentillifera*), based on their performance in the pond experiment and in long term culture at James Cook University.

This experiment ran for 30 days over April and May in the austral Autumn. The two size classes differed for *C. taxifolia* and *C. lentillifera* (due to their different morphologies) but were similar in that the larger size was approximately ten times the size of the smaller treatment. Average initial sizes (±1SD) for the experiment were *C. taxifolia* (small=1.9 g±0.4; large=12.6 g±2.0) and *C. lentillifera* (small=6.4 g± 1.3; large=60.3 g±10.6). Because growth was previously limited at the shallowest (4 cm) depth (Section 2.3), this experiment assessed persistence and growth of *Caulerpa* at two different depths (15 cm and 30 cm) at four of the previously used positions (blocks) around the settlement pond (3 of the previous 7 blocks, e.g. Section 2.3, were not used because of excessive cover by bloom-forming algae). Two replicates of each species by size were attached to a 15 cm tray and a 30 cm tray at each of three blocks on the pond periphery (a fourth block only had a single replicate of species by size per depth). Two replicates of each species by size were also deployed at 15 cm and 30 cm depths in the inlet channel to serve as an effluent control.

2.5. Nitrogen and carbon analysis

Fresh samples from both tank and pond experiments were weighed and oven-dried at 65 °C for approximately 48 h or until no further weight change. Nitrogen and carbon contents (% dry weight) were quantified by isotope analysis using a Carlo–Erba elemental autoanalyser (Environmental Biology Group, Australian National University, Canberra).

2.6. Data analysis

Growth is reported as specific growth rate (SGR), a log-function of the proportionate change in size (weight) over time (SGR=(lnWf−lnWi)*100/*t*; where lnWf=the natural logarithm of the final weight, lnWi=the natural logarithm of the initial weight, *t*=time (days) between lnWf and lnWi). This function incorporates any changes in growth that are due to the initial size of the alga. For experiments in which treatment averages represented a net loss in biomass (and any remaining thalli were healthy), these data were calculated using the above equation but referred to as "% change day^{-1}".

All experiments were designed to be analysed by multiple factor blocked (mixed model) ANOVAs. Growth and nutrient content of algae from the two different controlled systems were analysed separately by 3-factor blocked ANOVAs. The full table of analysis is listed in Results, but note that "Tank" is the blocking factor and the "Tank(System)*-Species" term is the MS error term for all of the fixed factors and their interactions. Dummy values for three lost samples (two *C. serrulata* and one *C. racemosa* var. *laetevirens*) were included to balance the blocked ANOVA and degrees of freedom adjusted accordingly from 41 to 38. Fig. show average growth calculated from tank means with corresponding standard errors (*n*=4 per species per tank).

Due to poor persistence in the pond, growth data from the in situ trials (Sections 2.3 and 2.4) were not analysed statistically, although relevant mean growth rates are reported. For Section 2.3, pond growth rates reflect only those individuals that were alive at the end of the experiment and had in fact grown. Persistence (% remaining) of each species in the pond was determined for each tray at each depth (i.e. a maximum of two individuals possible=100%) and averaged between blocks (*n*=7) around the settlement pond. For the second pond trial (Section 2.4), growth data included all individuals that had persisted. Persistence in Section 2.4 was analysed using chi-square testing the effect of fragment size by species, fragment size by depth and species by depth.

For the tank cultures, nitrogen content of one replicate of each species from each tank (out of the possible two) was determined and analysed by ANOVA (as per above, but modified for a single replicate species per tank). This enabled us to quantify the variation in nitrogen content, per unit fresh weight (fw) and dry weight (dw), under different environmental conditions. Pond samples were analysed where possible (see Results).

3. Results

3.1. Field sampling

The presence of the five members of the genus *Caulerpa* on shallow subtidal reefs varied between the five sites, despite their close proximity (i.e. adjacent reefs and separation of less than 10 km). *C. taxifolia* dominated the two mainland sites, Kissing Point and The Strand, and two other species *C. racemosa* var. *laetevirens* and *C. lentillifera* were collected at Kissing Point, although both were far less abundant than *C. taxifolia*. Two different species were encountered at the three Magnetic Island sites — *C. racemosa* and *C. serrulata*. *C. serrulata* was found at all three island sites, Cockle Bay, Picnic Bay and Nelly Bay. *C. racemosa* and *C. lentillifera* were collected at Cockle Bay and both *C. racemosa* and *C. racemosa* var. *laetevirens* were collected from Nelly Bay. Interestingly *C. taxifolia* was not found at any of the three Magnetic Island sites, despite being separated from Kissing Point by only 10 km.

3.2. Tank culture

There was a significant interaction between the growth rates of different *Caulerpa* species and the tank system in which they were cultured ($P<0.001$, Table 2; Fig. 2a), specifically, four of the five algae grew better under high nutrients (high N) whereas one alga *C. lentillifera* did not, and actually grew better under low N (Fig. 2a). Growth rates of both varieties of *C. racemosa* were very high under high N (SGR of ~7.5% day^{-1}), and after 15 days each variety was more than four-times its initial mean size. Even the slower growing *C. taxifolia* and *C. serrulata* had

Table 2
Outputs for 3-factor split-plot, partly nested ANOVAs testing the effect of two different tank-based culture systems on the growth (specific growth rate) and nitrogen content (fresh weight) of five different species of *Caulerpa*

Source	*df*	MS	*F*	*P*
Growth				
System	1	112.121	29.78	0.002
Tank(System)	6	3.764	2.29	0.056
Species	4	36.791	17.38	<0.001
System*Species	4	24.063	11.37	<0.001
Species*Tank(System)	24	2.117	1.29	0.239
Error	37	1.644		
Nitrogen content				
System	1	0.0107	36.08	<0.001
Tank(System)	6	0.0003	1.35	0.275
Species	4	0.0361	163.39	<0.001
System*Species	4	0.0030	13.73	<0.001
Error	24	0.0002		

Table 3
Mean values (and standard errors) for fresh to dry weight ratio, %N dry weight, %C dry weight of five species of *Caulerpa* cultured in two tank culture systems, high nutrient (high N) and low nutrient (low N)

System	Species	Wet:dry		%N dry mass		%C dry mass	
		Mean	SE	Mean	SE	Mean	SE
High N	*C. lentillifera*	31.7	2.5	1.203	0.103	11.10	0.80
Low N	*C. lentillifera*	25.4	1.5	**0.720**	0.221	15.54	4.00
High N	*C. serrulata*	12.8	0.4	2.293	0.138	22.82	2.25
Low N	*C. serrulata*	12.7	1.0	1.308	0.102	27.20	1.36
High N	*C. taxifolia*	12.0	0.5	**2.378**	0.041	28.10	0.95
Low N	*C. taxifolia*	**9.8**	1.0	1.165	0.030	**29.22**	0.61
High N	*C. racemosa*	**33.6**	3.3	0.958	0.180	12.53	2.37
Low N	*C. racemosa*	33.3	4.0	0.825	0.056	15.66	1.42
High N	*C. racemosa* (*laetevirens*)	31.6	2.6	0.878	0.045	**10.87**	0.41
Low N	*C. racemosa* (*laetevirens*)	20.9	1.2	0.583	0.083	15.47	1.50

Highest/lowest values (**bold**) are highlighted (n=7–8 per species × system for wet:dry ratios, n=4 per species × system for %N and %C). Note: similar data were not analysed for the pond trial due to poor persistence of all species, however, where possible average values of remaining fragments are reported in the text (Section 3.3).

doubled in size over this time in the high N system (3.6% and 4.1% day^{-1}, respectively). In contrast most species had very low specific growth rates in the low N tanks, in particular *C. taxifolia* (1.1% day^{-1}, Fig. 2a).

There was also an interactive effect of system (nitrogen level) and *Caulerpa* species on nitrogen content ($P<0.001$, Table 2). *C. serrulata* and *C. taxifolia* increased internal content of nitrogen per unit fresh weight by almost 100% from the low N to high N system (0.11% to 0.18%, and, 0.12% to 0.20%, respectively) whereas both *C. racemosa* varieties had no obvious changes in content (Fig. 2b). Variation in the wet to dry weight ratio for *Caulerpa* spp. generally increased from low to high N culture, and this increase was substantial for both *C. racemosa* var. *laetevirens* (50% increase) and *C. lentillifera* (25% increase) (Table 3). Variation in the wet to dry weight ratios between *Caulerpa* spp. meant that % nitrogen per unit fresh weight is the best comparative measure of nitrogen removed from the tank system per unit of growth (e.g. Fig. 2b). However, dry weight (dw) nitrogen content illustrated an even more pronounced difference between the two systems (Table 3). For example, the nitrogen content *C. taxifolia* of 2.38% dw under high N was more than double that of the low N system (1.17% dw; Table 3). The lowest overall nitrogen content was for *C. racemosa* var. *laetevirens* in the low N system where only 0.58% of dw was nitrogen (Table 3).

3.3. Pond culture

During the course of the pond experiment it became apparent that other factors were influencing algal culture in the settlement pond, the most conspicuous being smothering by filamentous green algae. Persistence (Fig. 3a; % of fragments remaining after 6 weeks) between the seven settlement pond blocks was zero at the shallowest (4 cm) depth treatment for all species other than *C. serrulata* (19%±9SE). However, in most cases persistence increased markedly for all *Caulerpa* spp. from a culture depth of 15 cm to 30 cm. For example, persistence doubled for *C. taxifolia* and *C. racemosa* from 15 cm to 30 cm, whereas *C. lentillifera* and *C. racemosa* var. *laetevirens* were

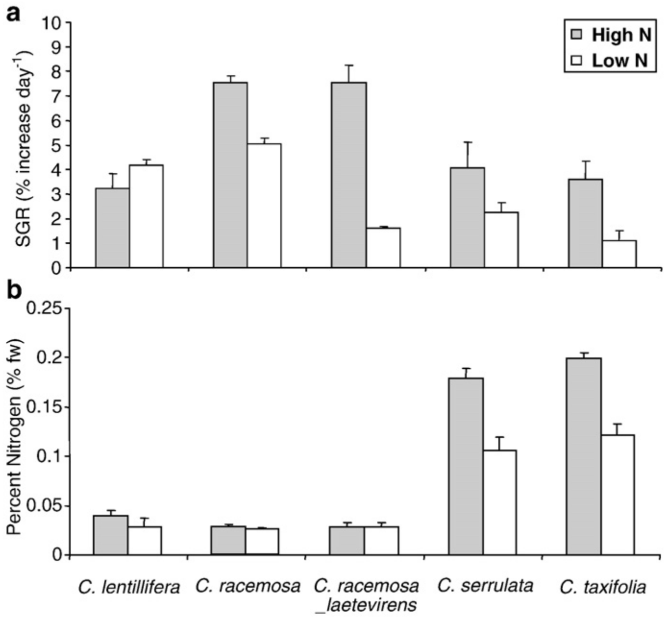

Fig. 2. Growth (a) and nitrogen content (b) of *Caulerpa* species cultured in two tank-based systems with marked difference in nutrients; System 1 = high nutrient, System 2 = low nutrient (means + 1SE, n=4 tanks per system). *Caulerpa* species had varied responses in regards to growth and nitrogen content when cultured under different conditions (ANOVA: species*system, $p<0.001$).

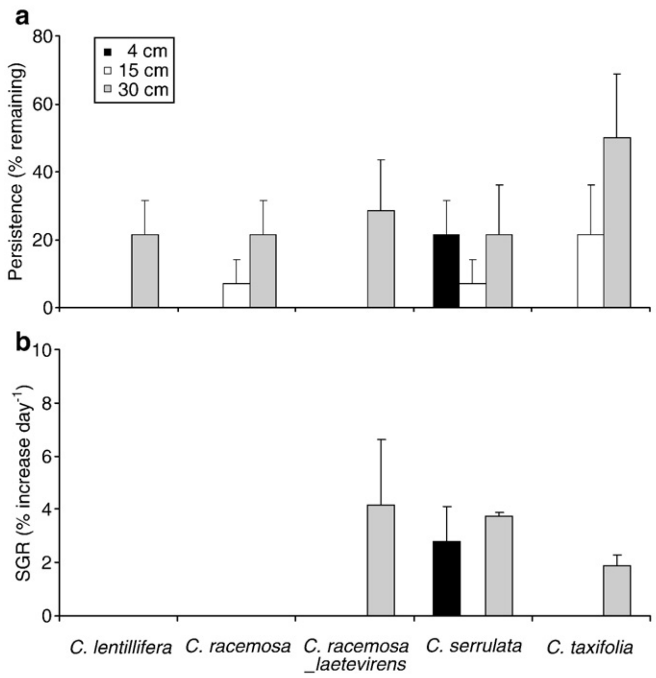

Fig. 3. Persistence (a) and growth (b) of *Caulerpa* species in situ in the settlement pond of a fish farm. (a) Persistence means (+1SE) calculated from tray means for each depth (4 cm, 15 cm and 30 cm) at 7 positions (blocks) around the pond (note: control site at inlet channel not included). (b) Growth of fragments in situ at fish farm settlement pond (n=7 trays per depth). Only two depths had fragments that persisted and increased in size (4 cm and 30 cm). Fragments that decreased in size were not included in calculations.

only present at the 30 cm culture depth. *C. taxifolia* was the most hardy of all *Caulerpa* species at these depths with persistence of 25%±13.3SE at 15 cm, and 56.2%±17.5SE at 30 cm.

Due to poor persistence, calculated growth rates reflect only individuals that were both alive and had in fact grown during the 6 week period (in order to assess possible growth maxima). Growth (SGR) varied between *Caulerpa* spp. in the pond. Growth of *C. serrulata* was as high at the shallowest depth (where few other species survived) as at the deepest (30 cm), averaging ~3% day^{-1} (Fig. 3b). Two of the remaining species grew well at the lowest depth (30 cm), in particular *C. racemosa* var. *laetevirens* which had a high but variable growth rate (~4% day^{-1}) and *C. taxifolia*, at a lower growth rate of 2% day^{-1} (Fig. 3b). The only species that persisted and grew well in the inlet (control) channel was *C. lentillifera*, with a growth rate of 3% (2.95±0.9 SE, $n=3$), averaged across the 15 cm ($n=1$) and 30 cm ($n=2$) culture depths. The only other species that persisted on the control trays was *C. taxifolia* (15 cm=−1.35% day^{-1}, $n=1$; 30 cm=−2.07% day^{-1}±2.21 SE, $n=2$).

The desired comparisons of nitrogen between species and depths could not be formally analysed because of poor persistence in the pond. However, some observational data from the remaining fragments indicate that nitrogen values varied between species but did not vary between depths. For example, *C. serrulata* had a nitrogen content (dry weight, dw) of 2.62% (±0.13 SE) at 4 cm very close to the 2.65% (±0.10 SE) at 30 cm. The highest values of % N dry weight were obtained for *C. taxifolia* ($n=7$) % N=2.88 dw±0.10SE, which was higher than the equivalent dry weights in the high N tank culture (see Section 3.2: 2.38%±0.04SE; Table 3). The only other species to record substantial growth in situ was *C. racemosa* var. *laetevirens* and it had an average nitrogen content of 1.95% dw (±0.04SE), more than double that of the high N tank culture in Section 3.2 (0.88%±0.05SE; Table 3). The lowest nitrogen content (0.83% N dry weight±0.03SE, $n=3$) came from *C. lentillifera* cultured in the control inlet channel (pre-effluent, i.e. indicating low nutrients in the inlet water) but this was higher than the corresponding low N tank cultures (see Section 3.2: 0.72%±0.22SE; Table 3).

3.4. Influence of seeding size and culture depth in ponds

Overall, *C. taxifolia* had higher persistence in the pond (21 out of 28 fragments, 78%) than *C. lentillifera* (11 of 28, 47%; chi-square=7.29, $P=0.007$), and there was no interactive effect on persistence between species and initial size (chi-square=1.85, $P=0.174$) or between species and culture depth (chi-square=0.01, $P=0.907$). A higher tolerance of *C. taxifolia* for pond conditions is consistent with the previous pond experiment (Section 3.3).

Growth data could not be analysed in a factorial manner due to missing values at each block, and means±standard errors are reported only for the deeper (30 cm) culture trays (Fig. 4a) to permit comparisons with the previous pond results (Section 2.3). Growth was low for all combinations of species, fragment sizes and culture depths, with a maximum growth rate of just more than 1% per day for large pieces of *C. taxifolia* (Fig. 4a). However, many fragments decreased substantially in size, and larger fragments of *C. lentillifera* had the lowest growth rates of all treatments (Fig. 4a). No direct comparisons can be made between *C. taxifolia* and *C. lentillifera* as there were different starting masses based on morphology, but *C. taxifolia* grew better on average (across sizes) in deeper trays (30 cm; 0.7% day^{-1}) than shallower trays (15 cm; −0.2% day^{-1}) whereas *C. lentillifera* growth rates were essentially similar (−5.5% day^{-1} versus −5.2% day^{-1}).

In the inlet channel (pre-effluent control), fragments remained on the trays for the duration of the experiment, however, growth was negative for all replicates (Fig. 4b). Growth of larger fragments was more than five-times lower, on average, than smaller fragments for both species (Fig. 4b). It is notable that the trend of a decrease in growth rate of small *C. lentillifera* fragments moving from the control to pond blocks (−1.23%±0.63SE versus −3.78%±0.47SE; *t*-test, $P=0.083$, $n=2$) is similar to a decrease in growth rate moving from low N to high N tank-based culture (Section 3.2).

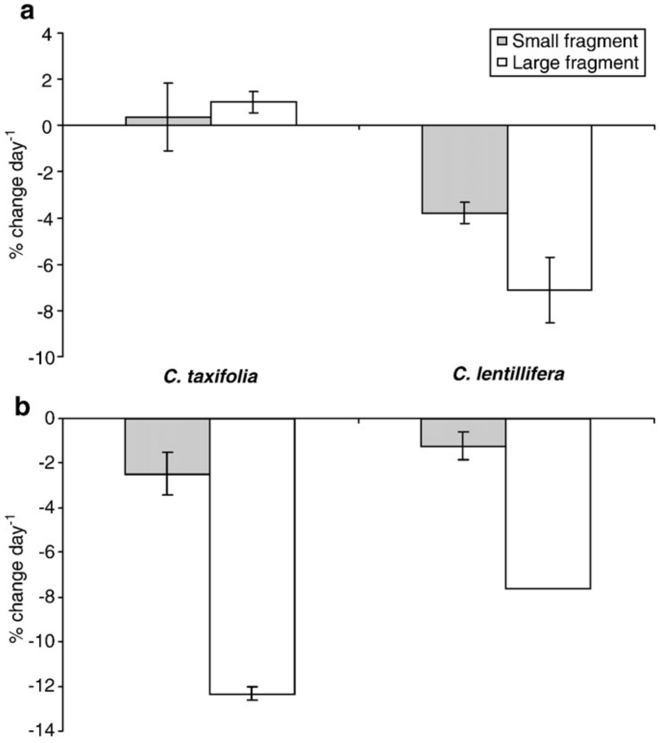

Fig. 4. Percentage change in biomass per day of two *Caulerpa* species (*C. taxifolia* and *C. lentillifera*) seeded at two different initial sizes (small and large). (a) Rate of change in biomass in settlement pond at 30 cm depth after 30 days. Means (±1SE) calculated from all remaining fragments (*C. taxifolia*, $n=5$ fragments per size; *C. lentillifera*, large fragments $n=4$, small fragments n=2). (b) Rate of change in biomass in a control inlet channel, $n=2$ per treatment (except *C. lentillifera* large, $n=1$).

4. Discussion

Common local seaweeds from the genus *Caulerpa* collected from nearshore reefs in northern Australia grew well and have promise as biofilters in integrated tank-based systems that are relevant to many forms of intensive aquaculture. Furthermore, some species of *Caulerpa* (*C. taxifolia* and *C. serrulata*) had luxury uptake of nitrogen, equating to a greater amount of nitrogen removed per unit mass when cultured in a high nutrient environment. However, there are clear pitfalls for the integrated culture of *Caulerpa* spp. in nutrient-rich settlement ponds of fish farms, as persistence and growth was largely restricted to only one species, *C. taxifolia*. This highlights the difference between the relatively clear water tank-based systems and an intensive aquaculture settlement pond, which was essentially a eutrophic mesocosm. The mesocosm used in these experiments (an operational settlement pond) contained bloom-forming, filamentous (‘green tide’) algae which had a major impact on the culture of *Caulerpa* and potentially any other large fleshy macroalgae common to integrated practices (e.g. *Gracilaria* spp.). The incidental culture of green tide algae will strip nutrients from aquaculture effluent – the intended role for integrated seaweed culture – but managing green tide algae will require ecological data, some of which can be obtained from studies of eutrophic estuaries (e.g. Raven and Taylor, 2003). Regardless, the use of green tide algae warrants serious consideration given their ability to thrive in aquaculture pond mesocosms.

Our assumption that *Caulerpa* would be suitable for integrated aquaculture was based on its ability to fragment, grow in high density stands and, through phenotypic plasticity, adapt to new environments (Collado-Vides and Robledo, 1999; Smith and Walters, 1999). This assumption was wrong with respect to growth in their present format in pond-based tropical aquaculture. The nutrient-rich settlement pond may have been too different to local reef conditions, and the overwhelming dominance of filamentous algae was a major factor limiting growth of *Caulerpa*. Furthermore, only a small portion of *Caulerpa* fragments persisted in the pond (no more than 50% of fragments of any one species at any culture depth). This is not an efficient or reliable form of biofiltration, especially as failed fragments may return nitrogen to the system through decomposition (e.g. Hanisak, 1993). The loss of *Caulerpa* fragments in the settlement pond is unlikely to be due to the fragmentation process, as fragments of *Caulerpa* much smaller than those used here are viable propagules (Smith and Walters, 1999; Khou et al., 2007) and few fragments failed to grow in the tank-based experiments.

All *Caulerpa* species grew well in the tank-based systems, especially the high nutrient system, as have most algal species tested for integrated aquaculture to date (e.g. Matos et al., 2006; Schuenhoff et al., 2006). Growth rates of more than 5% SGR for *Caulerpa* are higher than some species commonly used in integrated aquaculture (Msuya et al., 2006; Hernández-González et al., 2007) and comparable to others (Nagler et al., 2003; Hernández et al., 2006; Pereira et al., 2006), but not as high as the bloom-forming filamentous algae (Raven and Taylor, 2003). However, direct comparisons of algal potential for integrated aquaculture should not be based solely on growth because luxury uptake of nitrogen occurs in some algae (reviewed by Harrison and Hurd, 2001). High growth and high nitrogen content were not mutually exclusive for *Caulerpa*, as certain species had much higher nitrogen content in high nutrient culture than in low nutrient culture (doubling for *C. taxifolia* and *C. serrulata*). This is important to integrated aquaculture, as selecting species with high growth rates and high nitrogen content will maximize removal of soluble nitrogen from effluent. Our data indicate that growth rate comparisons between algae in the same genus cannot solely be used to predict the biofiltration potential due to interspecific variation in the extent of luxury uptake of nitrogen.

Environmental variables other than nitrogen will limit targeted culture of *Caulerpa* in integrated aquaculture. For instance, some types of *Caulerpa* appear to have season specific responses (e.g. Ceccherelli and Cinelli, 1999). But given consistently high temperatures in the tropics (Townsville sea temperatures remains between 22 and 31 °C; Kenny, 1974), it is more likely that interactions between environmental variables will affect nitrogen removal capabilities, as demonstrated for other integrated seaweed culture (e.g. Pereira et al., 2006). Some evidence for this is a lack of growth and persistence of *Caulerpa* fragments at the shallowest culture level in the pond (only *C. serrulata* did well in this treatment), despite that all original samples were collected from shallow (often intertidal) conditions in nature. Manipulating environmental factors, such as light, may facilitate growth of *Caulerpa* in the pond, but would need to be done in tandem with mechanisms to limit smothering by filamentous algae, for example maintaining high target seaweed density.

We have highlighted the promise of targeted growth of *Caulerpa* in tropical aquaculture, but also pitfalls associated with their culture in settlement ponds. Poor pond culture occurred despite *Caulerpa* being produced commercially in disused shrimp farms in the Philippines (Horstmann, 1983). But it seems that competition between resident green tide algae in the settlement ponds of operating farms makes culture of large fleshy macroalgae difficult, and analogous problems exist for other attempts of integrated aquaculture (Haglund and Pedersén, 1993; Blouin et al., 2007). Some effort to protect seaweeds with commercial interest has been made, for example, fouling by epiphytes was limited by copper dosing (Haglund and Pedersén, 1993) or pulsed input of nutrients (Capo et al., 1999). For similar reasons the culture of valuable “sea grape” *Caulerpa* varieties (*C. racemosa* and *C. lentillifera*) could justify future investment in refined culture techniques, examining the effects of larger scale cultures rather than smaller fragments.

Two clear outcomes for integrated aquaculture in the tropics come from this study, the suitability of *Caulerpa* to tank-based cultures or modified settlement ponds with staged or tiered filtration to remove competing organisms, and, the potential role for filamentous green tide algae in pond-based tropical aquaculture. Integrated tank-based culture of *Caulerpa* should focus on species with high growth rates and high nitrogen content but also assess the culture of valuable species, possibly culturing different *Caulerpa* species in tandem given their unique responses to different culture systems. For example, *C. taxifolia* will be best in high nutrient environments whereas *C. lentillifera* will be best in low nutrient environments. Because bloom-forming green tide algae have high growth rates and are free-floating, these seaweeds offer to be an excellent option for culture in settlement ponds, the most common bioremediation infrastructure in tropical aquaculture. The concept of a robust and cost-effective means to remove nutrients is vital to tropical pond-based aquaculture, in particular in northern Australian, as waste management will severely limit industry productivity if suitable options for integrated aquaculture are not developed.

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