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Integrating filamentous ‘green tide’ algae into tropical pond-based aquaculture

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‘Green tide’ algae bloom in eutrophic environments with fast growth rates and efficient nutrient uptake. These same characteristics are sought after for algae in integrated aquaculture systems. We examined the effect of two key variables, salinity and total ammonia nitrogen (TAN), on the growth of three filamentous ‘green tide’ algae, *Cladophora coelothrix*, *Chaetomorpha indica* and *Ulva* sp. Survival and growth were first determined across the extremes of salinity in ponds (0 to 45‰). Subsequently, the interactive effects of salinity (15, 36 and 45‰) and TAN (0–700 μmol l^{-1}) were quantified using a factorial design. All species have a broad tolerance to salinity (ranging from 5 to 45‰) with each having a different optimum for growth (*C. coelothrix* 30‰, *C. indica* 20‰ and *Ulva* sp. 15‰). A significant interaction (salinity*TAN*species) further demonstrated that responses vary between algae. *C. indica* and *Ulva* sp. have their highest growth rates of ~14% day^{-1} and ~25% day^{-1} respectively at higher TAN levels (>70 μmol l^{-1}) but at different salinities. Growth of *C. coelothrix* was optimum at lower TAN levels (35 μmol l^{-1}) and 36‰, but surprisingly was inhibited at the highest TAN. *C. coelothrix* and *C. indica* were also cultured in an operational bioremediation pond. High growth rates of *C. coelothrix* were maintained *in situ* (~6% day^{-1}), while *C. indica* performed poorly. Finally, TAN removal rates were calculated using natural densities and *in situ* growth rates demonstrating that ‘green tide’ algae have broad application across the environmental variables that characterize tropical pond-based aquaculture.

1. Introduction

Integrated aquaculture, the sequential use of species to optimize nutrient use, can produce sustainable and cost-effective operations that reduce the environmental impacts of aquaculture effluents (Chopin et al., 2001; Neori et al., 2004; Lu and Li, 2006; Crab et al., 2007). The incorporation of algae in these systems to remove dissolved nutrients is highly efficient with model bioremediation genera such as *Ulva* (Chlorophyta, Ulvaceae) removing up to 80% of dissolved ammonium in aquaculture wastewaters (Neori et al., 2000). A range of algal species have also been targeted, often based on their ability to value-add an additional product (Hernandez et al., 2002; Martínez-Aragón et al., 2002; Matos et al., 2006; Schuenhoff et al., 2006; Zhou et al., 2006; Yang et al., 2006). However, the diversity of species trialed in bioremediation systems to date is very limited and the majority of these studies have focused on temperate aquaculture systems. Consequently the focus of bioremediation has also been on temperate species of algae (Chopin et al. 2001; Neori et al. 2004; Blouin et al. 2007). The potential use of integrated systems in tropical aquaculture is enormous, particularly as the majority of production is pond-based with a resultant point-source discharge.

While the scale and structure of tropical aquaculture provides exceptional opportunity for integrated development to minimize nutrient discharge, the operational parameters affecting the selection and implementation of algae are significantly different from those affecting temperate systems. Pond-based systems also provide different operational considerations to cage and tank-based systems that have been the focus of integrated systems. Tropical pond-based aquaculture systems are typically characterized by large fluctuations in salinity and nitrogen availability, while in contrast to temperate systems, light and temperature are relatively stable. Seasonal (monsoonal wet season) rainfall and high levels of evaporation (dry season) result in salinity changes from 5‰ to 45‰ while management practices such as feeding rate, water flux and exchange rates (also significantly affected by seasonal rainfall) result in variations of nutrient levels in effluent, in particular total ammonia nitrogen (TAN) (Boyd, 2003; Metaxa et al., 2006). Ultimately any algal species to be used in tropical integrated aquaculture systems must have a broad tolerance to fluctuating environmental conditions. Furthermore, species with promise need to be assessed *in situ* in bioremediation ponds, in the early stages of the evaluation process, as the transfer from controlled conditions to ponds can provide contrasting outcomes (Paul and de Nys, 2008).

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One mechanism to select suitable species for integration into tropical pond-based aquaculture is to capitalize on the process of "natural" selection in the ponds themselves, especially as flow-through facilities deliver unfiltered water, with associated biota from adjacent coasts, to the bioremediation ponds. Abundant endemic species present in pond-based mesocosms offer a wide pool of suitable species to select and develop for bioremediation that may not be obvious in local nutrient-poor environments. Two of these species, *Cladophora coelothrix* and *Chaetomorpha indica*, are often referred to as 'green tide' algae because of their excessive nuisance growth under eutrophic conditions (Taylor et al., 2001; Raven and Taylor, 2003). They have fast growth and efficient nutrient uptake, as well as a tolerance of low dissolved oxygen, low pH and high levels of nutrients in the water (Morand and Merceron, 2005), all of which are essential for pond-based integrated aquaculture. However, the implementation of such species into integrated aquaculture systems remains dependent on their success (growth) under *in situ* environmental parameters (Chopin et al., 2001; Troell et al., 2003).

Given the need to identify algal bioremediation species that tolerate and grow well across the broad range of salinity and nutrients characteristic of tropical pond-based aquaculture we quantify the efficacy of 'green tide' algae in the bioremediation of aquaculture effluent. The tolerance and growth of three species of filamentous green algae, *Cladophora coelothrix* Kützing, *Chaetomorpha indica* (Kützing) Kützing and *Ulva* sp. are measured in response to a broad salinity gradient, and subsequently in response to the interaction between salinity and nitrogen (TAN) in laboratory studies. We then quantify growth *in situ* under operational conditions. Data from controlled environment and field are then used to calculate potential seaweed productivity and nitrogen extraction from aquaculture effluent under operational conditions.

2. Materials and methods

2.1. Study organisms: 'green tide' algae

Three species of 'green tide' filamentous algae (all Ulvophyceae) were used in experiments. *Cladophora coelothrix* Kützing and *Chaetomorpha indica* (Kützing) Kützing (hereafter *Cladophora* and *Chaetomorpha*) are related algae in the family Cladophoraceae. *Cladophora* species form dense mats composed of entangled branched filaments on the water surface, and ascending filaments from the water column that are loosely attached to the benthos. *Chaetomorpha* species have unbranched filaments as masses of long strands, or are entangled with other seaweed species. These two algae co-exist in floating mats on-site at Good Fortune Bay Fisheries Ltd. (Fig. 1A). Both species have uniseriate cell growth patterns (Womersley, 1984). *Ulva* sp. (hereafter *Ulva*) (Ulvaceae) is part of a broad species complex with the genus *Enteromorpha* (Hayden et al., 2003) and has diffuse cell growth forming foliose and tubular green filaments, which are attached or free floating (Womersley, 1984).

2.2. Sample collection

Algal samples were collected from a sedimentation pond of Good Fortune Bay Fisheries Ltd., a land-based, intensive salt water barramundi (*Lates calcarifer* Bloch) farm, Bowen, Australia (Latitude: 20.02 S Longitude: 148.22 E) from February to May of 2007 (Fig. 1A). The site has a mean annual temperature of 30 °C. *Cladophora* and *Chaetomorpha* co-exist and were collected from extensive floating mats within the pond. *Ulva* was collected from the same pond attached to the bottom and rocks. Algae used for experiments were transported in plastic bags filled with seawater and kept in the outdoor seawater recirculation system tanks with constant aeration at James Cook University — Marine and Aquaculture Research Facilities Unit. Isolated cultures of each species were maintained during the period of the experiments.

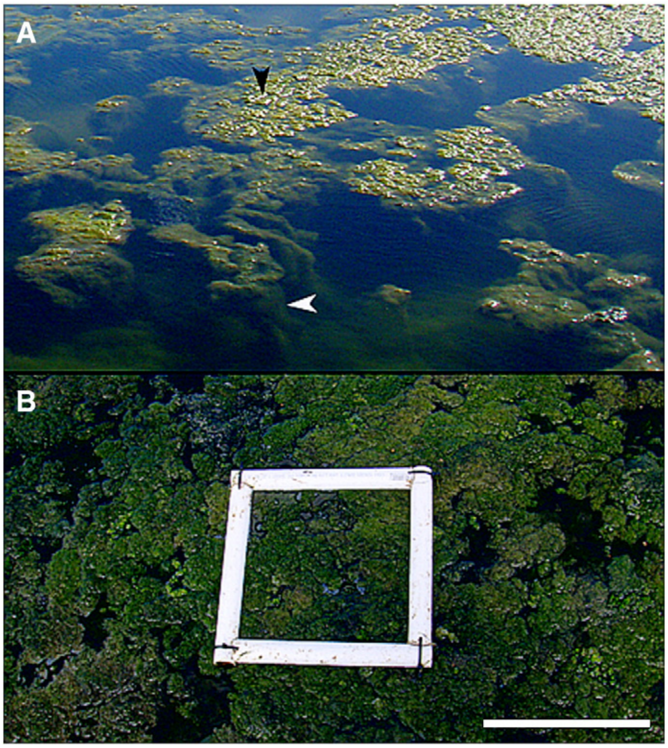

Fig. 1. Images of 'green tide' algae and bioremediation pond. (A) Floating mats (black arrow) at the pond surface. Mats can be thick (white arrow) indicating the large biomass that can be contained per unit area. (B) Quadrat used to sample biomass per unit area for growth calculations in the logistic model (Section 3.4). Density of the 'green tide' mats was up to 3000 g FW m^{-2}. Scale bar, 25 cm.

2.3. General culture conditions and methods for growth experiments in controlled environment

Filaments of each species were rinsed with filtered seawater to remove any visible epiphytes. Small pieces of algae were cultured in Petri dishes in a controlled temperature room (30±3.0 °C) and mean light intensity of 100 μmol photons m^{-2} s^{-1}. The dishes were placed under light and were repositioned daily to reduce any potential effect of light variation. Growth was measured using images taken on a dissecting microscope (Leica MZ125) at the beginning and end of each experiment. Due to morphological characteristics (refer to Section 2.1), length (mm) was used to quantify the growth of the uniseriate filaments of *Cladophora* and *Chaetomorpha*, whereas area (mm^2) was used to quantify the growth of the diffuse thallus of *Ulva*. Both length and area were measured using the software Leica Image Manager 5.0. Specific growth rates (SGR) were calculated from initial length or area (Li) and final length or area (Lf) using the following equation:

$$\mathrm{SGR} = 100\,[\ln(\mathrm{Lf}/\mathrm{Li})/t] \quad (1)$$

Culture experiments were run for a minimum of 5 days. This timeframe is suitable given the ability of filamentous algae to grow rapidly under a broad range of environmental conditions (Cohen and Fong, 2004) and provides for large effects (see Results).

2.4. Effect of salinity on growth

As 'green tide' algae can tolerate environments ranging from fresh to marine water (Taylor et al., 2001), growth rates of the three species were examined across a range of salinities, from 0‰ to 45‰ with increments of 5‰. Similar sized pieces of algae with a mean length of 5 mm for *Cladophora* and *Chaetomorpha*, and a mean area of 1.5 mm^2 for *Ulva*, were excised from the seaweed samples. Filtered sterile seawater (37‰) (as measured by refractometer) was used as basal culture medium. Salinity treatments less than 37‰ were prepared by diluting this medium with distilled water, while higher salinity treatments were made through the addition of artificial seawater salt (Aquasonic®). The medium for the 0‰ treatment was distilled water.

All algae were cultured in plastic culture dishes containing 5 ml of medium (n=5 replicates of each species per salinity treatment). Nutrients (nitrogen as NH_4Cl and phosphate as $NaH_2PO_4{\cdot}H_2O$) were added to the culture dishes daily to ensure that growth was not limited. Distilled water (0.4 ml) containing 370 µmol l^{-1} of TAN (NH_3^-/NH_4^+) and 22 µmol l^{-1} of PO_4^- was added daily as a source of nutrients, and this also served to adjust for increases in salinity resulting from calculated evaporation rates. The photoperiod used was 14L:10D, which is the annual average *in situ*. Growth (SGR) was measured after 6 days of culture.

2.5. Interactive effects between nitrogen (TAN) and salinity on growth

High variations of TAN in tropical aquaculture systems occur due changes in feed supplied to the main cultured species and water exchange. Due to the potential interactive effects of nitrogen and salinity on growth *in situ*, a broad range of nitrogen concentrations were examined 0, 7, 35, 70 and 700 µmol l^{-1} (equating 0, 0.1, 0.5, 1, 10 mg l^{-1} respectively) under three levels of salinity. These were 15‰ (the level at which *Chaetomorpha* and *Ulva* performed well and also the lower limit for *Cladophora*), 36‰ (seawater), and 45‰ (the highest salinity level tested in Section 2.4).

Filaments of a similar size (mean length of 5 mm for *Cladophora* and *Chaetomorpha* and a mean area of 1.5 mm^2 for *Ulva*) were cultured in 75 ml culture vessels containing 30 ml of seawater media (n=6 replicate vessels for each species*salinity*nutrient treatment). Larger containers were used in this experiment to limit the effect of evaporation on the salinity of the culture medium which occurred previously. A photoperiod of 14L:10D was used. Growth (SGR) was measured over 6 days.

2.6. Growth and survival in aquaculture effluent under a controlled environment

As an intermediate step between controlled culture conditions and *in situ* trials we determined the survival and growth of *Cladophora*, *Chaetomorpha* and *Ulva* in water (effluent) sampled directly from an operational bioremediation pond (36‰, 130 µmol l^{-1}–1.8 mg l^{-1} TAN level) and returned to controlled culture conditions. Fragments of *Cladophora*, *Chaetomorpha* and *Ulva* were generated (5 mm and 1.5 mm^2, as described above) and cultured in filtered (0.45 µm) aquaculture effluent for 5 days. Survival (alive/dead) and growth (SGR of surviving fragments) were quantified. These data permitted us to make direct comparisons with laboratory cultures in modified seawater, and to compare growth in aquaculture effluent to data from *in situ* experiment (Section 2.7).

2.7. Growth experiments in situ

To assess bioremediation efficiency, growth rates of the 'green tide' species (*Cladophora* and *Chaetomorpha*) were quantified under standard farm operating conditions (36‰, 130 µmol l^{-1}–1.8 mg l^{-1} TAN level). *Ulva* was not used in this experiment because it requires substratum to attach and grow. Mesh floating cages (7 cm diameter), containing either 0.1 or 0.3 g of each species, were attached to floating frames constructed from PVC pipes. These weights were used as they either sparsely (0.1 g), or completely (0.3 g), cover the water surface in the cage. Ten floats (experimental blocks), each containing two cylinders with 0.1 or 0.3 g of each species (eight cylinders per float), were positioned across the operational bioremediation pond. Specific growth rates (SGR) were calculated after 10 days.

2.8. Biomass measurements in situ

To calculate the potential removal of nitrogen from the effluent in the settlement pond using a growth and harvest scenario the ambient density of algal biomass was assessed to determine natural biomass carrying capacities. Samples were randomly selected from existing algal mats from around the pond. Samples were taken during the wet (monsoonal summer, n=20 in February) and dry seasons (n=8 in August) from the pond using a quadrat (0.067 m^2) to estimate the maximum standing biomass per hectare at each time (Fig. 1B). The samples were only obtained from the floating mats as a conservative estimate, and to provide information on the harvestable (floating) portion of the algae.

The relative abundance of *Cladophora* and *Chaetomorpha* were assessed in each pond sample by viewing sub-samples (n=4) under the microscope. This was necessary as even though these species co-exist in the floating mats they had different growth properties in experiments, which would affect the input into any model. Fresh weights for samples were recorded at the collection site after drying using a salad spinner, and were subsequently oven dried (65 °C for 24 h) to acquire the dry weight for each sample. Percentage nitrogen of the dry mass for each pond sample (n=20) was quantified by isotope analysis using a Carlo-Erba elemental autoanalyzer (Environmental Biology Group, Australian National University, Canberra). This allowed for the calculation of the amount of nitrogen per unit of fresh weight, and also the amounts of nitrogen accumulated as with algal growth.

2.9. Data analysis

Statistical analyses were performed using the software SYSTAT 10. A two-way ANOVA was used to compare the effect of different salinity treatments on the growth of the three species (Section 2.4). The interactive effect of salinity and nitrogen concentrations on the growth rates of the 3 species was analyzed using a 3-factor fixed-effect ANOVA (Section 2.5). The effects of initial size on the growth rates of 2 species at 10 experimental blocks within the pond were analyzed using a 3-factor mixed model ANOVA (Section 2.7). For the ANOVA the assumptions of homogeneity of variance and normality were assessed by scatter plots of residuals and normal curves of residuals, respectively (Quinn and Keough, 2001).

Calculations for the bioremediation potential (nitrogen removal) were made by logistic models using the average seaweed biomass per unit area (carrying capacity) for the more abundant species (*Cladophora*) (Section 2.7), approximated field experiment growth rates (Section 3.3), and the average percentage of nitrogen in the tissue of the samples. Calculations are outlined in detail in Results, including suggested harvesting regimes based on relevant variables for the monsoonal summer, and the dry season (Section 3.4).

3. Results

3.1. Effect of salinity on growth

The growth responses of the three species followed different trends across the range of salinities (Fig. 2), as demonstrated by a significant interaction between the species and salinity (species*salinity, $P<0.001$; Table 1). Optimum growth rates (SGR) occurred at different salinities for each of the species. Growth rates of *Cladophora* were optimum at 30‰ with a specific growth rate greater than 20% day^{-1}. Above this salinity growth decreased as salinity increased, to a minimum at 45‰ (Fig. 2). *Chaetomorpha* had the highest specific growth rate of more than 30% day^{-1} from 15 to 20‰, while *Ulva* had an optimum growth rate of 25% day^{-1} at 15‰ (Fig. 2). In general, all the species had a broad range of salinity tolerance. *Cladophora* grew from 15 to 45‰, and *Chaetomorpha* and *Ulva* had an even broader tolerance

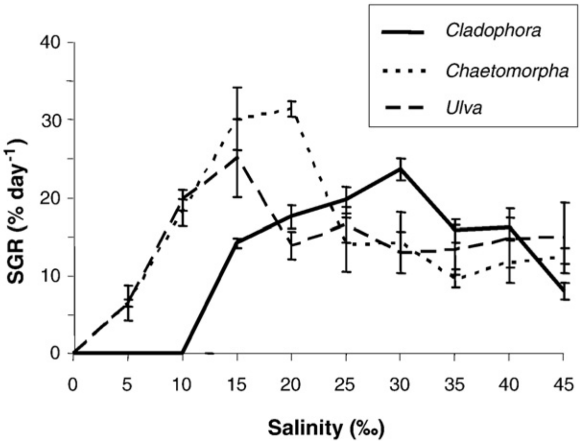

Fig. 2. Mean specific growth rates (SGR) (+1SE) of *Cladophora*, *Chaetomorpha* and *Ulva* in a range of salinities (n=5 for each salinity treatment*species).

growing from 5 to 45‰ (Fig. 2). Freshwater (0‰) represented the lower limit, with no species growing at this salinity (Fig. 2).

3.2. Interactive effects of salinity and nitrogen (TAN) on growth

There was a significant interaction between salinity and nitrogen on growth for the different species of 'green tide' algae (species*salinity*TAN, $P<0.001$; Table 2). This was again (similar to Section 3.1) driven by different optimum growth rates of each species, this time in a specific combination of salinity and TAN level. General growth responses showed that all algae had a broad tolerance across all salinity*TAN treatments, but with clear differences in the growth response curves (Fig. 3A–C). In general, the growth rates of *Cladophora* peaked at median TAN levels (35–70 µmol l^{-1}) but decreased for all salinities at the highest TAN (700 µmol l^{-1}) (Fig. 3A). In contrast, *Chaetomorpha* had a uniform positive growth trend with increasing TAN for all salinities, although at 15‰ the peak was at 35 µmol l^{-1} TAN (Fig. 3B). The growth response of *Ulva* was most variable, increasing almost exponentially with increasing TAN up to 70 µmol l^{-1} at higher salinities, yet the reverse was true at the lower (15‰) salinity (Fig. 3C).

The individual combination growth optima of each species also differed. *Cladophora* had the highest growth rate (~14% day^{-1}) at 36‰ salinity and higher levels of TAN (35 and 70 µmol l^{-1}) (Fig. 3A). The highest growth rates for *Chaetomorpha* (15% day^{-1}) were associated with the highest levels of nitrogen (700 µmol l^{-1}) and were indistinguishable between 36 and 45‰ (Fig. 3B). *Ulva* clearly grew fastest (~25% day^{-1}) at high salinity (45‰) and high TAN (70 and 700 µmol l^{-1}) (Fig. 3C).

3.3. Growth and survival in aquaculture effluent and in situ

Growth rates of *Cladophora* and *Chaetomorpha* were very high when cultured directly in aquaculture effluent (~44% day^{-1}) under controlled conditions (Fig. 4A). Both *Cladophora* and *Chaetomorpha* grew significantly faster than Ulva (23% day^{-1}) (Tukey's HSD, ANOVA, $F_{2,47}$=71.191, $P<0.001$). However, survival of generated fragments varied markedly between all three 'green tide' species, from 100% for *Cladophora*, to 40% for *Ulva*, and to a low 26.6% for *Chaetomorpha* (χ^2=39.3, df=2, $P<0.001$) (Fig. 4B).

Table 1
Output for 2-factor ANOVA testing the response of the three species of 'green tide' algae to changes in salinity

Source	*df*	MS	*F*	*P*
Salinity	8	1052.77	13.97	<0.001
Species	2	2947.46	39.11	<0.001
Salinity*species	16	908.04	12.04	<0.001
Error	108	75.36		

Table 2
Output for the 3-factor ANOVA testing the interactive effect of salinity and TAN (total ammonia nitrogen) on growth of the three species of 'green tide' algae

Source	*df*	MS	*F*	*P*
Species	2	430.06	22.71	<0.001
Salinity	2	31.85	1.68	0.189
TAN	4	154.51	8.16	<0.001
Species*salinity	4	201.09	10.62	<0.001
Species*TAN	8	86.93	4.59	<0.001
Salinity*TAN	8	74.61	3.94	<0.001
Species*Salinity*TAN	16	51.19	2.70	<0.001
Error	152	18.93		

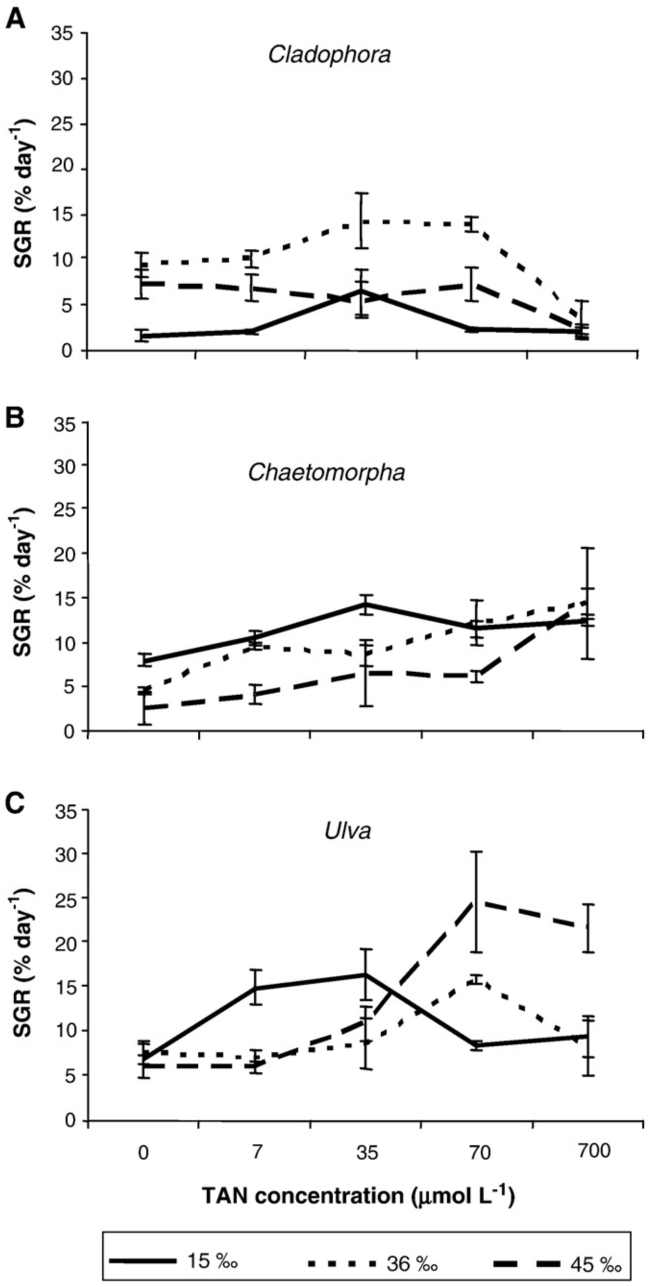

Fig. 3. Mean specific growth rates (SGR) (+1SE) of *Cladophora* (A), *Chaetomorpha* (B) and *Ulva* (C) cultured in different combinations of salinity and total ammonia nitrogen (TAN) concentrations (n=6 for each salinity*TAN*species).

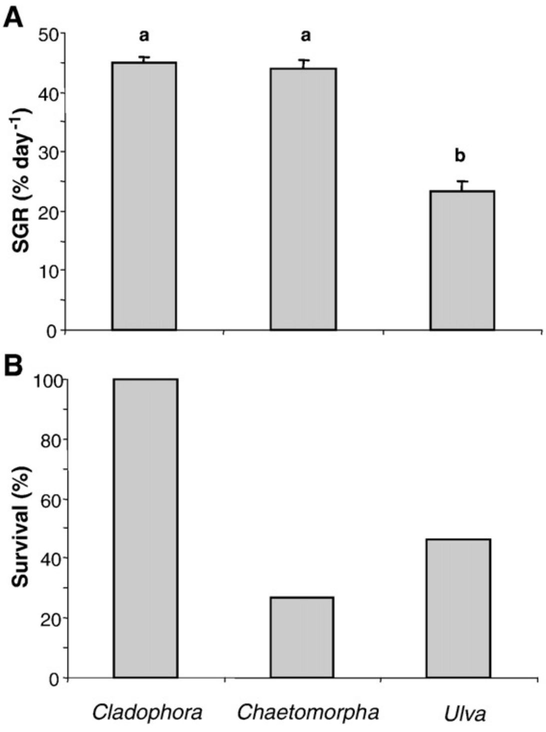

Fig. 4. Mean specific growth rates (SGR) (+1SE) (A) and survival (B) of 'green tide' algae cultured in aquaculture effluent under laboratory conditions (*Cladophora* $n=30$; *Chaetomorpha* $n=8$; *Ulva* $n=14$). Different letters denote significant difference $\alpha=0.05$.

Growth was substantially lower for the *in situ* trial than any of the controlled condition experiments. *In situ* growth rates of *Cladophora* (6.6±2.1% day^{-1} for 0.1 g, 4.9±1.9% day^{-1} for 0.3 g) were much higher than *Chaetomorpha* (−2.4±2.5% day^{-1} for 0.1 g, −6.7±2.7% day^{-1} for 0.3 g) (Fig. 5). In fact, positive growth rates for *Chaetomorpha* were only recorded in 3 of the 40 samples (a maximum individual growth rate of 7.54% day^{-1}), as most of the samples had visibly senesced. Because of the loss of *Chaetomorpha* replicates in the majority of experimental blocks (creating an unbalanced design), we could not analyze growth rates using the desired 3-factor mixed model ANOVA. However, the growth of *Cladophora* could still be analyzed separately, testing the effect of initial size on growth in the 10 blocks around the pond in a 2-factor mixed model ANOVA. There was no effect of initial size on growth of *Cladophora* ($F_{1,8}=0.10$, $P=0.766$; Table 3), however, there was a significant effect of block ($F_{8,15}=5.11$, $P<0.001$). Within-pond (block) variation in growth of *Cladophora* resulted in individual growth rates ranging from −8.5 to 13.6% day^{-1}.

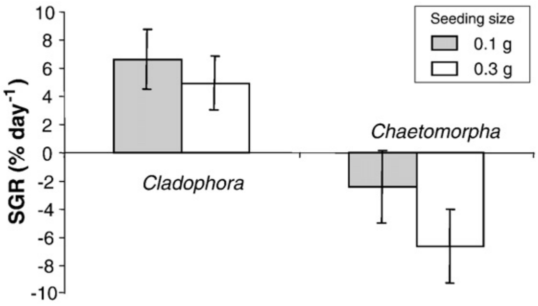

Fig. 5. Mean specific growth rates (SGR) of *Cladophora* and *Chaetomorpha* at two different seeding sizes *in situ* in the bioremediation pond (*Cladophora* 0.1 g samples $n=15$ and 0.3 g samples $n=15$; *Chaetomorpha* 0.1 g samples $n=9$ and 0.3 g samples $n=6$). Different letters denote significant difference $\alpha=0.05$.

Table 3
Output for the 2-factor mixed model ANOVA testing the effect of initial size on growth of *Cladophora* at different blocks (sites) around an operational bioremediation pond

Source	*df*	MS	*F*	*P*
Initial size	1	2.15	0.10	0.766
Block	8	111.59	5.11	<0.001
Size*block	8	22.58	1.04	0.453
Error	15	21.82		

3.4. Bioremediation potential

Estimates of natural carrying capacity based on the average density of floating mats of algae, measured as kilograms of fresh weight per square meter (kg FW m^{-2}), varied between the monsoonal wet season (1.5±0.816 kg FW m^{-2} 1SD) and dry season (1.0±0.786 kg FW m^{-2} 1SD). This equates to seasonal pond (1 ha) carrying capacities of 15,000 kg (wet season) and 10,000 kg (dry season), and represents a conservative value as some monsoonal samples contained more than 3.0 kg FW m^{-2} (giving a maximum potential capacity of 30,000 kg). Because the majority of samples across both seasons (>90%) were solely *Cladophora*, growth rates for this species were used to calculate potential nitrogen removal rates. Data used for logistic regression (below) are the approximate mean (7% day^{-1}) and upper (15% day^{-1}) growth rates of *Cladophora* from the *in situ* experiments (Section 3.3) and a mean nitrogen content of 'green tide' algae sampled from the pond of 0.58% FW (±0.139 1SD) calculated from 20 samples (mean±SD: % N dry weight=2.90±0.97, C:N=9.18±3.17).

The calculation of nitrogen removal uses the basic logistic equation: Biomass $(B)_{t+1}=B_t+r*B_t\,((K-B_t)/K)$, and assumes a constant growth rate ($r=15\%$, maximum for any block) and maximum carrying capacity ($K=15{,}000$ kg in monsoon). Growth is maximal over the linear portion of the harvestable biomass curve (5500–9500 kg) and harvest is

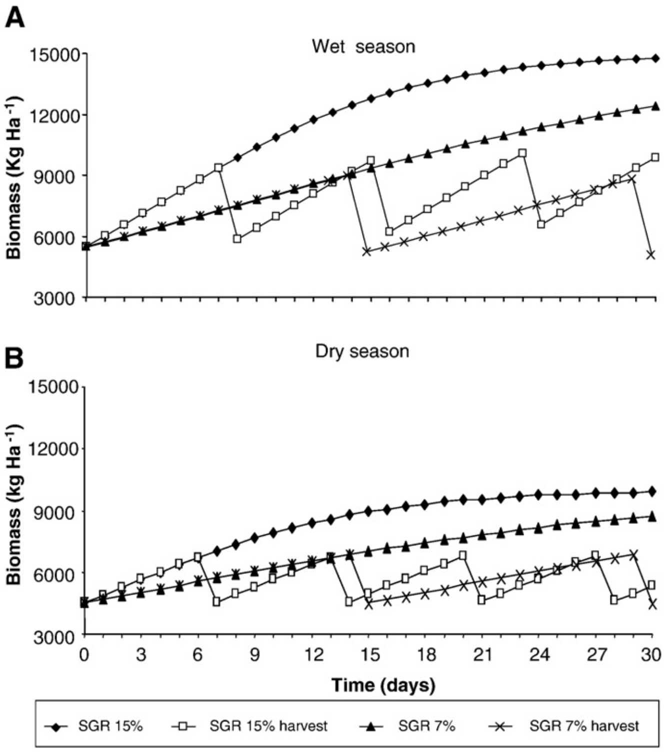

Fig. 6. Logistic growth of 'green tide' algae in monsoonal wet season (A) and dry season (B) at high (15% day^{-1}) and average (7% day^{-1}) growth rates. Models include a 4-tonne fresh weight (FW) harvesting component. Optimum stocking density (5500 kg FW wet season, 4500 kg dry season) uses the linear portion of the curves with the minimum number of harvests per unit time.

calculated for a 7-day cycle (Fig. 6A). These parameters were selected on the basis of maximal growth within the linear range of the curve and an appropriate management regime. This then equates to an approximate 4-tonne harvest from a standing stock (day 0) of 5500 kg in the pond after 7 days (Fig. 6A) with nitrogen removal of 23 kg N harvest^{-1} (equating to 3.3 kg N day^{-1} ha^{-1}).

Changes in growth rates will alter the amount that can be harvested. For example, sub-optimal growth at 7% results in a 15 day 4-tonne harvesting cycle (Fig. 6A). Furthermore, differences in the natural carrying capacity between seasons will also affect yield with decreased biomass and subsequent nitrogen removal (Fig. 6B). Each model illustrates the best case scenario based on optimum standing stock relating to growth, and limiting the frequency of harvest (monsoonal summer = 5500 kg, dry season = 4500 kg). This model also allows the addition of data from relevant growth experiments under controlled conditions to assess potential variation in productivity and nitrogen removal.

4. Discussion

The filamentous ‘green tide’ algae trialled have excellent potential for implementation in tropical integrated aquaculture systems. This is based on their ability to survive, and grow, under the conditions that characterize tropical pond-based aquaculture. Growth of *Cladophora* in particular proved to be high *in situ*. Moreover, high growth rates and tolerance to variation in salinity and TAN in the controlled environment demonstrate its potential for broader application. This study is one of the first to consider the potential role of what are essentially fouling organisms, or ‘green tide’ species, in integrated pond-based aquaculture. These forms of algae have largely been overlooked in terms of their application in bioremediation, and this is understandable given that their economic value is unclear. However, there are economic drivers which can be applied to these species, in particular where legislative control of water quality affects the productivity of aquaculture farms within limited infrastructure. This is the case for the aquaculture industry in Northern Queensland, Australia, where aquaculture adjacent to the world heritage listed Great Barrier Reef Marine National Park is regulated in terms of the nitrogen load within discharge waters (maximum total nitrogen of 3 mg l^{-1}, equating to 210 µmol l^{-1}), and the total amount of nitrogen discharged per day (1 kg ha^{-1} day^{-1}) (EPA, 2005). Any removal of nitrogen from aquaculture effluent prior to discharge provides the opportunity to increase feed inputs, and therefore productivity of fixed infrastructure, with concurrent increases in profitability. The ability to ameliorate nitrogen through a farm management plan incorporating algal removal through controlled harvest can have tangible economic benefits. Furthermore, harvested algae have potential commercial uses ranging from fertilizers (Cavallo et al., 2006) to aquaculture feeds (Valente et al., 2006).

However, any algae that can be incorporated into a management plan for aquaculture must be suitably resilient to produce efficient and reliable removal of nitrogen waste. A broad environmental tolerance was observed for all ‘green tide’ species, yet each had an optimum salinity (*Cladophora* 30‰, *Chaetomorpha* 20‰ and *Ulva* 15‰) that could serve to aid bioremediation in fluctuating environments, in particular if they are utilized as a species consortium. Notably, tropical aquaculture species (crustaceans and fish) are normally cultured between 25 and 40‰, where all three algal species had growth rates over 10% day^{-1}. Under climatic extremes the salinity of ponds can fluctuate from 5‰ to 45‰ in response to either high rainfall (wet season) or high levels of evaporations (dry season), and again these conditions are within the spectrum for growth, albeit sub-optimal. Similar high tolerance has been documented for *Cladophora*, *Chaetomorpha* and *Ulva* in salinities up to 50‰ in other regions, supporting their broad utility (Birch et al., 1981; Lavery and McComb, 1991, Cohen and Fong, 2004).

Given the highly variable nature of tropical pond-based aquaculture systems the interaction between the key environmental parameters of nitrogen and salinity will affect productivity. The results presented in this study and those for other resilient species, such as *Rhizoclonium riparum* (Chao et al., 2005), *Cladophora albida* (Gordon et al., 1981), *Ulva* species and *Chaetomorpha linum* (Taylor et al., 2001), confirm that a diversity of factors interact to affect growth. However, the transfer of these results into operational systems is the key to species selection. The performance of *Chaetomorpha* in this study highlights the difficulty of transferring controlled environment outcomes to operational farm systems. *Chaetomorpha* has excellent growth under controlled conditions approximating the temperature (28 °C ± 2.0 °C), nitrogen load (130 µmol l^{-1}), and salinity (36‰) of the operational pond, but failed to grow (essentially died) under short-term *in situ* trials. In contrast, *Cladophora* performed well under these same conditions and this may be due to its regenerative capabilities upon fragmentation. While there was variable growth of *Cladophora* across locations (−8.5 to 13.6% day^{-1}) within the pond due to variation in water quality, the survival of *Cladophora* upon fragmentation was high in all trials (100%). This is in contrast to the *Chaetomorpha*, the other uniseriate filamentous algae, for which only 25% of fragments regenerated. Survival and growth were considered in tandem for the *in situ* growth trial, a factor that influenced the highly negative growth of *Chaetomorpha*. However, given the high growth rate of *Chaetomorpha* in all controlled experiments, further research to develop fragmentation methods to improve survival is warranted.

One of the traits that bioremediation species often have is high levels of internal nitrogen (this may be organic but also inorganic forms that are stored in vacuoles) (Harrison and Hurd, 2001). Given that bioremediation potential is a function of growth and nitrogen assimilation, *Cladophora* is an excellent candidate. The mean tissue nitrogen level of 2.9% (of dry weight) is similar to that of the bioremediation species *Ulva rotundata*, 2.9%, *Enteromorpha intestinalis*, 3.2%, and *Gracilaria gracilis*, 3.4% (Hernández et al., 2002). However, *Chaetomorpha* also offers potential given its high growth rate at very high nitrogen concentrations. Under this scenario the potential also exists for higher (luxury) uptake similar to that of *Porphyra amplissima*, with high growth and nitrogen content when cultured in a high nitrogen environment (Carmona et al., 2006).

In summary we have demonstrated that ‘green tide’ algae can be cultured in ponds with high productivity and nitrogen assimilation. The optimization of growth under controlled conditions can be managed in an aquaculture context, and data can be used for the development of models for nitrogen stripping. These can be tailored to suit the activities of aquaculture facilities that typically work in cycles relating to maintenance and grow-out of stock. Providing functional growth-bioremediation models to aquaculture facilities will be critical in developing the integration of algal bioremediation in the tropics with mesocosm-like ponds, and these models can be developed to incorporate new components for more complex environmental scenarios.

The inability of traditionally high value genera such as *Gracilaria*, *Porphyra* and more recently targeted high yield species such as *Asparagopsis* (Dawes et al., 1999; Matos et al., 2006; Schuenhoff et al., 2006), to survive the broad environmental fluctuations of tropical pond-based systems provides an impetus for the selection of alternative bioremediation species. The “natural” selection and development of algae endemic to tropical pond-based mesocosms is effective, in this instance, in delivering bioremediation species. The high productivity and nitrogen removal of *Cladophora*, allied with a broad tolerance to the tropical pond-based environment, supports the integration of filamentous ‘green tide’ algae into tropical pond-based aquaculture to promote sustainability.

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