Reviews in Aquaculture (2012) **4**, 21–31 doi: 10.1111/j.1753-5131.2012.01057.x

Experiments on an integrated aquaculture system (seaweeds and marine fish) on the Red Sea coast of Saudi Arabia: efficiency comparison of two local seaweed species for nutrient biofiltration and production

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Received 17 July 2011; accepted 22 December 2011.

Abstract

Seaweeds absorb inorganic nutrient wastes from mariculture and reduce their undesirable environmental effects. Mariculture in Saudi Arabia is increasing rapidly, thus, to exploit aquaculture wastes and to reduce coastal pollution risks, local seaweeds were cultured using mariculture effluents in integration on the Red Sea coast. The aim of the present study was to test integrated aquaculture of seaweed and marine fish (*Oreochromis spilurus*) for the first time in Saudi Arabia and to determine the seaweeds, *Ulva lactuca* and *Gracilaria arcuata*, biomass production and inorganic nutrient bioremediation capabilities. Results showed that *G. arcuata* grew at a significantly higher rate (2.71% wet weight day^{-1}) than *U. lactuca* (1.77% wet weight day^{-1}). The biomass yield (42.38 g wet weight m^{-2} day^{-1}) and net yield (91.11 g wet weight day^{-1}) of *G. arcuata* were also significantly higher than *U. lactuca* (27.39 g wet weight m^{-2} day^{-1} and 58.89 g wet weight day^{-1}, respectively). *Gracilaria arcuata* removed 0.45 g m^{-2} day^{-1} of total ammonia nitrogen (TAN) with 80.15% removal efficiency and 1.03 g m^{-2} day^{-1} of soluble phosphate with 41.06% efficiency. *Ulva lactuca* removed 0.42 g m^{-2} day^{-1} of TAN with 83.06% removal efficiency and 1.07 g m^{-2} day^{-1} of soluble phosphate with 41.11% efficiency. Total tissue carbon of both species reached 25.1–26.9% and nitrogen content reached 3.0–3.2% of dry weight. The C/N ratio for both seaweeds was <10, indicating that nitrogen was not a limiting factor in culture. Both seaweeds are suitable for integrated aquaculture and bioremediation, but *G. arcuata* has relatively higher growth potential.

Key words: coastal integrated aquaculture, marine fish, Red Sea, Saudi Arabia, seaweeds.

Introduction

Aquaculture activities can cause several environmental impacts including coastal eutrophication and habitat modification (Read & Fernandes 2003). The discharge of nutrients produced by fish farming is a problem of considerable concern in many parts of the world (Gowen & Bradbury 1987; Folke & Kautsky 1989; Ackefors & Enell 1990; Neori *et al.* 2007). Aquaculture effluents laden with feed wastage, fish excretion and faeces may significantly contribute to the nutrient loading of coastal waters because the effluents are rich in inorganic nitrogen (N) and phosphorus (P) (Kautsky *et al.* 1997). These nutrients are derived from the bacterial release of inorganic N and P from non-consumed animal food, and from excretory waste products of the cultured animals (Beveridge 1987; Chopin *et al.* 2001). The detrimental effects of eutrophication include blooms of harmful phytoplankton and unwanted macroalgae (Cuomo *et al.* 1993; Naylor *et al.* 2000), as well as the development of hypoxic and anoxic conditions (Sfriso *et al.* 1992; Bonsdorff *et al.* 2002).

In this context, the promotion of more sustainable aquaculture practices for coastal aquaculture has been strongly emphasized (Naylor *et al.* 2000; Wurts 2000; Troell *et al.* 2003; Neori *et al.* 2004). The integration of finfish aquaculture with macroalgal (seaweeds) culture is one such practice for the bioremediation of the waste-laden effluents; in this set-up seaweeds are grown downstream from the animals (Chung *et al.* 2002; McVey *et al.* 2002). Eutrophic inputs of N and P from finfish farming can be reduced using an integrated approach that combines the aquaculture of marine macroalgae with finfish (Folke *et al.* 1994; Krom *et al.* 1995; Fei *et al.* 1998; Chopin *et al.* 2001; Yang & Fei 2003; Neori *et al.* 2004). The marine macroalgae benefit from the co-culture with finfish because the algae require dissolved N and P, which are waste products from the finfish aquaculture.

In addition to the ecological aspect, integrated aquaculture also has economic incentives because the nutrients contained in effluents such as N and P could be channelled into the production of valuable products that are otherwise flushed from the system (Chopin *et al.* 2001). The use of macroalgae as nutrient strippers in integrated aquaculture is an excellent example of ecotechnology (e.g. Neori *et al.* 2004). Modern integrated mariculture systems, seaweed-based in particular, are bound to play a major role in the sustainable development of coastal aquaculture (Neori *et al.* 2004).

The best seaweed to integrate into an animal aquaculture operation is one characterized by rapid growth, the accumulation of N and P to high levels in tissue, and some added value (Neori *et al.* 2004). Species of the genus *Ulva* are usually preferred in biofiltration studies owing to a high biomass production and biofiltering efficiency (e.g. Neori *et al.* 1996). The genus *Gracilaria* (Rhodophyta) has also been shown to be a most attractive candidate for intensive culture because of its ability to achieve high yields and produce commercially valuable products (e.g. Buschmann *et al.* 1996). *Gracilaria* species, being an efficient nutrient pump, offer both high bioremediation efficiency and commercial value in established markets, such as agar–agar, human consumption and fodder for other high-valued aquaculture organisms such as abalone (Chopin *et al.* 2001; Fei 2004; Neori *et al.* 2004). Gracilarioid species (mainly *Gracilaria*, but also *Gracilariopsis*) can contribute to the efficient removal of dissolved P and N wastes from intensive fish farms, increasing the economic output of the activity (Buschmann *et al.* 1996; Troell *et al.* 1997; Alcantara *et al.* 1999; Jones *et al.* 2001).

Owing to the rapid growth of aquaculture in recent years on the coasts of Saudi Arabia, there are increasing concerns with regard to reducing the adverse environmental impact of aquaculture and environmental aspects are beginning to receive more attention. As part of an effort to develop an environmentally friendly integrated aquaculture technology, we have been evaluating a tank-based integrated system for bioremediation of effluents using the red alga *Gracilaria arcuata* and the green alga *Ulva lactuca*, which are locally available in the Red Sea off the Jeddah coast of Saudi Arabia. A possible reduction in nutrient concentrations in seawater effluents and diversification of production in changing market conditions might be considered to be additional sources of income by aquaculture entrepreneurs in Saudi Arabia. This issue is highly relevant for a growing aquaculture industry in Saudi Arabia (FAO 2010) to reduce the environmental risk to an oligotrophic sea that has high biodiversity (Khalil & Abdel-Rahman 1997; Baars *et al.* 1998). The overall aim of this research is to select appropriate species and to test and develop integrated aquaculture innovations relevant to local conditions prior to increasing the scale. The present study is a small step forward before a thorough assessment of the costs and benefits (financial, economic, social, environmental) is undertaken as a comparative assessment of integrated aquaculture relative to other resource uses.

Materials and methods

Integrated aquaculture system

An integrated coastal aquaculture system using fibreglass tanks was installed at the Fish Farming Center of the Ministry of Agriculture, Obhur (Jeddah), Saudi Arabia, as per the design depicted in Figure 1. The system was composed of three components: a fish culture tank, followed

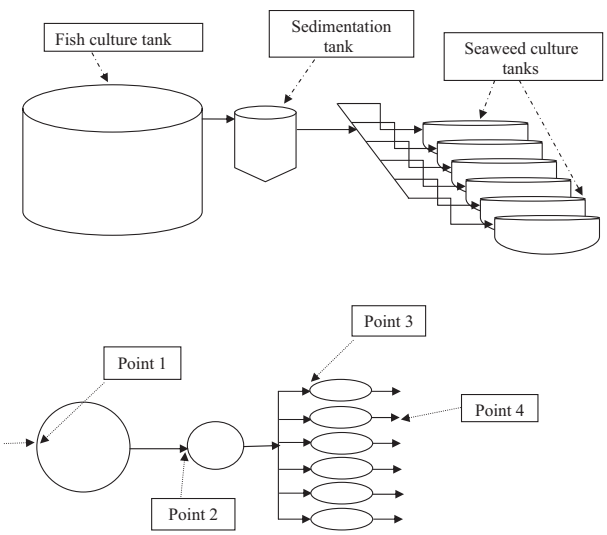

Figure 1 Diagrammatic presentation of the layout of the integrated tank-based aquaculture system for the marine fish and seaweed culture experiments. The figure at the base indicates the water-sampling points in the culture system (see Table 1). Water sampling points 3 and 4 correspond to paired samples for each seaweed culture tank.

by a sedimentation tank and then seaweed culture tanks. The fish culture tank was round with a conical bottom (3.1 m in diameter, 1.65 m depth in the centre and 1.4 m depth on the periphery; total volume approximately 11 m^3). The sedimentation tank was round with a conical bottom (1.3 m in diameter, 1.25 m depth in the centre and 1 m depth on the periphery; total volume approximately 1.44 m^3). There were six oval round bottom seaweed culture tanks (each with a total length of 1.85 m, a width of 1.24 m and filled to a total water volume of 1 m^3 with a surface area of 2.15 m^2 per tank). Moderate aeration was provided to the aquaculture tanks using polyethylene tubes (2.5 cm in diameter) placed at the bottom of the seaweed tanks and connected to an air blower. Holes measuring 2.5–3 mm were spaced at 10-cm intervals along the pipes, so that the bubbles produced stirred and moved the seaweed inside each culture unit.

Experimental set-up

To test the suitability of local seaweeds in integrated aquaculture, two local seaweed species, *Ulva lactuca* and *Gracilaria arcuata*, were selected to test their biofiltration capacity and growth. Seaweeds were collected from a common habitat in shallow water off the Jeddah coast and taken to the experimental site in buckets. The inoculums were washed and cleaned of debris and associated algae or any possible epiphytes before stocking.

The experimental fish culture tank received 10 m^{-3} seawater from a well located adjacent to the coast and was stocked with 200 kg (at a stocking density of 200 fish m^{-2} and a rate of 20 kg m^{-3}) of *Oreochromis spilurus* with an average weight of 101.29 ± 4.03 g. The fish were fed twice daily (at 08.00 and 13.00 hours) with a 30% protein diet (derived from fish meal) at 2% of the biomass (1% at each feeding) and the total feed given per day was recorded. A centre drain was used to remove solids from the cone-bottom tank. Fish effluents from the sedimentation tank were allowed to flow by gravity to the six seaweed culture tanks. Three seaweed tanks were stocked with 3 kg of *U. lactuca* per tank and the other three tanks were stocked with 3 kg of *G. arcuata*; that is, each seaweed species was stocked in three replicated tanks. The water flow in each tank was set to approximately 225 L h^{-1}, which was equivalent to 3.75 L min^{-1}, so that each seaweed tank had 5–6 water turnovers per day. The airflow was adjusted to be strong enough to allow the rotation of seaweed at a rate of three to fourfold per minute in the tanks. The experiment was designed to run for 30 days and every 10 days the seaweed biomass were re-stocked to the initial density in each tank; thus, the experiment was repeated three times.

Water quality and other parameters

The water-quality parameters of temperature, pH, dissolved oxygen and salinity were measured using a WTW multiline P4 multimeter (WTW, Weilheim, Germany) and ammonia-N, nitrite-N, nitrate-N and phosphate-P were analysed using a Hach spectrophotometer (DR2800; Hach, Düsseldorf, Germany) from samples collected at four different points in the system at different frequencies as shown in Table 1 and Figure 1. Light irradiance was determined with a quantum scalar irradiance meter (QSL-101; Biospherical Instruments, San Diego, California, USA).

Fresh weights of the seaweeds were determined after removing any visible epiphytes or other undesired algae at the initial point and after every 10 days using drainage procedure (i.e. by taking all of the seaweed from one tank and letting the water trickle for 5 min, then shaking the seaweed up and down 3–5 times and then measuring the total wet weight. The seaweed stocking was adjusted in

Table 1 Summary of the variables that must be monitored during the seaweed culture experiments using fish effluents

Variables	Measurement points	Frequency
Seaweed biomass	Seaweed tanks	Every 10 days
Temperature	Points 1, 3 and 4	Daily – early morning, noon and late afternoon
Salinity	Points 1, 3 and 4	Daily at noon
Dissolved oxygen	Points 1, 3 and 4	Daily at noon
pH	Points 1, 3 and 4	Daily – early morning, noon and late afternoon
Nutrient concentration	Points 1, 3 and 4	Days 2, 5 and 9 of each experiment – early morning, noon and late afternoon
Organic material	Points 1, 2 and 3	Days 2, 5 and 9 of each experiment and taken at noon
Sludge	Point 2	At the completion of each experiment (every 10 days)
Carbon, hydrogen, nitrogen	Seaweed tissues	At the start (day 1) and end (day 10) of each experiment
Water flow	Point 3	Daily
Seaweed aeration control	Seaweed tank	Weekly
Fish feeding	Point 1	Daily
Fish biomass and density	Fish tank	Every 10 days

triplicate for both seaweeds to the original 3 kg in each tank every 10 days by taking out any excess seaweed.

Fish growth

The daily weight gain (DWG) expressed as g fish day^{-1}, net production (NP) expressed as kg m^{-3} day^{-1} and feed conversion ratio (FCR) were calculated using the following formulae:

$$\text{DWG} = (\text{final weight} - \text{initial weight})/\text{no. fish}/\text{time (days)}$$

$$\text{NP} = (\text{final biomass (kg m}^{-3}) - \text{initial biomass (kg m}^{-3}))$$

$$\text{FCR} = \text{total dry feed fed (kg)}/(\text{final fish biomass (kg)} - \text{initial fish biomass (kg)}).$$

Seaweed growth

The seaweed specific growth rate (SGR) (% wet weight day^{-1}), biomass yield (Y) (g wet weight m^{-2} day^{-1}) and net yield (NY) (wet weight day^{-1}) were determined according to Evans (1972) and calculated as:

$$\text{SGR (\%)} = 100 \times [\ln W_t - \ln W_0)]/t$$

$$Y \text{ (g wet weight m}^{-2} \text{ day}^{-1}) = [(W_t - W_0)/t]/\text{SA}$$

$$\text{NY (g wet weight day}^{-1}) = [\text{final biomass (m}^3) - \text{initial biomass (m}^3)]/\text{time (days)}$$

where W_0 and W_t are the initial and final wet weights in grams, t is the time in days and SA is the surface area of each seaweed tank.

Biofiltration efficiency of the seaweeds

Twice per week, water samples were taken at the inflows and outflows of the seaweed tanks to measure TAN, and to analyse nitrite and nitrate. The average reduction in TAN concentration between the inflows and the outflows of the tanks ($n = 3$ for each culture condition) is expressed as a percentage and defined as TAN uptake efficiency or TAN removal efficiency. The amount of TAN removed per unit of time per unit of area by the seaweeds represented the nitrogen uptake rate or TAN removal rate and was calculated using a formula adapted from Evans and Langdon (2000):

$$\text{TAN uptake efficiency (\%)} = (S_\text{i} - S_\text{o})/S_\text{i} \times 100$$

$$\text{TAN uptake rate (g m}^{-2} \text{ day}^{-1}) = Q(S_\text{i} - S_\text{o})/A/T$$

where Q is the flow rate, S_i is the TAN inflow (g L^{-1}), S_o is the TAN outflow (g L^{-1}), A is the tank surface (m^2) and T is time. The same formulae were used to calculate phosphate uptake efficiency and phosphate uptake rate.

Seaweed tissue composition

At the end of each experimental repetition, seaweed tissues from each tank in the integrated system were ground to a fine powder to determine the percentage of carbon (C) and nitrogen (N) present in the seaweed tissues. Fresh tissue (50 g) from both seaweed species was collected at the start and end of each of the three culture trials. The tissues were dried at 65°C for 24 h and then stored in a dry place until C and N determinations at King Abdulaziz City for Science and Technology's Environment Technology Laboratory. Samples were kept in the oven (40°C) for 24 h to eliminate any residual moisture, ground to make a powder and analysed for total C and N tissue content using a Perkin Elmer Model 240c CHN analyser (Perkin Elmer; Waltham, Massachusetts, USA).

Statistical analysis

The growth, daily variations in nutrient uptake and uptake efficiency, and C and N contents of the two species of seaweeds over the culture period (30 days) were compared using a two-way ANOVA after assessing normality and homogeneity using Shapiro–Wilk's and Levene's tests, respectively. One-way ANOVAs were used to test the significance of differences among means of the biomass parameters after assessing the normality and homogeneity of the data. All statistical tests were carried out using IBM SPSS Statistics version 19 (SPSS 2009).

Results and discussion

Culture conditions

Daily daylight irradiance ranged from 950 to 1200 μmol photon m^{-2} s^{-1} with almost all days sunny during the experiment. Water temperature, dissolved oxygen, pH, total ammonia nitrogen (TAN), nitrite (NO_2–N), nitrate (NO_3–N) and phosphate (PO_4–P) values in the fish culture tank are listed in Table 2 and in the inflows and outflows of the seaweed culture tanks in Table 3. Seawater temperature fluctuated during the experiment and ranged between 20.5 and 30.6°C; however, salinity showed no fluctuations and was always approximately 42 g L^{-1}. Dissolved oxygen (DO) in the fish tank ranged from 0.85 to 6.2 mg L^{-1}, whereas the values of DO in the inflow and the outflow of the seaweed tanks was 2.92 and 5.59 mg L^{-1}, respectively. The mean pH value in the fish culture tank was 7.57 and in the seaweed tanks it was 8.04, showing additional CO_2 available for photosynthesis as a result of fish respiration. In the fish culture tank,

Table 2 Water quality in the system in the morning, afternoon and evening at fish culture tank supply (point 1)

Parameters	Fish culture tank							
	Morning		Afternoon		Late afternoon		Mean	
	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)
Water temperature (°C)	20.5–30	28.13 (2.61)	21.8–30.6	29.00 (2.51)	21–29.8	28.22 (2.41)	20.5–30.6	28.45 (2.51)
Dissolved oxygen (mg L^{-1})			0.85–6.2	2.71 (1.71)			0.85–6.2	2.71 (1.71)
pH	7.03–7.81	7.54 (0.19)	7.26–7.85	7.57 (0.18)	7.22–7.98	7.60 (0.18)	7.03–7.98	7.57 (0.18)
Salinity (g L^{-1})		42		42		42		42
Ammonia-N (mg L^{-1})	0–0.01	0.001 (0.003)	0–0.05	0.006 (0.017)	0–0.13	0.018 (0.04)	0.01–0.13	0.008 (0.02)
Nitrite-N (mg L^{-1})	0–0.6	0.08 (0.02)	0–2.8	0.36 (0.92)	0–1.7	0.27 (0.56)	0.001–2.8	0.45 (0.50)
Nitrate-N (mg L^{-1})	0–1.8	0.3 (0.62)	0–0.3	0.06 (0.11)	0–2.2	0.57 (0.93)	0.001–2.2	0.48 (0.55)
Phosphate-P (mg L^{-1})	0.13–1.4	0.79 (0.45)	0–1.5	0.81 (0.61)	0.03–1.21	0.75 (0.42)	0.03–1.5	0.89 (0.49)

SD, standard deviation.

TAN values ranged between 0.01 and 0.13 mg L^{-1}, nitrite ranged from 0.001 to 2.8 mg L^{-1}, nitrate fluctuated between 0.001 and 2.2 mg L^{-1} and phosphate ranged from 0.03 to 1.5 mg L^{-1}. Mean values of TAN, nitrite, nitrate and phosphate were 0.23, 0.69, 0.77 and 0.75 mg L^{-1}, respectively, in the inflow of the seaweed tanks (Table 3). The mean values of the same parameters in the outflow of the seaweed tanks were 0.72, 0.81, 0.74 and 0.58 mg L^{-1}, respectively, showing a threefold increment in TAN and a slight increment in nitrate owing to fish excretion and feed decomposition.

Fish growth

Fish growth data are summarized in Table 4. The initial and final average weights for the fish during the experiment in the integrated system were 101.3 and 146.1 g $fish^{-1}$, respectively. Initial biomass in the fish-rearing tank was 197 kg and the final fish biomass was 265 kg. The daily weight gain was 1.55 g fish day^{-1} and the total weight gain and net production were 68 kg and 6.8 kg m^{-3}, respectively. The value for FCR was found to be 1.85 and survival was 92.97% during the experimental period of 1 month.

Marine tilapia, *O. spilurus*, was chosen because of its hardiness, good growth rate and ease of culture, in addition to the tolerance of this species to seawater. Commercially, fish weighing 30–120 g are reared at a stocking rate of 200–300 fish m^{-3}, fed a 30–34% protein diet and production is reported to be 30–40 kg m^{-3} (FFC 2007). Similar stocking rates and feeding were used in our study to indicate the relevance and reality of the outcome. Preliminary work on this species by Al-Amoudi (1987) indicated the potential of this species for marine culture in Saudi Arabia based on its tolerance to seawater. Vine (1980) also reported culture of *O. spilurus* in seawater cages in the Red Sea in Saudi Arabia and recommended it for commercial farming.

During the 29 days of rearing in the integrated system, fish were fed daily with 2% body weight following Al-Ahmad *et al.* (1988) who studied growth and production of *O. spilurus* in seawater tanks, raceways and cages in Kuwait and determined a daily feed ration of 2% body weight to be optimum for fish ranging in size from 70 to 130 g. According to Al-Ahmad *et al.* (1988), the growth rate of *O. spilurus* in seawater tanks was 1.28 g fish day^{-1} with a production of 6.1 kg m^{-3} $month^{-1}$, feed conversion ratio of 1.37 and survival ranging from 93.0% to 98.7%. These results are comparable to our results, except that daily weight gain (1.55 g fish day^{-1}) is much better in our integrated system; however, their FCR is superior. Cruz *et al.* (1990) also reared *O. spilurus* in flow-through seawater tanks and reported much higher daily weight gain, ranging from 2.07 to 3.49 g day^{-1}, with a feed conversion ratio ranging between 1.47 and 2.13 and survival rates from 94.99% to 97.71%.

Seaweed growth and production

The red seaweed *G. arcuata* showed a significantly ($F_{1,12} = 9.294$, $P < 0.01$) higher SGR (2.71 % wet weight day^{-1}) compared with the green seaweed *U. lactuca* (mean SGR = 1.77% wet weight day^{-1}) (Fig. 2; Table 5). There was no significant change in growth between the three series of 10 days each ($F_{2,12} = 0.249$, $P > 0.78$). In addition, the interaction of species and time did not show any significant differences ($F_{2,12} = 0.291$, $P > 0.752$), indicating that at all times *G. arcuata* maintained a higher growth rate than *U. lactuca*. The biomass yield (42.38 g wet weight m^{-2} day^{-1}) of *G. arcuata* also showed a significantly ($F_{1,16} = 10.632$, $P < 0.005$) higher value than *U. lactuca* (27.39 g wet weight m^{-2} day^{-1}).

Table 3 Water quality of the effluents from the sedimentation tank (inflow to the seaweed tanks) (point 3) and the effluents (outflow) from the seaweed tanks (point 4) in the morning, afternoon and evening

Parameters	Inflow to the seaweed tanks					Outflow from the seaweed tanks				
	Morning Mean (SD)	Afternoon Mean (SD)	Late afternoon Mean (SD)	30 day Range	30 day Mean (SD)	Morning Mean (SD)	Afternoon Mean (SD)	Late afternoon Mean (SD)	30 day Range	30 day Mean (SD)
Water temperature (°C)	27.48 (2.61)	28.31 (2.51)	27.79 (2.41)	20–30.4	27.86 (2.45)	25.9 (2.54)	27.09 (2.09)	26.63 (2.02)	18.4–29.8	26.54 (2.28)
Dissolved oxygen (mg L^{-1})	–	2.92 (1.29)	–				5.59 (1.52)			
pH	7.55 (0.14)	7.60 (0.19)	7.56 (0.39)	7.1–8.1	7.57 (0.27)	7.97 (0.39)	8.06 (0.26)	8.07 (0.19)	7.27–8.9	8.04 (0.3)
Salinity (g L^{-1})					42					42
Ammonia-N (mg L^{-1})	0.20 (0.51)	0.21 (0.48)	0.21 (0.57)	0.01–0.6	0.23 (0.17)	0.07 (0.29)	0.03 (0.2)	0.03 (0.16)	0.0–0.3	0.07 (0.06)
Nitrite-N (mg L^{-1})	0.56 (3.7)	0.59 (5.9)	0.47 (2.7)	0–0.6	0.69 (1.05)	0.52 (3.3)	0.49 (3.4)	0.42 (3.5)	0.0–3.5	0.81 (1.76)
Nitrate-N (mg L^{-1})	0.44 (2. 3)	0.86 (4.5)	0.48 (2.7)	0–4.5	0.77 (0.89)	0.60 (3.7)	0.58 (3.9)	0.34 (1.6)	0.0–3.9	0.74 (0.95)
Phosphate-P (mg L^{-1})	0.62 (1.27)	0.84 (1.9)	0.70 (2.64)	0–2.6	0.75 (0.51)	0.51 (1.24)	0.66 (2.63)	0.56 (1.33)	0.06–2.6	0.58 (0.66)

SD, standard deviation.

Table 4 Fish (*Oreochromis spilurus*) stocking and growth data in the integrated system

Parameters	Mean ± standard deviation
Average initial fish weight (g)	101.3 ± 4.03
Total initial fish biomass (kg)	197
Total initial fish number	1950
Average final fish weight (g)	146.1 ± 4.9
Total final fish biomass (kg)	265
Total final fish number	1813
Fish survival rate (%)	92.97
Culture period (days)	29
Weight gain (g fish day^{-1})	1.55
Total gain (kg)	68
Net production (kg m^{-3})	6.8
Feeding rate (%)	2
Total feed consumed (kg)	125.2
Feed conversion ratio	1.84

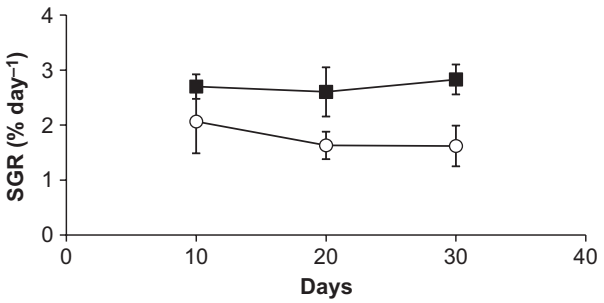

Figure 2 Mean (± standard deviation; $n = 3$) of the specific growth rates (SGR) of *Ulva lactuca* (○) and *Gracilaria arcuata* (■) during the 30 day experiment.

These production values allowed us to obtain a net yield of 91.11 g wet weight day^{-1} for *G. arcuata* that was also significantly ($F_{1,16} = 10.637$, $P < 0.005$) higher than the yield for *U. lactuca* (58.89 g wet weight day^{-1}) (Table 5). Although the growth rate of *G. arcuata* in the present study is less than that reported in previous studies (e.g. Ryther *et al.* 1975 for *G. foliifera*), the biomass production value of *G. arcuata* (91.11 g wet weight day^{-1}) is in the range of previous studies in Florida (62.2 g wet weight day^{-1}; Hanisak 1987) and in Chile (70.6 g wet weight day^{-1}; Ugarte & Santelices 1992). As these first experimental trials have a longer term to be optimised and with the technological developments described by Neori *et al.* (2004), we expect to increase these values in future, reaching above 150 g wet weight day^{-1} as found in some previous studies (Lapointe *et al.* 1976).

Nutrient uptake rate and removal efficiency

Gracilaria arcuata removed a significant amount of TAN, ranging from 0.34 to 0.55 g m^{-2} day^{-1}, which is

Table 5 Specific growth rate, biomass yield, net yield, nutrient uptake rates, nutrient uptake efficiencies and nutrient removal efficiencies (± standard deviation) of the seaweeds *Gracilaria arcuata* and *Ulva lactuca*

Parameters	*G. arcuata*	*U. lactuca*	*F*-values (df)	*P*-values
Biomass yield (g wet weight m^{-2} day^{-1})	42.38 ± 7.95	27.39 ± 11.26	10.632 (1,18)	0.0049
Net yield (g wet weight day^{-1})	91.11 ± 17.1	58.89 ± 24.21	10.637 (1,18)	0.0049
TAN uptake rate (g m^{-2} day^{-1})†	0.45 ± 0.37	0.42 ± 0.34	0.062 (1,27)	0.8040
TAN uptake efficiency (%)†	80.15 ± 28.17	83.06 ± 30.38	0.118 (1,27)	0.7334
Phosphate uptake rate (g m^{-2} day^{-1})†	1.03 ± 0.37	1.07 ± 0.34	0.010 (1,27)	0.9197
Phosphate uptake efficiency (%)†	41.06 ± 25.5	41.11 ± 27.72	<0.001 (1,27)	0.9960

TAN, total ammonia nitrogen.
†Values recorded 1 h after the second feeding (14.00 hours). The water flow was approximately 5.4 m^3 day^{-1} $tank^{-1}$ (5–6 water turnovers per day).

Table 6 Daily variation in uptake rates (g m^{-2} day^{-1}) and uptake efficiencies (%) of the seaweeds *Gracilaria arcuata* and *Ulva lactuca* in the morning, afternoon and late afternoon

Parameters	*G. arcuata*			*U. lactuca*		
	Morning	Afternoon	Late afternoon	Morning	Afternoon	Late afternoon
TAN uptake efficiency (%)	66.47 ± 35.62[a]	80.15 ± 27.22[b]	86.92 ± 16.66[b]	58.13 ± 37.40[a]	83.06 ± 16.26[b]	84.74 ± 27.58[b]
Phosphate uptake efficiency (%)	29.99 ± 23.9[a]	41.06 ± 29.32[a]	31.65 ± 23.68[a]	35.24 ± 28.99[a]	41.11 ± 28.37[a]	30.97 ± 24.13[a]

TAN, total ammonia nitrogen.
Values represent mean ± standard deviation and different superscript letters indicate statistically significant differences (*post-hoc* Tukey's honestly significant difference test; $P < 0.05$).

equivalent to a removal efficiency of 66.47–86.92%. This amount was not statistically significantly different to the other algal species tested (*U. lactuca*), which showed values ranging from 0.28 to 0.42 g m^{-2} day^{-1}, equivalent to 58.13–84.74% of removal efficiency (Table 5). In the case of soluble phosphate an average uptake of 1.03 g m^{-2} day^{-1} was achieved for a removal efficiency of 41.06% for *G. arcuata*, values that were not statistically different from those achieved by *U. lactuca* (Table 5). These results indicated that both seaweeds are equally suitable as a seaweed/fish integrated mariculture system model for bioremediation. Previous studies using *U. lactuca* have shown a mean ammonia-N removal rate of 49–56%, and fluxes of 4.8–5.2 g m^{-2} day^{-1} using marine fishpond effluents (Cohen & Neori 1991). An abalone and macroalgae culture system removed 55% of ammonia-N at a flux of 4 g m^{-2} day^{-1} (Neori *et al.* 1998). The TAN removal efficiency (64%) in the present study is similar to that reported by Schuenhoff *et al.* (2003) for an integrated fish and seaweed (*U. lactuca*) system. In the case of *Gracilaria vermiculophylla*, removal of 2.01 g m^{-2} day^{-1} of nitrogen and a removal efficiency of 80% have been reported (Abreu *et al.* 2011). The removal efficiency of orthophosphate in our macroalgae biofilter tank was similar to that reported in other studies (Cohen & Neori 1991; Neori *et al.* 1998; Schuenhoff *et al.* 2003). Nevertheless, as *G. arcuata* produced a higher biomass than *U. lactuca* under the environmental conditions provided in the existing study area it might be possible to get higher economic revenue by using this red alga.

The daily variations in the removal efficiencies of TAN and phosphate are presented in Table 6. For the case of the TAN removal efficiencies, no significant ($F_{1,75} = 0.149$, $P > 0.700$) differences between the species were found; however, TAN removal efficiencies varied significantly over time ($F_{2,75} = 4.297$, $P < 0.015$), showing lower values in the early morning for both species (Table 6). No significant variations in time or between species were found for phosphate uptake efficiencies (Table 6).

The seaweed cultivated in our tank systems appeared to be able to remove 1.8 g of ammonia per day, representing 75% of the ammonia produced by the fish. In addition, this study also showed a high maximum nitrate removal efficiency of 40% and a total nitrate removal efficiency of 17%. Therefore, the seaweed tanks were able to remove 1.8 g of ammonia per day, roughly 75% of the ammonia produced by *O. spilurus*. Evidently, nitrogen concentrations in a recirculating system can be maintained at low levels, but the control of phosphate concentration by seaweed is less efficient than that recorded in previous studies (e.g. Neori *et al.* 1991; Buschmann *et al.* 1996).

Carbon and nitrogen storage

The carbon content in the tissues did not vary significantly over time or between species ($F_{3,16} = 2.487$, $P > 0.098$ and $F_{1,16} = 0.132$, $P > 0.721$, respectively), showing values of approximately 20–22% of the dry weight (Fig. 3a). In the case of N, there was no significant difference between the species ($F_{1,16} = 0.134$, $P > 0.719$), but there was a significant ($F_{3,16} = 9.403$, $P < 0.001$) change in time (Fig. 3b). Tukey's HSD (Honestly Significant Difference) test showed that the N content was significantly ($P < 0.05$) lower and the initiation (1–2%) of the culture experiment with respect to the three later culture intervals were the nitrogen reached values above 3%. The C/N ratio showed significantly ($F_{3,16} = 7.236$, $P < 0.002$) higher values at the beginning of the experiment (varied between 15 and 20 on average) and reached average values of eight by the end of the experiment (Fig. 3c). No significant ($F_{1,16} = 0.022$, $P > 0.883$) differences were found between *U. lactuca* and *G. arcuata* (Fig. 3c). As C/N ratios are an indication of the nutrient uptake capacity of seaweeds they show whether or not the seaweeds are limited by N (Hanisak 1990). The results found in the present study indicate that the *U. lactuca* and *G. arcuata* plants collected in nature were N limited because their C/N values were above 15 (Fig. 3c). The C/N ratio inversely correlates with N-enriched fish effluents that allowed reducing the C/N values down to 8, which are similar to those obtained for *U. lactuca* by Neori *et al.* (1991). In the case of *Gracilaria*, it has been shown that C/N values below 10 will not influence the N uptake rate (D'Elia & DeBoer 1978; Abreu *et al.* 2011). The fact that both studied species showed a linear increment in N in the tissues when cultivated using fish effluents appears to be an indication of improved growth conditions, including high water movement and a continuous supply of ammonia without temperature and light limitation (Hurd 2000; Harrison & Hurd 2001; Abreu *et al.* 2011).

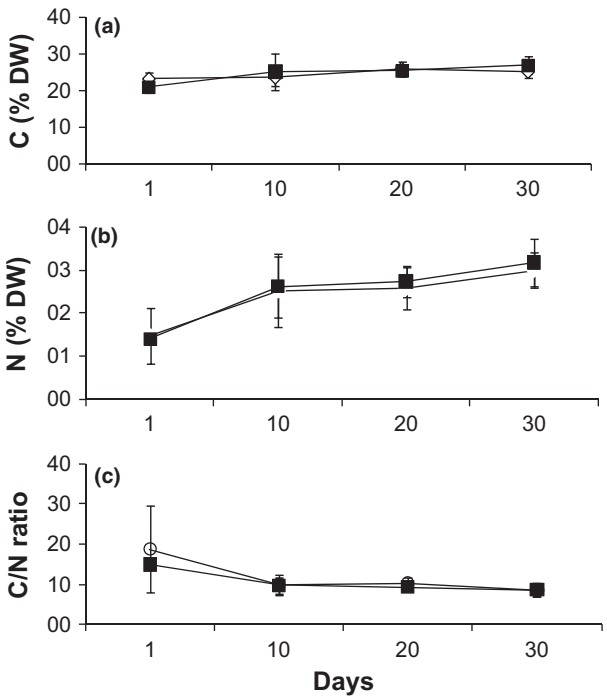

Figure 3 Mean (± standard deviation; $n = 3$) of the percentage values of (a) carbon and (b) nitrogen in the tissues [based on dry weight (DW)] and (c) the C/N ratio of *Ulva lactuca* (○) and *Gracilaria arcuata* (■) during the 30 day experiment.

Applicability of this research

The Kingdom of Saudi Arabia is rich in marine resources with 1600 km of coastline along the Red Sea and 500 km along the Arabian Gulf; this coastline includes sheltered bays, mangrove swamps, mud flats and onshore plains that provide suitable sites for either land-based farms or for cage and pen culture. The Kingdom has untapped potential to exploit this valuable resource with the development of a marine aquaculture industry. The Red Sea coast, in particular, has stable salinity (42–44 p.s.u.) and warm sea temperatures (21–31°C) that are more suitable for marine aquaculture than those of the Arabian Gulf (42–55 p.s.u. and 12–35°C). Stable salinity and warm temperatures throughout the year on the Red Sea coast present a promising proposition for diversifying and augmenting the aquaculture industry. Growing the marine aquaculture industry in the Kingdom must leverage modern scientific knowledge and engage in diligent monitoring in order to avoid the ecological pitfalls that have plagued conventional fish farms around the world (e.g. Naylor *et al.* 2000; Tett 2008; Buschmann *et al.* 2009; Burridge *et al.* 2010; Hargrave 2010). There is a strong possibility that the research work presented in this paper may have significant application in diversifying and developing sustainable aquaculture in Saudi Arabia.

Marine fish farms on the Red Sea coast are land based to produce marine fish and shrimp with essentially open systems with large volumes of effluent discharged to the sea. There are three main types of facilities used for marine finfish farming on the Red Sea coast: (i) large extensive ponds; (ii) semi-intensive ponds; and (iii) semi-intensive hatchery–nursery systems. Downstream from the rearing ponds, sedimentation ponds and long zigzag fashioned canals are used to reduce particulate matter exportation. These settling tanks or sedimentation basins readily collect particulate matter in effluents, but for dissolved material (organic and mineral nitrogen and

phosphorus), mechanical filtration is not efficient. Best management practices and policies are going to be increasingly restrictive for nitrogen and phosphorus discharge.

New technologies contributing to sustainable aquaculture development are nevertheless emerging around the globe including integration of seaweed culture with finfish farming. Hussenot and Lejeune (2000) developed a system of physical water treatment by foam fractionation adapted to open-air systems in France, to be put directly into canals or discharge ponds. A phytoplankton production system converting fishpond wastewater into nutrients for diatoms (Lefebvre *et al.* 1996; Hussenot *et al.* 1998) or seaweeds (Neori *et al.* 2004) can reduce inorganic dissolved nutrients in aquaculture effluents. Results from our research indicate that seaweed is a good candidate for seaweed/fish integrated mariculture for bioremediation and economic diversification. The integration can benefit the economy and environment in a sustainable manner in coastal waters of Saudi Arabia. As has been experienced in the present study, seaweed growth was very encouraging with biomass doubling in <2 weeks and bioremediation of significant amounts of nutrient wastes from the aquaculture effluents. It will be interesting to apply the results of this research at a larger scale to realistically report the ratios for the commercial application of integrating seaweed culture to traditional fish/shrimp aquaculture in coastal Red Sea farms.

Conclusions

The suitability of seaweed species for integrated aquaculture may differ depending on the type of culture operation and the local environmental conditions. Nelson *et al.* (2001) found that in Hawaii, *G. parvispora* was well suited to culture in pond effluent in extensive production systems: it became established in the effluent ditches and persisted as a dominant species without direct management. However, Neori *et al.* (1996) found that for treating fish culture effluent with tank-cultured seaweeds, the Chlorophyte *Ulva* was highly effective, but the Rhodophyte *Gracilaria* performed poorly. Various strategies for integrating seaweed cultivation with fish culture have been successful. Buschmann *et al.* (1994) found that effluents from intensive tank cultures of salmon in Chile were effective in the production of *G. chilensis* in tank cultures. In Sweden, Haglund and Pedersén (1993) found that *G. tenuistipitata* worked well in co-cultivation with rainbow trout, particularly during the warmer months of the year. In Israel, the green alga *U. lactuca* was found to be an attractive candidate for production with effluent from the culture of the gilthead bream *Sparus aurata* (Vandermeulen & Gordin 1990). According to Nelson *et al.* (2001), results obtained from one aquaculture system may not be applicable in different environments. In Israel, Friedlander *et al.* (1991) found that the result of fertilization on the growth of *G. conferta* varied considerably among seasons. Although seasonal variations in environmental conditions are much less pronounced on the Saudi Arabian Red Sea coast the improvement and optimisation of this integrated aquaculture culture unit will move into an optimisation phase in the near future.

In the present study, we have shown how the concept of integrated production can be applied in the management of commercial aquaculture systems in Saudi Arabia. As has been shown in many studies (Cohen & Neori 1991; Neori *et al.* 1991; Schuenhoff *et al.* 2003), seaweeds used for human consumption have relatively high economic value and can contribute substantially to the economic viability of integrated aquaculture systems. The integration of *U. lactuca* and *G. arcuata* farming with land-based aquaculture offers similar opportunities for the Saudi Arabian Red Sea coast and other areas with similar environmental conditions, which are particularly relevant for maintaining the oligotrophic conditions of this sea by reducing the environmental impacts of a growing local aquaculture industry.

Acknowledgements

The authors acknowledge financial support from King Abdulaziz City for Science and Technology, Riyadh, and the facilities provided by the Fish Farming Center of the Ministry of Agriculture, Jeddah, which enabled this research to be conducted.

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